ASSESSMENT OF ANTIPYRETIC AND ANALGESIC ACTIVITY OF HINGULESHVARA RASA IN EXPERIMENTAL ANIMALS

A Thesis

SUBMITTED TO THE

TILAK MAHARASHTRA VIDYAPEETH, PUNE

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY
In
RASASHASTRA EVAM BHAISHAJYA KALPANA

Under the Board of Ayurved Studies

Estd. 1921

Submitted by
DR SHARADINI ANIKET KARAMBELKAR
M.D. (Rasa Shastra)
(PRN NO.: 05614007209)

Under the Guidance of
DR. S. S. SAVRIKAR
M.D. (Ayurved), Ph.D.

DEPARTMENT OF AYURVEDA
TILAK MAHARASHTRA VIDYAPEETH, PUNE

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CERTIFICATE OF THE SUPERVISOR

It is certified that work entitled **"Assessment of antipyretic and analgesic activity of Hinguleshvara Rasa in experimental animals"** is an original research work done by **Dr Sharadini Aniket Karambelkar** Under my supervision for the degree of Doctor of Philosophy in **Rasashastra evam Bhaishajya Kalpana** to be awarded by Tilak Maharashtra Vidyapeeth, Pune. To the best of my knowledge this thesis embodies the work of candidate herself has duly been completed fulfils the requirement of the ordinance related to Ph. D. degree of the TMV up to the standard in respect of both content and language for being referred to the examiner.

Signature of the Supervisor

DECLARATION

I hereby declare that the thesis entitled **'Assessment of antipyretic and analgesic activity of *Hinguleshvara Rasa* in experimental animals'** completed and written by me has not previously been formed for the award of any degree or other similar title upon me of this or any other Vidyapeeth or examining body. I understand that if my Ph.D. thesis (or part of it) is found duplicate at any point of time my research degree will be withdrawn.

Place : Pune

Date : 13/03/2024

Signature of the Research Student

Dr Sharadini Aniket Karambelkar

PRN No. : 05614007209

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Abstract

Title:Assessment of antipyretic and analgesic activity of *Hinguleshvara Rasa* in experimental animals

Key words:Antipyretic,analgesic Hinguleshvara Rasa

INTRODUCTION

According to Ayurveda *Dehasantapa*/hyperpyrexia is the cardinal symptom of *Jvara. Jvara* the disease, described in Ayurveda classics, is not limited only to pyrexia. It is a disease marked with complex manifestations Number of Ayurvedic drugs are prescribed and widely used in the treatment of *Jvara* with an intention of bringing down the increased body temperature. However, their efficacy as an antipyretic drug in comparison with conventional antipyretic drugs such as Paracetamol, Aspirin etc. needs to be proved.

One of the formulations of *Hinguleshvara Rasa* mentioned in an Ayurvedic text named *Bhaishajya Ratnavali*[1] in the chapter of *Jvarachikitsa* 5/483), is composed of only three ingredients *Hingula* (Red Cinnabar: HgS), *Pippali* (Long Pepper: *Piper longum* Linn.), *and Vatsanabha (Aconitum balfourii* Stapf*)*

This formulation of *Hinguleshvara Rasa* is recommended in *Vatajajvara*. Small number of ingredients makes this formulation of *Hinguleshvara Rasa,* easy to prepare and found most popular among the Ayurvedic practitioners. Therefore, this formulation of *Hinguleshvara Rasa* was selected for the present study entitled **"An assessment of antipyretic and analgesic activity of *Hinguleshvara Rasa* in experimental animals."**

Although *Hinguleshvara Rasa* is prescribed in the treatment of *Vataja Jvara*, there is no such specific model available to assess specific *Doshaja Jvara* in experimental animal. Therefore, *Doshaja* predominance was not considered in this study. Only hyperpyrexia being *pratyatmalakshana* in all types of *Jvara* and not *Jvararoga* as a whole; was the subject of evaluation in the present study. Such a study will prove the efficacy of *Hinguleshvara Rasa* and facilitate the clinician to use the drug confidently in the treatment of *Jvara,* the Pyrexia.

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AIM AND OBJECTIVES

Aim: An assessment of Antipyretic and Analgesic activity of *Hinguleshvara Rasa* in experimental animal.

Primary Objective:

- Preparation of *Hinguleshvara Rasa.*
- Physicochemical analysis and characterization of *Hinguleshvara Rasa.*
- Assessment of antipyretic and analgesic activity of *Hinguleshvara Rasa.*

Secondary Objective:

- Assessment of acute toxicity study of *Hinguleshvara Rasa* and its ingredients.
- Assessment of antipyretic study of Shuddha Vatsanabh, *Shuddha Hingula , Pippali*
- Assessment of Analgesic activity of *Shuddha Vatsanabh, Shuddha Hingula , Pippali*

MATERIALS AND METHODS

Materials and Methods:

- This study was conducted in three stages:
- A] Preparation of *Hinguleshvara Rasa*
- B] Analytical Study
- C] Experimental Study

- **Preparation of *Hinguleshvara Rasa* includes following steps:**
- **Collection& authentication of raw materials**
- **Pharmacognosy of *Vatsanabh (Aconitum)***

- **Pharmacognosy of *Piper longum Fruits*:Pippali**
- **Estimation of Microbial load /viable count of *Hinguleshvara Rasa* and its ingredients**
- **Physicochemical analysis of *Hinguleshvara Rasa* and its ingredients as per A.P.I**

- **Characterization of** *Hingula* (Red cinnabar: HgS) **(Before and after *shodhana)* and *Hinguleshvara Rasa* by SEM, particle size analyzer, FTIR & X-ray Diffraction technique.**
- **Phytochemical Analysis:**

It includes High performance liquid chromatography for

1 **Estimation of Piperine content and total alkaloid content from all batches *Piper longum* Linn**

2 Estimation of Aconitine content and total alkaloid content from *Ashuddha* and *Shuddha Vatsanabh (Aconitum balfourii Stapf* Stapf)

3 Estimation of total alkaloid from all batches of fruits of *Piper longum* Linn, & *Aconitum balfourii* Stapf (before and after shodhana)

4 **Estimation of Aconitine and Piperine content of *Hinguleshvara Rasa.***

Preparation of *Hinguleshvara Rasa*

Hinguleshvara Rasa was prepared according to description in *Bhaishajya Ratnavali Jvara Chikitsa adhyaya* 5/483

Sr. No.	**INGREDIENTS**	**QUANTITY**	**EQUIPMENTS**
1	*Shodhit Vatsanabh*(roots of Aconitum balfourii Stapf) (Aconite processed with Cow urine)	250 gms	*Khalwayantra* (Mortar pestle) **mesh size 100**
2	*Shodhit Hingula (Red cinnabar: HgS) (Hingula processed in Zingiber officinalis))*	*250gms*	*Khalwayantra* (Mortar pestle) **Mesh Size 200,**
3	*Pippali* (*Piper longum Linn*)	250gms	*Khalwayantra* (Mortar pestle) **mesh**

			size 100

All three ingredients were subjected to mix in Ball mill machine.

Total 750 Gms of material was kept in drum of ball mill machine having 20 porcelene balls. Mixing was performed 8hrs daily (9am to 5pm.) for 3 days.

On fourth day uniform mixture of *Hinguleshvara Rasa* was obtained. This mixture was subjected to trituration in *Khalvayantra* (mortar pestle) for 7 days.7hrs (9am to4pm) trituration performed daily for 12 days. One day =12 hrs i.e. from sunrise to sunset, so for7 days trituration =84 hrs. So total 84 hrs trituration performed to get *Hinguleshvara Rasa.* This *Hinguleshvara Rasa was* then subjected to characterization by SEM, Particle size analyzer, FTIR and XRD, and physicochemical, phytochemical analysis.

Experimental study

This study was comprised of three parts:

- Study of Acute oral toxicity of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii Stapf), & Shuddha Hingula (*Red Cinnabar:HgS) by following OECD 423 guidelines
- Study of antipyretic activity of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*), & Shuddha Hingula (*Red Cinnabar:HgS) *& Pippali (Piper longum* Linn*)*
- Study of Analgesic activity of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii Stapf), & Shuddha Hingula (*Red Cinnabar:HgS) *& Pippali (Piper longum* Linn*)*

Study design adopted for determination of acute oral toxicity was as follows

MATERIALS AND METHODS

Acute oral toxicity of *Hinguleshvara Rasa*, *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf), & *Shuddha Hingula* (Red Cinnabar: HgS) by following OECD 423 guideline

a TEST Material

1) **Test Material : "*Hinguleshvara Rasa*"**
   Physical State : Solid
2) **Test Material : "*Shuddha Hingula (*Red Cinnabar) HgS"**

Physical State : Solid

3) **Test Material : "*Shuddha Vatsanabh (Aconitum balfourii* Stapf*)*"**

Physical State : Solid

b DOSE SELECTION AND PREPARATION:

Sample was stored at room temperature.

The dose of each test material selected for the study was 300 mg/kg at step 1 and repetition of 300 mg/kg at step 2 for test material *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*),& Shuddha Hingula (*Red Cinnabar:HgS)

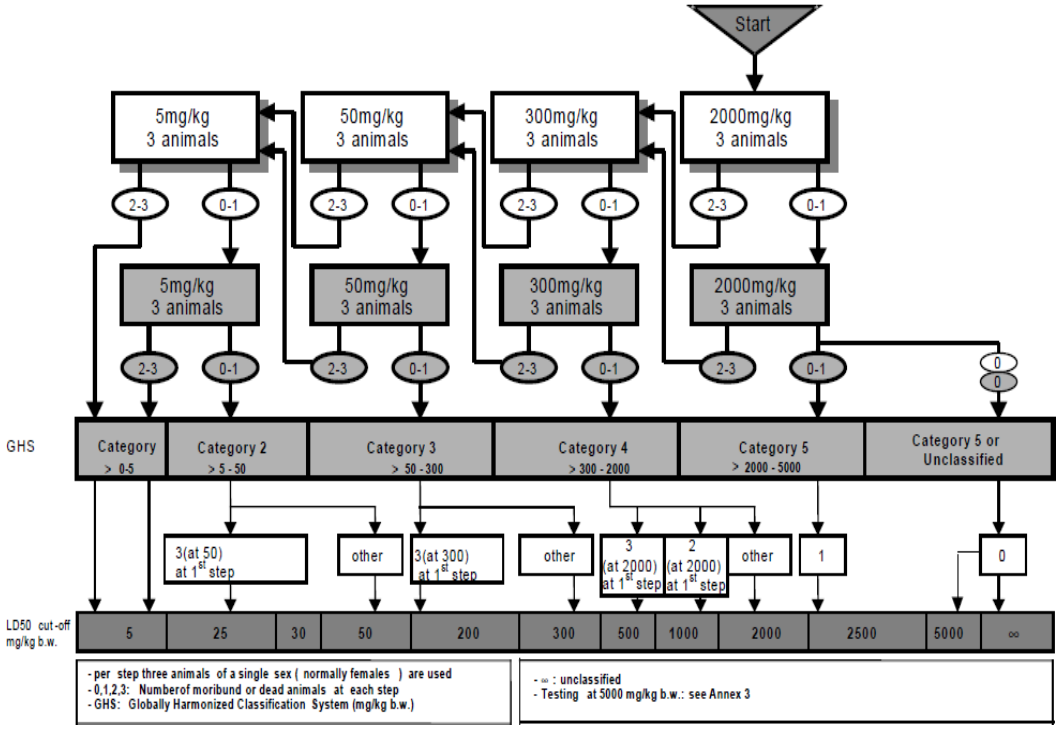
The doses of each test material selected for the study were 2000 mg/kg at step 3 for the test material *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii Stapf), &* ***Shuddha Hingula (*Red Cinnabar:HgS)** The Formulation of the test material was prepared shortly before dosing. The vehicle used for dose preparation was corn oil.

c TEST SYSTEM AND MANAGEMENT

1. Species	:	Mice
2. Strain	:	Swiss Albino
3. Source	:	APT Testing and Research Pvt Ltd, Pune
4. Age and Sex	:	Female, 06-08 weeks
5. Body weight range	:	25-30 gm
6. Identification	:	By unique identification number marked by writing on cage tag and by corresponding colour body markings.
7. No. of animals	:	Three animals were tested in per group.
8. Acclimatization	:	The mice were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
Husbandry		
9. Environmental Conditions	:	Room temperature maintained between 22 ± 3^0C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark.

10. Accommodation	:	Three mice per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet	:	Pelleted feed supplied by Supplier.
12. Water	:	Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

ANNEX 2d: TEST PROCEDURE WITH A STARTING DOSE OF 2000 MG/KG BODY WEIGHT



According to OECD Annex 2c, if the dose of a substance is unknown we can start the dose at 300 mg/kg if it shows mortality after 4-6hrs that dose will be considered fatal dose otherwise lower or higher dose have to be studied.

EXPERIMENT FOR ANTIPYRETIC ACTIVITY:-

MATERIALS AND METHODS

Antipyretic activity of Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii Stapf), Pippali (Piper longum Linn), Shuddha Hingula (Red cinnabar: HgS) was assessed by Brewer's yeast induced pyrexia model.

a MATERIALS AND METHODS

a. TEST MATERIAL

Test Material	**:**	***Hinguleshvara Rasa***
Physical State	**:**	**Solid**
Quantity	**:**	**100 gm**
Color	**:**	**Orange**

b. TEST MATERIAL

Test Material	**:**	***Shuddha Hingula* (Red cinnabar:HgS)**
Physical State	**:**	**Solid**
Quantity	**:**	**100 gm**
Color	**:**	**Orange**

c. TEST MATERIAL

Test Material	**:**	***Shuddha Vatsanabha***
Aconitum balfourii Stapf		
Physical State	**:**	**Solid**
Quantity	**:**	**100 gm**
Color	**:**	**Light brown**

d. TEST MATERIAL

Test Material	**:**	**Pippali (*Piper longum* Linn)**
Physical State	**:**	**Solid**
Quantity	**:**	**100 gm**
Color	**:**	**Light brown**

b DOSE SELECTION AND PREPARATION:

Sample was stored at room temperature.

The doses selected for the study was as mentioned in the study design for test material *Hinguleshvara Rasa* (Classical drug).

Dose Calculation[2]: Human dose of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*), Pippali (Piper longum* Linn), *Shuddha Hingula* (Red cinnabar: HgS) was converted to animal dose in accordance to Paget G E and Barnes, J.M.' Toxicity tests' In:" Evaluation of drug activities pharmacometrics" (Laurene, D.R. and Bacharach, A.L eds)Vol 1,Academic press London 1964 pp -135-165

Classical doses of Pippali 1 masha=750mg (Sha.Sa) [3]

As per *Sharangdharasamhita* Dose of *Pippali (Piper longum* Linn),churna is 1 masha1 ratti =125 mg

6ratti=1 masha[4]

Vatsanabh1/8 Ratti=15.6mg(RT) [5]

Hingula 1 Ratti=125mg (Ayu.Pra)[6]

Hinguleshvara Rasa Half gunja =62.5mg (Bhaishajya Ratnavali)[7]

Human to animal dose were converted by using Paget and Barner's

Scale as in given table:

These doses of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*), Pippali (Piper longum* Linn), *Shuddha Hingula* (Red cinnabar: HgS) were used for both in antipyretic study as well as analgesic study model.

EXPERIMENT FOR ANTIPYRETIC ACTIVITY:-

c ADMINISTRATION OF TEST MATERIAL

Test materials were administered orally.

1. Species	:	Rat
2. Strain	:	Wistar Rats
3. Source	:	APT Testing and Research Pvt Ltd, Pune
4. Sex	:	Both male and female
5. Body weight range	:	200-250g
6. Identification	:	By unique identification number marked by writing on cage tag and by corresponding colour body markings.
7. No. of animals	:	42 animals
8. Acclimatization	:	The rats were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
Husbandry		
9. Environmental Conditions	:	Room temperature maintained between 22 ± 3^0C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark.
10. Accommodation	:	One rat per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet	:	Pelleted feed supplied by Supplier.

12. Water	:	Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

STUDY DESIGN:

The antipyretic activity was conducted on Wistar Rats both male and female (weight range 200-250g gms) in 7 groups. Each group was comprised of 6 animals. (3M + 3F)

EXPERIMENT FOR ANTIPYRETIC ACTIVITY:-

Assessment of anti-pyretic activity in rats:

Purpose: Subcutaneous injection of Brewer's yeast suspension is known to produce fever in rats. A decrease in temperature can be achieved by administration of compound with antipyretic activity. Antipyretic test in rats can be regarded as a classical method in pharmacology.

Brewer's yeast induced pyrexia model·**[8-10]**

Before inducing pyrexia the initial rectal temperature of the animals was monitored. Animals fasted overnight was used. Pyrexia was induced by injecting 20% v/v Brewer's yeast suspension (10mg/kg S.C) into the animal dorsum region. 18 hrs after the injection, the rectal temperature of each rat was measured using digital thermometer. Only rats which show an increase in temperature at least 0.7^0 C was used for experiments. The treatment with *Hinguleshvara Rasa, Shuddha Hingula* (Red cinnabar: HgS), *Shuddha Vatsanabh (Aconitum Balfourii* Stapf*) & Pippali (Piper longum* Linn) was administrated orally at suitable dose. After 30 mins of drug administration the rectal temperature was monitored at 30min, 60 min, 2hrs, 4 hrs., 6 hrs. 8 hrs., 24 hrs. and 48 hrs

Groups of animals for antipyretic activity:-

S. No.	**Groups**	**No of rats**	**Drug**	**Route**

1	Normal Control	6	---	Oral
2	Disease control	6	Brewer's Yeast (10 mg/Kg, s.c)	Parenteral
3	Standard	6	Brewer's Yeast+ Paracetamol (150 mg/kg)	parenteral
4	Test(T1)	6	Brewer's Yeast+ *Hinguleshwara Rasa* (5.63 mg/Kg)	Oral
5	Test(T2)	6	Brewer's Yeast+ *Shodhita Hingula* (Red Cinnabar:HgS (11.25 mg/Kg)	Oral
6	Test(T3)	6	Brewer's Yeast+ *Shodhita Vatsanabh Aconitum balfourii* Stapf) (1.40 mg/Kg)	Oral
7	Test(T4)	6	Brewer's Yeast+ *Pippali* (*Piper longum* Linn) (67.5 mg/Kg)	Oral

EXPERIMENT FOR ANALGESIC ACTIVITY:-

a MATERIALS AND METHODS

a. **Test Material : *Hinguleshvara Rasa***

Physical State : Solid

Quantity : 100 gm

Color : Orange

b. Test Material : *Shuddha Hingula* (Red cinnabar:HgS)

Physical State : Solid

Quantity : 100 gm

Color : Orange

c.	**Test Material**	**:**	***Shuddha Vatsanabha***
	***Aconitum balfourii* Stapf**		
	Physical State	**:**	**Solid**
	Quantity	**:**	**100 gm**
	Color	**:**	**Light brown**

d.	**Test Material**	**:**	***Pippal i*(*Piper longum* Linn)**
	Physical State	**:**	**Solid**
	Quantity	**:**	**100 gm**
	Color	**:**	**Light brown**

b.DOSE SELECTION AND PREPARATION:

Sample provided was stored at room temperature.

The doses selected for the study was as mentioned in the study design for test material *Hinguleshvara Rasa* (Classical drug).

EXPERIMENT FOR ANALGESIC ACTIVITY:-

ADMINISTRATION OF TEST MATERIAL

c. Test materials were administered orally.

1. Species	:	Mice
2. Strain	:	Swiss albino mice
3. Source	:	APT Testing and Research Pvt Ltd, Pune
4. Age and Sex	:	Female, 06-08 weeks
5. Body weight range	:	18-22
6. Identification	:	By unique identification number marked by writing on cage tag and by corresponding colour body markings.
7. No. of animals	:	Six animals were tested in per group.
8. Acclimatization	:	The mice were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
Husbandry		
9. Environmental Conditions	:	Room temperature maintained between 22 ± 3^0C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark.
10. Accommodation	:	Three mice per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet	:	Pelleted feed supplied by Supplier.
12. Water	:	Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

EXPERIMENT FOR ANALGESIC ACTIVITY:-

The analgesic activity was conducted on Swiss Albino female mice (weight range 18-

22 gms) in 6 groups. Each group was comprise of 6 animals.

The groups are as follows:

S. No.	Groups	No of Mice	Drug	Route
1	Diseased Control	6	1) Writhing- 0.1 ml of 1% acetic acid solution, i.p 2) Formalin test- 20 µl of 1% formalin solution under right hind paw 3) Hot plate- No drug treatment	Parenteral
2	Standard	6	For- 1) Writhing and Formaline- Diclofenac (10mg/Kg) 2) Hot Plat- Pentazocine- 1.4 mg/kg	Parenteral
3	Test (T1)	6	*Hinguleshvara Rasa* *(8.125ma /kg)*	Oral
4	Test (T2)	6	*Shodhita Hingula* (Red Cinnabar:HgS) (16.25mg /kg)	Oral
5	Test (T3)	6	*Pippali* (*Piper longum* Linn) *(97.5 mg / kg)*	Oral
6	Test (T4)	6	*Shodhita Vatsanabh* *Aconitum balfourii* Stapf) *2.028mh / kg*	Oral

Following tests were conducted on (Swiss Albino female mice) for analgesic activity.

1Formalintest[11-12]

2Writhing assay[13-14]

3Hot plate test[15-18]

CONCLUSION

This study was aimed to assess antipyretic and analgesic activity *of Hinguleshvara Rasa*. Primary objective of this study included preparation of *Hinguleshvara Rasa*, physicochemical analysis and characterization of *Hinguleshvara Rasa*. Physicochemical parameters like total ash, moisture content, thin layer chromatography etc of *Hinguleshvara Rasa* were studied. Secondary objectives included the study of all three ingredients of *Hinguleshvara Rasa* and *Hinguleshvara Rasa* itself for an assessment of their antipyretic as well as analgesic activity.

The study was conducted in three steps 1. Preparation of *Hinguleshvara Rasa*, 2. Physicochemical analysis and 3. Assessment of Antipyretic and Analgesic activity in experimental animals.

Following conclusions can be drawn from the first step of study i.e. Preparation of Hinguleshvara Rasa:

1. *Hinguleshvara Rasa* was prepared with *Shuddha Hingula (*Red Cinnabar: HgS), *Shuddha Vatsanabh* (*Aconitum balfourii* Linn) *and Pippali (Piper longum* Linn) in equal proportion (250 g each) using method described in the classical text *Bhaishajya Ratnavali Jvara chikitsa (*5/72). The weight of final product was observed reduced by 14 g. (1.86%) may be due to loss incurred during manual handling in processing.
2. It was also observed that the formulation ingredients 20.72% of Hingula gets lost during its wet grinding in ginger juice during its shodhana process.
3. Approx 34.12% of Vatsanabh is lost during its *shodhana* in Gomutra(Cow urine) .

Following conclusions can be drawn from the physicochemical testing

1. Reduction of number of spots during comparison of Thin layer chromatographs of *Ashuddha and Shuddha Vatsanabha* indicating that some of the phytochemical ingredients are lost during its *shodhana.*
2. Aconitine content of Vatsanabha was observed markedly reduced following its *shodhana.*
3. while total alkaloid content was observed increased due to its processing with cow urine.

4. Increase in the Particle size of formulation ingredients was observed after comparison of the ingredient before and after the wet grinding. This was due the the coating of the *Bhavana Dravya* on the particles of the ingredients as well as due to agglomeration of the particles.
5. The product *Hinguleshvara Rasa* was found to contain alkaloids Piperine (0.059%) and total alkaloids (o.48%). The Aconitine content in *Hinguleshvara Rasa* was observed to be 0.0022%. The observation concludes that even after *shodhan of Vatasnabha,* some amount of Aconitine remains in *Vatsanabh,* which is transferred to the final product *Hinguleshvara Rasa*. Which underlines the importance of caution to be observed during its therapeutic use.

Following conclusions can be drawn from the experimental study of

Hinguleshvara Rasa

1. Acute oral toxicity study of *Shuddha Hingula, Shuddha Vatsanabh and Hinguleshvara Rasa* was performed as per OECD-423 guidelines. No toxic effects in the form of mortality or pathological changes in organs were observed following oral administration of ***Hinguleshvara Rasa*** in therapeutic dose in the experimental animals.
2. It can be concluded on the basis of antipyretic study that although in initial period the antipyretic activity of the standard drug Paracetamol is more pronounced than that of the test drug ***Hinguleshvara Rasa***. However, the activity is almost comparable in later stages (24 hrs) with the standard drug Paracetamol. Still, it can be said that the test drug ***Hinguleshvara Rasa*** can't substitute the standard drug Paracetamol as far as its antipyretic activity is considered.
3. Writhing test, Hot plate analgesia and Formalin induced licking tests were utilized for testing analgesic activity of the test drug. On the basis of these tests, it can be concluded that the analgesic effect of ***Hinguleshvara Rasa*** is as good as the standard drug Diclofenac for Writhing test and Formalin test, Pentazocin for Hot plate Test. On the basis of this study, it is concluded that ***Hinguleshvara Rasa*** is having significant antipyretic and analgesic activity. However, although its antipyretic activity is not comparable with the standard drug Paraetamol, ***Hinguleshvara Rasa*** is as effective as standard drugs Diclofenac and Pentazocin in it's analgesic effect.
4. It is also concluded that the ingredients ***of Hinguleshvara Rasa*** are found potent analgesic but weak in antipyretic effect.

Bibliography:

1 Govind Dasa, Bhaishajya Ratnavali, Hindi Commentry by Ambikadatta Shastri,14thEdn, Chaukhamba Sanskrit Sansthana,Varanasi(India)1999,pg 82

2 Paget G E and Barnes, J.M.'Toxicity tests' In:"Evaluation of drug activities pharmacometrics" (Laurene,D.R.and Bacharach,A.L eds)Vol 1,Academic press London 1964 pp -135-165

3. (sharangdharacharya,SharangdharaSamhita,GudharthdipikaChaukhambaorientalia Varanasi 4thedn 2000,Madhyam khandaadhyay 6/8 pg 178

4(sharangdharacharya,SharangdharaSamhita,GudharthdipikaChaukhamba orientalia Varanasi 4thedn 2000,pratham khandaadhyay 1/20&32 pg 7-9)

5. (Sadanand Sharmana, Rasatarangini Motilal Banarasidas 11thedn, Reprint 2000,24/66 pg 660.)

6.(Shri Madhav Ayurved Prakash commentary by Gulraj Sharma Mishra Chaukhamba Bharati Academy reprint 1999,2/76 pg 275)

7. (Ambikadattashastri, Bhaishajya Ratnavali,Chaukhamba Sanskrit Sansthana 9th Edn 1991,5/483,pg 82)

8. Junaid Niazi etal Antiinflammatory and antipyretic activity of aleuritis moluccana leaves ,Asian journal of pharmaceutical and clinical research volm 3 isue 1 Jan –March 2010,pg 35-37

9 Nirmal kumar etal Analgesic and antipyretic ativities of methanol extract and its fraction from the root of Schoenoplectus grossus Hindawi publishing corporation evidence based complementary and Alternative medicine volm 2016, 8 pages,article Id 3820704

10) H.G.Vogel,Drug discovery and evolution 2nd revised edn,Antipyretc action in rats,H.4.0.2,pg777

11. Ref: Hunskaar, Steinar, Odd-Geir Berge, and Kjell Hole. "Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic acid and indomethacin in the formalin test." Pain 25.1 (1986): 125-132

12. H.G.Vogel,Drug discovery and evolution 2nd revised edn formalin test in rats H.1.2.10,pg 702

13 Ref : S R Jaiswal,S D Sontakke, Experimental evaluation of analgesic and anti-

inflammatory activity of simvastatin and atorvastatin Indian Journal of pharmacology2012-ncbi.nlm.nih.gov

14 H.G.Vogel,Drug discovery and evolution 2nd revised edn Writhing Test,H.2.0.2,pg 716

15 Menéndez, Luis, Ana Lastra, Agustın Hidalgo, and Ana Baamonde. "Unilateral hot plate test: a simple and sensitive method for detecting central and peripheral hyperalgesia in mice." Journal of neuroscience methods 113, no. 1 (2002): 91-97.

16Tripathi k d essentials of medical pharmacology7th ednJaypee brothers medical publishers pvt ltd new delhi 2013

17. Ghosh m n fundamentals of experimental pharmacology3rd edn Kolkata Hilton and company 2005

18. H.G.Vogel,Drug discovery and evolution 2nd revised edn .Hot plate Method.H.1.2.4,pg 696

1. INTRODUCTION

Jvara is very common ailment usually taken as fever, the pyrexia, in the language of conventional medicine. Pyrexia is considered to be the salient feature of inflammation pathology in the body. According to Ayurveda *Dehasantapa*/hyperpyrexia is the cardinal symptom of *Jvara. Jvara* is known as *Sarvarogadhipati* i.e. king of the all the ailments.[1] *Jvara* the disease, described in Ayurveda classics, is not limited only to pyrexia. It is a disease marked with complex manifestations However, *dehasantapa* means increased body temperature is the *Pratyatmalakshana* i.e. cardinal symptom of *Jvara.*[2] Therefore, along with other symptomatic treatment, bringing down the increased body temperature is an important objective in the management of *Jvararoga* .Numbers of antipyretic drugs such as Paracetamol, Aspirin etc. are described in conventional medicine usually known as modern medicine for bringing down the body temperature. These, drugs have become so common in practice that any common man can get them as an over the counter (OTC) product without any prescription from registered medical practitioner. Apart from their antipyretic action these drugs are known to possess analgesic activity as well. Number of Ayurvedic drugs are prescribed and widely used in the treatment of *Jvara* with an intention of bringing down the increased body temperature. However, their efficacy as an antipyretic drug in comparison with conventional antipyretic drugs such as Paracetamol, Aspirin etc. needs to be proved. In this respect many Ayurvedic mercurial drug preparations such as *Tribhuvankirti Rasa*, *Anand Bhairav Rasa* etc. can be considered. *Hinguleshvara Rasa* is also one such formulation used as an antipyretic drug. It is a well-known Ayurvedic herbo-mineral drug formulation. *Hinguleshvara Rasa* is specially indicated in *Vatajajvara* (pyrexia resulting from inflation of *Vata*).

As the name *Hinguleshvara Rasa* suggests, *Hingula* (Red Cinnabar:HgS) is the main ingredient in the formulations. Different compositions of *Hinguleshvara Rasa* are mentioned in different Ayurvedic classical texts. These formulations are formulated with multiple ingredients. This multi-ingredient composition makes preparation and use of these formulations little complex and difficult to understand. One of the formulations of *Hinguleshvara Rasa* mentioned in an Ayurvedic text named *Bhaishajya Ratnavali* in the chapter of *Jvarachikitsa* 5/483), is composed of only three ingredients *Hingula*

(Red Cinnabar: HgS), *Pippali* (Long Pepper: *Piper longum* Linn.), *and Vatsanabha (Aconitum balfourii* Stapf*)*

This formulation of *Hinguleshvara Rasa* is recommended in *Vatajajvara*. Small number of ingredients makes this formulation of *Hinguleshvara Rasa,* easy to prepare and found most popular among the Ayurvedic practitioners. Therefore, this formulation of *Hinguleshvara Rasa* is selected for the present study

Pyrexia is considered as *Dehasantapa* (increased body temperature) as the *pratyatmalakshana of Jvara*. *Hinguleshvara Rasa* is prescribed for *Vatajajvara*, in Ayurvedic classics. However, being an experimental study conducted in animals, the antipyretic effect of *Hinguleshvara Rasa* is studied in *Jvara* irrespective of its nature in the present study. Although *Hinguleshvara Rasa* is prescribed in the treatment of *Vataja Jvara,* there is no such specific model available to assess specific *Doshaja Jvara* in experimental animal. Therefore, *Doshaja* predominance is not considered in this study. Only hyperpyrexia being *pratyatmalakshana* in all types of *Jvara* and not *Jvararoga* as a whole; is the subject of evaluation in the present study. Such a study will prove the efficacy of *Hinguleshvara Rasa* and facilitate the clinician to use the drug confidently in the treatment of *Jvara,* the Pyrexia.

The present study includes study of *Hinguleshvara Rasa* with reference to its preparation, Physico-chemical characterization using advance techniques like XRD, SEM Particle size analyzer (PSA), Fourier Transmission Infrared Spectroscopy (FTIR). The study is further supplemented with experimental study for assessment of its antipyretic and analgesic activity. Brewer's yeast induced pyrexia model[3-4] has been used to study Antipyretic activity of *Hinguleshvara Rasa*

Prior to antipyretic and analgesic study acute oral toxicity of *Hinguleshvara Rasa, Shuddha Hingula* (Red Cinnabar:HgS), *Shuddha Vatsanabh (Aconitum balfourii* Stapf) has conducted as per OECD-423 guideline, details are as described in Analytical reports.

To assess an analgesic activity of *Hinguleshvara Rasa, Shuddha Hingula* (Red Cinnabar:HgS), *Shuddha Vatsanabh (Aconitum balfourii* Stapf) and *Pippali (Piper*

longum Linn), following tests are conducted on (Swiss Albino female mice)
Formalin test [5-6]:
Acetic acid induced writhing method [7-8]:

Hot plate test [9-12]: These test are used to check central as well as peripheral analgesic activity of drug.

Acetic acid induced writhing test, formalin test are used to check peripheral analgesia while Hot plate test used to check central analgesia.

Antipyretic effect of *Hinguleshvara Rasa* is observed studied in the past by some scholars too. [13-15]

However, role and effect of individual ingredients of the formulation is not found studied. The formulation of *Hinguleshvara Rasa* described in *Bhaishajya Ratnavali* being studied in the present study, has only three ingredients *Hingula* (Red Cinnabar: HgS), *Vatsanabh* (*Aconite*) and *Pippali* (*Long pepper: Piperlongum* Linn). The formulation being easy to prepare and having minimum ingredients is selected for this study considering its utility in the treatment of *Jvara.*As described above *Vatsanabh* (*Aconite*) and *Hingula* (Red Cinnabar:HgS) both are having antipyretic activity.[16-17] Their individual effects in this respect need to be compared with the formulations in which they are accompanied by other ingredients. Such an attempt has made in the present study. Antipyretic and analgesic activity of *Hinguleshvara Rasa* is compared with individual *Vatsanabh* (*Aconitum balfourii* Stapf) and *Hingula* (Red Cinnabar:HgS) and *Pippali (Piper longum* Linn) independently. Although *Hinguleshvara Rasa* is known to possess a wide range of therapeutic activity such as *amapachakc (strongly digestive), swedajanan (Sudorific), etc, the* present study is restricted to evaluation of its antipyretic and analgesic activity.

Considering the therapeutic efficacy of each ingredient of *Hinguleshvara Rasa i.e. Shuddha Hingula* (Red Cinnabar:HgS), *Shuddha Vatsanabh (Aconitum balfourii* Stapf) *and Pippali (Piper longum* Linn.), it became essential to study whether the therapeutic activity of this formulation is a result of synergetic action of its ingredients or any of the ingredients is responsible to exert dominant therapeutic activity.

This study will become helpful in this perspective to describe therapeutic action of *Hinguleshvara Rasa* as a formulation itself and in comparison of its ingredients too.

REVIEW OF LITERATURE

2.1Hinguleshvara Rasa

Many numbers of herbo-mineral formulations are described for the treatment of *Jvara* (Pyrexia) in Ayurvedic classics. *Tribhuvankirti Rasa*, *Anand Bhairav Rasa*, *Mrityunjaya Rasa* are few of them. *Hinguleshvara Rasa* is also a well-known herbo-mineral Ayurvedic mercurial preparation used in the treatment of *Jvara* (Pyrexia). It is indicated in many disorders like *Grahani (*malabsorption), *Aamvata* (rheumatoid arthritis), and *Jvara* (pyrexia). Different compositions of *Hinguleshvara Rasa* are found in different classical texts. As the name suggests itself, *Hingula* (Red Cinnabar:HgS) is the main ingredient of all these formulations. Another important ingredient of *Hinguleshvara Rasa* is *Vatsanabh* (Aconite). Most of the formulations of *Hinguleshvara Rasa* are prescribed for *Vataja Jvara* (Pyrexia due to inflation of *Vata*). The *Hinguleshvara Rasa* from classical text *Rasatarangini is* prescribed for *Aamvata* (Rheumatoid Arthritis) and *Jvara (*Pyrexia**).**[18]** However, formulation of *Hinguleshvara Rasa* from classical text *Rasa Raja Sudhakar is* not much used in *Jvara*, it is prescribed in *Grahani***[19]** Ingredients and indications of *Hinguleshvara Rasa* described in classical texts *Bruhat Rasarajsundar* and *Rasamanjiri* and *Bhaishajya Ratnavali* are similar except difference is prescribed dose. In *Bhaishajya Ratnavali***[20]** dose of *Hinguleshvara Rasa* is described as half gunja *(*62.5mg), whereas, its dose is mentioned as 2 *gunja* (250mg) in *Bruhat Rasarajsundar* and *Rasamnjiri***.[21-22]** Variation in ingredients as well as change in their proportion is also found in different classical texts. Ingredients of *Hinguleshvara Rasa* from various classical texts are as follows: [23]

Sr. No.	**Text**	**Name of Ingredients**						
		Shodhit Hingula	***Shuddha Vatsanabh***	***Pippali***	***Shuddha Jay pal***	***Shuddha Parada***	***Shuddha gandhak***	***Tamra bhasmas***
1	B.R	+	+	+				
2	RT	+	+	+				
3	RK	+	+	+				
4	RRS	+			+	+		
5	RRS	+					+	+
6	RM	+	+	+				
7	BRRS	+	+	+				

B.R Bhaishajya Ratnavali, RT (Rasatarangini) RK (Rasakamdhenu) RRS (Rasaratnasamucchaya) RRS (Rasa Raja Sudhakar)RM (Rasamanjiri) BRRS (Bruhat Rasarajsundar)

Hinguleshvara Rasa is a well-known *Kharaliya Rasayana* (formulations prepared by trituration of herbomineral ingredients in mortar and pestle).

For preparation of these *Kharaliya Rasayana, Bhavana sanskaras* (process of wet grinding) plays an important role in reduction of particle size to facilitate high therapeutic efficacy. *Rasarajsudhakar* mentioned *Shalmali sattva* (Essence of Salmalia malberica) as *bhavanadravya*[24] while *Rasaratnasamucchaya* mentioned *Danti* kvath (decoction of Baliospermum montanum) as *bhavanadravya* of *Hinguleshvara Rasa*.**[25]** *Anupana* (vehicle) of *Hinguleshvara Rasa* is *Madhu* (honey)as described by many classical texts. Only in *Rasaratnasamucchaya, Sita* (candy sugar) is mentioned as *Anupana.* It is named as *Vruddhajvarankush Rasa* in *Rasaratnasamucchaya*. In 1st shloka of *Bharata Bhaishajya Ratnakar, Dhanyakakvath* (decoction of *Coriandrum sativum*), *Jeerakkwath* (decoction of *Cuminum cyminum* seeds), are mentioned as *Anupana* along with *Madhu* (honey)**[26]** The formulation of *Hinguleshvara Rasa* described in classical text *Bhaishajya Ratnavali* has only three ingredients *Shuddha Hingula* (Red Cinnabar:HgS), *Shuddha Vatsanabh (Aconitum balfourii* Stapf*)* and *Pippali* (*Piper longum* Linn). Considering its utility in *Jvara* and having ease in preparation this formulation is used widely by Ayurvedic practitioners.

Hinguleshvara Rasa has been studied widely for its therapeutic activity. *Hinguleshvara Rasa* was studied by Koteshvara (2005) for its preparation, Physico chemical analysis and clinical efficacy on *Aamvata*w.s.r.to Rheumatoid arthritis. This study concluded that *Hinguleshvara Rasa* has definite activity in reducing the cardinal symptoms of *Aamvata*.**[27]**

Hinguleshvara Rasa was studied (by Sarashetty & Bagewadi) **[28]**

for its antipyretic activity against paracetamol as a standard and propylene glycol as control. Antipyretic activity was assessed by yeast induced hyper pyrexia in Albino rats. Significant antipyretic activity was observed with *Hinguleshvara Rasa.* Antipyretic activity was noted from 1st hr. which sustained up to 9th hrs. in test drug *Hinguleshvara Rasa.* While in standard drug Paracetamol, it was up to 5 hrs. In phytochemical analysis presence of alkaloid, carbohydrates, and proteins was observed.

As, Bi, Hg and S were detected in ICP. Antipyretic activity was assessed as mention in *Sahasrayogam.*

Effect of *Hinguleshvara Rasa* was also studied on *pratishyay; Jvara by*. Thakur S K.[29] Role of *Bhavana* process in the antipyretic effect of *Hinguleshvara Rasa* was studied.[30] Comparison between '*Hinguleshvara Rasa* with *Bhavana*' and *'Hinguleshvara Rasa* without *bhavana'* shows that *Hinguleshvara Rasa* with *bhavana* has got better antipyretic activity profile. In fact it has been produced moderate and statistically significant reduction in the rectal temperature after yeast suspension injection while *Hinguleshvara Rasa* without *bhavana* produced only a weak non-significant effect. Longer period and sustained activity was noted in *Hinguleshvara Rasa* with *Bhavana* group.

In vitro anti-inflammatory and antioxidant activities of *Hinguleshvara Rasa* were also studied.[31] This study was an interventional investigation in three samples: In the first sample, *Hinguleshvara Rasa* (HR1) was prepared as per described in *Rasatarangini u*sing *Shuddha Hingula (10 g), Shuddha Vatsanabh (10 g), and Pippali (10 g).* In the second and third sample, *Hinguleshvara Rasa* was prepared by replacing *ShuddhaHingula* with *Kajjali (Black sulphide of Mercury)* where *Kajjali (Black sulphide of Mercury)* made from *Hingulottha Parada* (Mercury extracted from Cinnabar) and *Sodhita Parada* (Hg) considered two varieties of *Hinguleshvara Rasa,* i.e. HR2 and HR3. In vitro antioxidant activity was studied using 2,2-diphenyl-1-picrylhydrazyl, and the observation was recorded at 517 nm. For evaluating the in vitro anti-inflammatory studies, the inhibition of albumin denaturation technique was performed. The results showed that the formulation of *Hinguleshvara Rasa* has shown dose-dependent activity which was observed in 100ug concentration. HR1, HR2, and HR3 showed 36.11, 17.22, and 16.11% radical scavenging activity. This study concluded that the changes made in the formulations did not affect the in vitro anti-inflammatory and anti-oxidant effects of the herbomineral formulations.

Pharmaceutical–Analytical study of *Hinguleshvara Rasa* concluded that the pharmaceutical processing of *Hinguleshvara Rasa* is easy and economical All analytical results are well within the safety limits[32]

Analgesic activity of *Bruhat Hinguleshvara Rasa* was evaluated. Significant and

sustained analgesic activity was observed with *Bruhat Hinguleshvara Rasa* and standard drug**.**[33]

Antipyretic activity of *Bruhat Hinguleshvara Rasa* was also evaluated. Significant antipyretic activity was observed with *Bruhat Hinguleshvara Rasa* in comparison with control and standard drug**.**[34]

The formulation of *Hinguleshvara Rasa* described in *Bhaishajya Ratnavali* has only three ingredients *Hingula* (Red Cinnabar:HgS), *Vatsanabh* (*Aconite*) and *Pippali* (*Piper longum* Linn). The formulation being easy to prepare and having minimum ingredients was selected for this study considering its utility in the treatment of *Jvara***.** *Vatsanabh* (*Aconite*) and *Hingula* (Red Cinnabar:HgS) both are having *Jvaraghna* (antipyretic)[35-36]activity. Their individual effects in this respect need to be compared with the formulations in which they are accompanied by other ingredients. Such an attempt was done in the present study. Antipyretic and analgesic activity of *Hinguleshvara Rasa* was studied in experimental animals (Wistar Rats & Swiss Albino Mice respectively) in this study. It was compared with individual *Vatsanabh* (*Aconitum Balfourii* Stapf) and *Hingula* (Red Cinnabar:HgS) and *Pippali* (*Piper longum,* Linn.) independently.

Being principal ingredients of most of the antipyretic-analgesic drugs as well as *Hinguleshvara Rasa* review of activity of *Shuddha Hingula* (Red Cinnabar:HgS), *Shuddha Vatsanabha (Aconitum balfourii* Stapf)*) and Pippali (Piper longum* Linn) are cited here in this reference.

2.2HINGULA (Red Cinnabar:HgS)

'Hinguleshvara Rasa', the name itself suggests that *Hingula* (Red cinnabar:HgS) Red Sulphide is one of the principal ingredient of *Hinguleshvara Rasa.*

Since years together *Hingula* (Red Cinnabar:HgS) is used in *Rasashastra* (Iatrochemistry of Ayurveda) for medicinal purpose. It is one of the important drugs used in the preparation of Ayurvedic mercurials. Different authors of classical texts have classified it as *Maharasa, Uparasa, Sadharana Rasa etc. Rasaratnasamucchaya* being most popular classical text, classification according to it, is followed by followers of *Rasashastra*. So *Hingula* (Red Cinnabar:HgS) is included in group *of Sadharanrasa Rasa.*[37]

Sr.No.	Classical texts	*Maharasa*	*Uparasa*	*Sadharana Rasa*
1	*Rasarnava*[38]	+		
2	*Rasaratnasamucchaya*[39]			+
3	*Rasendra Sara sangraha*[40]		+	
4	*Ayurved Prakash*[41]		+	
5	*Rasajalanidhi*[42]			+
6	*Bhavaprakash*[43]		+	
7	*Anandkanda*[44]		+	
8	*Rasachandanshu*[45]			+
9	*Rasendra Chudmani*[46]		+	

Synonyms of *Hingula* (Red Cinnabar:HgS)**are**

Sr. No.	Classical texts	Synonyms
1	**(***Ayurved Prakash*[47]	*Hingula, Darada, Mleccha, Churnparada, Suranga, Rasagarbha, Barbaram* and *Rakta*.
2	***Dhanvantari Nighantu***.*[48]*	*Hingula, Darada, Mleccha, Churnparada, Suranga, Rasagarbha, Barbaram* and *Rakta Charmanuranjana*
3	*Rasarnava*[49]	*Manirag* and *Laghukanda*
4	*Mahaushdhi Nighantu*[50]	*Rasasthana, Rasagarbha, Rasodbhava* a *Hingula,Darada, Mleccha, Churnparada, Suranga, Rasagarbha, Barbaram*and *Rakta*.
5	In *Kaiydev Nighantu [*[51]	*Atiraktam, Valutiktam, Kraunchlohitam, Kuruvinda* are few additional synonyms than other *Rasagranthas.*
6	***Shrimadvag bhatacharya virachit Ashtang Nighantu, Viprakirna prakarana27/292*** *[52]*	*Rasabhu, Raktarenu, Shreebhushan, Naagsambhav*

7	*Rasendra Sara sangrah*[53]	*Hingula, Hingulu, Darada, Shuktunda, Daityaraktak, Rasagandhaksambhut*
8	*Rasatarangini*[54]	*Hingula, Hingulu, Darada, Mleccha, Churnparada, Suranga Rasodbhava* a, *Rasasthana, Suranga, Rakta, Kapisheershkam, Raktakaya, Chitrang, Ranjana etc*
9	*Bhavaprakash*[55]	*Hingula, Darada, Mleccha, Churnparada,*

Types of *Hingula* (Red Cinnabar:HgS)

There are three types of *Hingula* (Red Cinnabar:HgS)

1. *Charmara*
2. *Shuktundakhya*
3. *Hansapada*

Among three types subsequent variety of *Hingula* (Red Cinnabar:HgS) is more superior than other. So *Hansapada* is the best among three**. (Ayu.Pra2/70)**

Charmar refers to white cinnabar. *Shuktunda* refers to parrot's beak, which is red in colour. *Hansapada* refers to swan or goose feet, which are pink in colour. The variety is also compared with a flower of *Hibiscus rosasinencis*, which is pink in colour and hence mentioned as *japakusumsankasho*.[56-57] According to classical texts, *Rasaratnasamucchaya Hingula* (Red Cinnabar:HgS)is of two types. *Shukatunda* and *Hansapada. Shukatunda* and *Charmar* are considered inferior as compared to *Hansapada.*The *Hingula* (Red Cinnabar:HgS) which resembles to colour of coral and having white shining lines on it, is called *Hansapada*.[58]

Rasatarangini also mentioned two types of *Hingula* (Red Cinnabar:HgS) *namely* [59]

1. *Khanija*
2. *Krutrim*

Therapeutic **Properties of** *Hingula* (Red Cinnabar:HgS)***:***

Ayurved Prakash[60]	*Hingula* (Red Cinnabar:HgS) is *Tikta* (bitter), *Kashaya* (astringent) and *katu* (hot) in R*asa*, it is *Netramayaghna* (Ophthalmic diseases), *kaphapittahari* (Controls vitiation of Kapha and Pitta), *Hrullasa* (Nausea), *Kustha* (Skin diseases), *Jvara* (Fever), *Kamla* (Jaundice), *Pliha* (Spleen disorders), *Aamavata* (Rheumatoid arthritis), it Destroyes *Garavisha*.
RasaRatna Samucchay ***[61]***	According to *Rasaratna Samucchaya Hingula* (Red Cinnabar:HgS) is *Sarvadoshaghna* (Controls vitiation of all doshas), *Deepana* (Appetizer), *Atirasayana, Sarvarogahara* (Cures all diseases), *Vrishya (Aphrodisiac), and* very exclusive *for Parada Jarana* (special processing of mercury to enhance its therapeutic efficacy).
Mahaushadhi Nighantu[62]	According to *Mahaushadhi Nighantu* all properties are same as per *Rasaratna Samucchaya*, few are different these are *Hingula* (Red Cinnabar:HgS) is *Vataghna (Pacifies Vata), Kamala (Jaundice), Pliha (Splenic diseases) and Hrullashar (suppress Nausea/Anorexia).*
Rasarnava[63]	According to *Rasarnava*, *Hingula* prepared from *Parad*a and *Gandhak* is *Tikta* (bitter), *ushna* (hot), *Balavardhak* (increase strength), *Medhavardhak (*increases intelligence), *Jatharagnideepak* (Appetizer), *Mehahar* (DM), *Kushthahara* (Cures skin disorders). Author of Rasarnava mentioned it as **Divyam.** Rasarnav7/51
Dhanvantari Nighantu [64]	*Hingula* (Red Cinnabar:HgS) is having *Ushna Veerya (hot), Kushthahar (recover skin diseases), Vishahar (to treat poisonous), Visarpahar* properties and indicated in *Twakdoshas (Herpis &other skin diseases)* as per *Dhanvantari Nighantu*. It destroys *Tridoshjanya Jvara, Dvidoshaj Jvara. (Pyrexia caused by 3 or 2 doshas)*
Bhavaprakash Nighantu[65]	Synonyms, types, and properties of *Hingula* (Red Cinnabar:HgS) mentioned which are as similar as mentioned in *Rasaratna Samucchaya*
Rasendra Sara Sangraha[66]	*Hingula* (Red Cinnabar:HgS) resembles to *Bimbiphala* (Coccinia grandis L) Voigt for its red colour.

Parada (Mercury:Hg) extracted from *Hansapada Hingula* is having properties as similar as *Gandhakjeerna parada*.**RRS3/151**[67]

Grahya lakshanas (selection criteria) of *Hingula* (Red Cinnabar:HgS)**:**[68]
Cinnabar should be as red as flower of *Hibiscus rosa Scinencis*. It should be heavy to touch and on trituration it is bright red in colour with metallic lusture.

Effect of *Ashudha* *Hingula* (Red Cinnabar:HgS):[69]
Ashuddha Hingula (Red Cinnabar:HgS) causes *Andhya* (Blindness), *Kshainya* (wasting), *Klama* (Fatigue), *Bhrama* (Giddiness), *Moha (*stuper), *Meha* (polyuria). Therefore *Hingula* (Red Cinnabar:HgS) should be used only after its *Shodhana.* **Ayu Pr.2/73**

***Shodhana (*therapeutic purification*)* of** *Hingula* (Red Cinnabar:HgS)**:**
Hingula (Red Cinnabar:HgS) is wet grinded with *Ardrak Swaras* (Juice of *Zingiber officinalis*) or *Lakuchswarasa* (juice of *Atrocarpus lakuch*) for seven times to make it *Shuddha.*[70]

Hingula (Red Cinnabar:HgS)*)* is wet grinded seven times with *Meshaksheer* (Goatmilk) and *Amladravya* (juice or liquids with sour taste) to make it *Shuddha.* When it is consumed it attains a saffron complexion. Uniformly grinded *Hingula* (Red Cinnabar:HgS) resembles to gold which heated several times in fire.[71]

In *Rasarnava,* it is mentioned that *Shuddha Hingula* (Red Cinnabar:HgS) has properties to convert white gold into saffron colored gold as it is heated in *teevra Atapa* (scorching sun heat). [72]

According *to Rasendrasaarsangraha* apart from *Meshaksheer* (Goatmilk) and *Amlavarga (drugs sour taste) dravyas,* it is grinded seven times with *Mahishksheera (buffalo milk)* and dried to make it *Shuddha* One more procedure of *Hingula* (Red Cinnabar:HgS) *shodhana* is found in *Rasendrasaarsangraha,* where *Hingula* (Red Cinnabar:HgS)is boiled with *Jambeersvarasa* (*Citrus Bergamia* Risso) in *dolayantra* and subjected to trituration with *Ajamutra* (Goat urine)for seven times to make it *Shuddha.*[73-74]

Sattva patana of Hingula (Red Cinnabar:HgS):

It is the traditional process of Mercury extraction from Cinnabar known as *Sattvapatana*. *Sattva* means essence.

Mercury (Hg) is considered as *Sattva* of *Hingula* (Red Cinnabar:HgS) is heated with each namely *Gomansa* (Cow meat), *Mahishmutra* (Buffalo urine), *Dadhyamla* (Curd water) and *Teelataila* (Sesame oil) for three days. Then it is triturated with *Shikhipitta* (Peacock bile) and subjected to *Patana* (distillation) to obtain its S*attva* i.e. *Parada* (Mercury:Hg)[75]

1. *Hingula* (Red Cinnabar:HgS) is triturated with *Nimbapatraswarasa* (*Azadirectaindica*) or *Nimbuswarasa (Citrus lemon* Linn) for one *yam* (3Hrs) and subjected to *urdhvapatana* (distillation) in *Damaruyantra* (Instrument used for distillation in ancient times which resembles to musical instrument Damaru) to get its *Sattva*i. e. *Parada*. This *Parada* is free from all seven *doshas.* There is no need to perform its *Shodhana.[76]*

2. According to *Rasatarangini* powder of *Hingula* (Red Cinnabar:HgS) is *bundled* in *pottali* (bundle) and its *Swedana* (sudation) is performed in *Dolayantra* filled with each namely *Mahishmutra (Buffalourine), Teelataila (*Sesame oil*),* and Suramla *(*Weak acetic acid prepared traditionally)for seven days. *Kramagni* (gradually increasing heat) is given. (Dolayantra is specially designed instrument used to perform sudation of parade (Mercury:Hg).

3. After that it is subjected to trituration with *Nimbuswarasa (Citrus lemon* Linn *Juice)* followed by *urdhvapatana (*distillation) to obtain its *Sattva*i. e. *Parada:Hg*.[77]

Review of Research on *Hingula* (Red Cinnabar:HgS)**:**

Comparative XRF study *of Hingula* (Red Cinnabar:HgS) purified in two different media was conducted by Dr. Nalin D Shah & etal.[78] In both methods of *shodhana (Bhavana* by two different media, *Nimbuk (Citrus lemon* Linn and *Ardrak (Zingiber officinale* Rosc), it was observed that *Hingula* (Red Cinnabar:HgS) was reduced to very fine powdered form. In both method of *shodhana*, Arsenic level shows decreasing pattern. *Ashuddha Hingula* (Red Cinnabar:HgS) contain Hg,S,As.But after *Shodhana* with *Citrus lemon* Linn juice the elements Ca and Fe were identified. They kept on increasing quantitatively with subsequent *Bhavanas* and finally showed a reduction after *prakshalana*. Similar was the case after s*hodhana* with *Zingiber officinale* Rosc)

juice and was supported by the research work done on *Zinziber officinalis* Rosc **(Shirin** Adel P R Jamuna Prakash, Chemical composition and antioxidant properties of ginger root (*Zinziber officinalis*) Journal of medicinal plant research 2011,pg2674-2679)

Pb however is never found in *Zinziber officinalis* rhizomes naturally. But present in Sample, in which shodhana is performed by Ardrak swarasa (*Zinziber officinalis* Rosc Juice). But to find a definitive cause behind this shows scope for further study.

Hingula (Red Cinnabar:HgS)**,** was subjected to *shodhana* process with *Lakuch (Atrocarpus Lakuch* fruit juice) *phalaswarasa* by the method of *Bhavana* (wet grinding).[79] Analytical study was carried out to notice the changes before and after *shodhana* w.s.r to heavy metals. The study revealed significant role of media used for *Shodhana*. Specially to detect the heavy metal analysis of raw and *Shodhita Hingula* (Red Cinnabar:HgS)**:** using Lakucha phaal swarasa (*Atrocarpus Lakuch* fruit juice) Induced Coupled Plasma-Atomic Absorption Spectroscopy (ICP,AAS) analysis was carried out on samples collected before and after *Shodhana* of *Hingula* (Red Cinnabar:HgS)**,**

Elements	**Ref. limit in** *Hingula* (Red Cinnabar:HgS)**, as per API Quantity (in ppm)**	***Ashuddha*** *Hingula* (Red Cinnabar:HgS)**, Quantity (in ppm)**	***Shuddha*** *Hingula* (Red Cinnabar:HgS)**, Quantity (in ppm)**
Lead	NMT 10 ppm	28.99 ppm	0.63 ppm
Cadmium	NMT 0.3 ppm	ND	ND
Arsenic	NMT 3 ppm	4.62 ppm	0.18 ppm
Mercury	NMT 1ppm	5 ppm	0.5 ppm

The method of *Shodhana* proves effective in removal of heavy metals up to prescribed limits. All of the heavy metals' quantities reduce to lower levels than that of mentioned limits as per Ayurvedic Pharmacopoeia of India. In this way, it is concluded that impact of *Shodhana* on *Hingula* (Red Cinnabar:HgS)**,** with *lakoocha phala swarasa* (juice of *Atrocarpus lakoocha*) is significant as per the need w.s.r. heavy metals.

Acute and sub-acute toxicity study of Ashuddha and Shuddha *Hingula* (Red Cinnabar:HgS)**,** reported that Ashuddha *Hingula* (Red Cinnabar:HgS)**,**is proved to be

having toxic effect while Shuddha *Hingula* (Red Cinnabar:HgS)**,** at 250 mg is a non-toxic and safe drug.[80]

Hingula (Red Cinnabar:HgS)**,** is one of the important drugs used in Ayurvedic pharmaceutics to prepare number of formulations. It is observed that it is one of the ingredients of most of the Ayurvedic antipyretic drugs. It is indicated in many therapeutic conditions. It is need of hour to conduct research project on this drug thoroughly for it's different therapeutic activity.

2.3PIPPALI (Piper longum Linn*)*

Since years together, *Pippali, Piper longum Linn* used in Ayurvedic medicine by Indian community. It is second important ingredient of *Hinguleshvara Rasa.*

It is used for many ailments. It is prominently used for the diseases of respiratory system. It is usually used in combination as *Trikatu, Chaturushana, Panchkol, and Shadushana* for *Deepana (digestive stimulant)* properties. Many researchers studied this drug for vivid range of its potent physicochemical, phytochemical and therapeutic activity. It is very interesting to find that, this drug proved to be boon to mankind by its therapeutic potency as described in *ancient* texts. There is tremendous scope to study it for its number of proven still unknown beneficial properties. References of this drug are found in Ayurveda literature from *Bruhattrayee* to *Nighantu* and recent books on dravyaguna. Many researchers from conventional resources are also trying to study this drug thoroughly by conducting various projects to assess its medicinal properties. In this study an attempt is made to collect more and more information regarding multidimensional aspect of *Piper longum*, Linn.

Varieties of Pippali (*Piper longum,* Linn.)**[81]**

There are four varieties of *Piper longum,* Linn

1) *Pippali* –which is found in Indian places like *Magadh, Videha* etc.

2) *Gajapippali*-it is an ambiguous drug. Many people call *Chavika'*s fruit as *Gajapippali*. (*Chavikayah phalapradnyaikathita Gajapippali).*

3) *Simhalee*-This variety is imported from Srilanka, Singapore and other foreign countries.

4) *Vanapippali*- Grows in jungles. It grows in the abundance in Bengal, but it is small, slender, and thin and less *Tikshna* (sharp). This **Pippali** (*Piper longum,* Linn.*)* is now to be imported. The big one should be Sinhalese and the small one should be from Bengal.

The Pippali from Paanveli is available under the name of Paan Pippali.

Description of Pippali (*Piper longum,* Linn.*)*[82]

Macroscopic: Fruit greenish black to black, cylindrical 2.5-5 cm long and 0.4-1cm thick consisting of minute sessile fruits, arranged around an axis, taste pungent producing numbness on the tongue.

Odor-aromatic

Microscopic:

Catkin shows 6 to 12 fruits arranged in circle on a central axis each having an outer epidermal layer of irregular cells filled with deep brown content and covered externally with a thick cuticle, mesocarp consist of larger cells usually collapsed, irregular in shape, and thin walled, a number of stone cells in singles or in groups present. Endocarp and seeds coat fused to form a deep zone, outer layer of this zone composed of thin walled cells and colourless, inner layer composed of tangentially elongated cells, having reddish-brown content, most endocarp filled with starch grains, round to oval measuring 3 to 8 micron in diameter.

Powder-Deep moss green shows fragments of parenchyma oval to elongated stone cells, oil globules and round to oval starch grains, measuring 3to8 micron in diameter.

Habitat of Pippali (*Piper longum,* Linn.*)*:[83]

***PIPPALI (Piper longum* Linn.)** is grown chiefly in the following places, Bengal, Bihar, Assam, Travancore, Nepal Bhutan, Terai region of Himalayas, Island of Philippines Malaysia, Singapore. It is cultivated in Bangladesh.

Distribution Pippali (*Piper longum,* Linn.*)***of** :[84]

Plant occurs through the country extending up to 1800 meters altitude (m.a.s.l) especially in sub montane tracts. it is cultivated to some extent in Karnataka,

Tamilnadu, Uttarpradesh, Bihar, and West Bengal. In tropical regions of India, it is cultivated ; Madras (Annamli Hills) and Assam, East Nepal, Konkan, Travancore etc.

The **Pippali** (*Piper longum,* Linn.*)* is cultivated on large scale in limestone soil, 450-500metrers (below the Cheranpunji region). It is cultivated mainly by layering of mature branches or by suckers planted at the beginning of the rainy season. The vines are well matured with cow dung cake and start bearing three or four years after planting. The spikes are harvested in January, while still green and unripe, as they are most pungent at this stage.

They are dried in the sun when they turn grey. The yield increases from 560kg per hectare in the first year to 1,680 kg (per hact) in third year and then decreases.

Chemical constituents of Pippali (*Piper longum,* Linn.*)*[85-86]

Major-The alkaloid piperine, piper longumine (Piplartine)2, piperlongumine 2-3,and also methyl1-3,4,5-trimethoxycinnamate

Others

Sesamine, a lignan5, dihydrostigmasterol and two low melting unstable compounds, one of which appeared to be isobutyl amide of an unsaturated acid, n-isobutyl-deca-trans-2-trans-4-dieneamide6, essential oil consisting of n-hexadecane, n-heptadecane, n-octadecane, n-nonadecane, n-cicosane, n-hencosane, alpha-thujene, Terpinolene, zingiberine, p-cymene, p-methoxyacetophenone, dehydrocarveol and two monocyclic sesquiterpenes.

The presence of L-tyrosine, L-cysteine hydrochloride, DL-serine and L-aspartic acid as free amino acids also has been reported in the fruits The seeds contain sylvatine dieudesmin.

In addition to palmitic, hexadecenoic, stearic, linoleic, oleic, linolenic, higher saturated acids, arachidic and behenic acids are also reported.

Two alkaloids piper longumine and piper longuminine characterized as N-(3,4,5-trimethoxy cinnamoyl)-piperidine -2-one and isobutyl amide of piperic acid

respectively (stem and root), n-hexadecane, n-heptadecane, n-octadecane, n-nonadecane, n-eicosane, n-heneicosane, alpha-thujene, terpinolene, zingiberene, p-cymene, P-methoxyacetophenone, traces of dihydrocaveol, phenylethyl alcohol and two sesquiterpenes (essential oil for dried fruits), piperine, piplartin, triacontane, dihydro-stigmasterol, an identified steroid, reducing sugars, glycosides, sesamine and methyl-3,4,5, trimetoxycinnamate (roots), major alkaloid piperine, and Sesamine, a, (stem and fruits), Sequeterpine hydrocarbon, caryophyllene, a sequeterpene, alcohol, carbonyl compound (essential oil), N-isobutyldecatrans 2-tran-4 dienamide, piperine, pipertine, and align and sesamin, two piperidine, alkaloids-pipernonaline, and piperundecalidine, alkaloids-pipernonaline and piperundecalidine (fruit) sylvatine, sesamine, and dieudesmin (seed).

Classification of *Pippali* (*Piper longum,* Linn.*)* as per different Ayurvedic texts is as follows:[87-88]

Sr. No.	***Samhita /Nighantu***	***Gana/varga***
1	*Charak Samhita*	*Shirovirechana, Vamana, Deepaniya, Kanthya, Truptighna, Asthapanopaga, Shirovirechanopaga, Hikkanigrahana , Kasahara, Shoolaprashamana*
2	*Sushruta Samhita*	*Pippalyadi Gana, Trikatu, Urdhwabhagahara, Shirovirechana, Amalkyadivarga.*
3	*Ashtang Sangraha*	*Shirovirechana, Vamana, Deepaniya, Kanthya, Truptighna, Asthapanopaga, Shirovirechanopaga, Hikkanigrahan, Kasahara, Shoolaprashamana, Sheetashamana, Pippalyadi Gana*
4	*Ashtang Hrudaa*	*Vamanopayogi, Vatsakadi Gana*
5	*Ashtang Nighantu*	*Pippalyadigana*
6	*Abhidhan Ratnamala/ Shadrasa Nighantu*	*Katukskandha*
7	*Kaidevnighantu/ pathyaapathyavibodhaka*	*Aushadhivarga*

8	*Dravyagunasngraha*	*Lavanadivarga*
9	*Dhanvantari Nighantu*	*Shatapushpadivarga*
10	*Nighantu shesh*	*Latakanda*
11	*Paryayratnamala*	*Panchkol*
12	*Bhavaprakash*	*Haritakyadivarga*
13	*Madanpala Nighantu*	*Shunthyadi varga*
14	*Madhav dravyaguna*	*Vividhaushadhi Varga*
15	*Mahaushadhi Nighantu*	*Mahaushadhi varga*
16	*Shodhit Nighantu*	*Shatapushpadi Varga*
17	*Hridaydeepak Nighantu*	*Catuspada varga*
18	*Raja Nighantu*	*Pippalydi varga*
120	*Saraswati Nighantu*	*Chandanadi varga*
20	*Nighantu Adarsh*	*Pippalydi varga*
21	*ShaligramNighantu*	*Haritakyadi varga*
22	*Priya Nighantu*	*Pippalydi varga*
23	*Gunaratnamala*	*Haritakyadi varga*
24	*Yadav Trikamji Acharya*	*Pippalyadivarga*

Properties of *Pippali* (*Piper longum*, Linn.)[89-90]

Pippali (*Piper longum*, Linn.) is used in ardra (fresh) as well as in shushka (dry) form. It has different therapeutic properties, which are as follows:

Properties of Ardra* (fresh) *Pippali (*Piper longum*, Linn.)*:*

Rasa:Madhura

Vipaka:Madhura

Veerya: Sheeta

GunaSnigdha: Guru,

Doshghnata:Pittaprashamaniya

Properties in *Shushka* (dry) *Pippali* (*Piper longum*, Linn.*):*

Rasa Katu(Pungent)

Vipaka Swadupaka (Sweet)

Veerya, Anushna

Guna- Snigdha (unctuous), Laghu (light).

Doshghnata :Vatashleshmahara (Pacifies Vata Kapha),

Pittaprakopini (Aggrevate Pitta).

In Ayurvedic pharmaceutics there are certain rules regarding collection of raw material. There is concept of Nava (fresh) and Purana (dried) dravyas. This concept is specifically applicable to *Pippali (Piper longum Linn), Vidang (Embelica ribes), Sarpi (Cow Ghee), Guda (Jaggery), and Guggulu (Commiphera mukul).*[91]

One year old dravyas is consider to be the Purana dravyas [92] Consequently another concept of *Ardra* (fresh)and *Shushka* (dried) dravyas is followed by *Nava* and *Puranadravya.*Authors are working on this perspective to find truth behind this concept of *Ardra/Nava* (Fresh) and *Shushka/Purana* (old) dravyas. Results will publish on completion of study.

Root of Pippali (*Piper longum*, Linn.*)* is also known as *Pippalimula*.–[93]

Synonyms of root **Pippali** (*Piper longum*, Linn.*)* are *Granthik, Pippalimulam, Ushanam, Chatakashir*.

Properties of *Pippalimula* (Root of *Piper longum* Linn) are[94]

It is Included in combination as *Trushana, Chaturushana, Panchakol, Shadushana.*

Properties of *Pippalimula*:

*Rasa : Katu (*pungent)

*Guna: Laghu (*light*) Ruksha (*dry), *Bhedi (*penetrating)

Veerya :Ushna (hot*)*

*Vipaka: Katu (*pungent)

*Doshghnata: Pittakar (*aggrevate *pitta), Kaphapaha, Vatapaha (pacifies Vata and Kapha)*

Therapeutic indication:

Aanaha (flatus), Pliha (splenic disease), Gulma (inflammatory condition of viscera), Krumi (as antihelmethic), Shwas (Respiratory disorders), Kshay (tuberculosis) etc

Acute toxicity study of *Pippali (Piper longum* Linn.)[95]:

Pippali (*Piper longum* Linn) fruits, one of the important drugs of Ayurvedic Pharmacopoeia have been used in approximately 324 compound formulations, as one of the ingredient. It is widely used as spices or as a food ingredient in Indian kitchen. In spite of this frequent use, the classical text of Ayurveda advocates not to use this drug in higher dose and also for a long time period. In order to assess the safety of the drug an acute and sub chronic toxicity studies of fruit of **Pippali** (*Piper longum,* Linn.*)* was carried out on Wistar strain albino rats It was observed that at the dose of 3750mg/kg at five times higher dose than therapeutic equivalent dose, there was no mortality in acute toxicity study. And in sub chronic toxicity study even at three time higher dose than therapeutic equivalent dose i.e. 2250mg/kg no serious toxicity was observed on different parameters studied.

Piperine was the first Amide isolated from piper species and has anti –inflammatory properties.

The study was conducted to assess the analgesic effect of piperine combined with evaluation of its antipyretic and ulcerogenic effect. Analgesic effect of piperine was determined using acetic acid writhing test and hot plate method. The acetic acid writhing test is a non discriminatory antinociceptive model. Intraperitoneal injection of acetic acid was given to mice ,nerve ending were excited due to the painful response and acute inflammation, because of release of endogenous substances in the peritoneal area. Aspirine and other nonsteroidal anti inflammatory drugs can reduce number of writhes in this model by blocking cyclo oxygenase enzyme in peripheral tissues, obstructing the transduction in primary afferent non ciceptors. Thus the analgesic effect of piperine may be due to blockage of the local level of prostaglandins. However the determination of this writhing test alone does not confirm that this effect is related with central analgesic substances. Furthermore hot plate test is extensively applied method in analgesic investigations for several decades in determining the action of drugs through central nervous system. This test along with the writhing test, usually differentiate between central and peripheral effects. A significant analgesic action was

shown by piperine in hot plate method after 30 minutes administration. The results showed significant analgesic effect in acetic acid writhing response and hot plate reaction test by piperine. This confirms that analgesic effect of piperine is resultant of both peripheral and central acting mechanism.

Fever is for natural nonspecific immune response against various ailments. Antipyretic are drugs which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point is elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature. The antipyretic effect was investigated by yeast inducing pyrexia test in mice. In yeast induced fever or pathogenic fever, production of prostaglandin occurs which set the thermoregulatory center at a higher temperature and is regulated by hypothalamus. Antipyretic activity is mainly due to inhibiting effect on prostaglandin formation. Administration of antipyretic compounds after 15 to 18 hrs of yeast injection is a common method used by many researchers to investigate their pyretic effects. Mice were administered with piperine (20 and 30 mg/kg) and indomethacine (10mg/kg) after yeast injection. The result obtained using piperine showed a significant ($p<0.05$) reduction in rectal pyrexia, similar to standard drug indomethacine. Thus it can be concluded that piperine possesses significant antipyretic effect in yeast included elevation of body temperature in mice, and this can also be due to its anti-inflammatory effects. Since it is well known that anti-inflammatory agents that exhibit their activity through the inhibition of prostaglandin biosynthesis may induce gastric ulceration. Appearance of gastric lesion and thus ulcer is a common side effect associated with nonsteroidal anti-inflammatory compound. Indomethacine is observed to possess ulcerogenic effect as proved by previous studies. In this study after fasting appearance of ulcers was observed in control group of rats but when mice were administered with piperine there was a significant ($p<0.05$) dose dependent reduction in the ulcer index as compared to the control group of mice .Moreover gastric lesion were seen in indomethacine treated mice.

This study concluded that piperine has antipyretic, analgesic, and anti ulcerogenic properties, however further studies are required to elucidate the mechanism of piperine to confirm these activities. [96]

2.4VATSANABH (ACONITE):

Since years together use of poisonous drugs in Ayurveda is very common. *Rasashastra* is a separate branch which comprises of *Rasaushadhies* (ie. herbomineral, metalo-mineral formulations) prepared from *Mrutalauha* (metallic bhasmas), *Kandavisha* (poisonous drugs) and *Sutam* (mercurials**).** [97]

So use of poisonous drugs only after the peculiar therapeutic processing/*sanskaras t*o enhance their therapeutic value at the same time reduce their toxicity is uniqueness of this branch of medicine.

***Visha*/poisonous described in different texts of Ayurveda:** [98]

Sr no	Name of Classical Text	*Visha* described
1	*Abhidhaan Kamdhenu*	*Visha, Haalahal, Brahmputra, Saurashtra Pradipana, Daarad, Kaakolam, Shauktikeya, Ahichhatra, Mesh Shrung, Kushth, Haimavat Pippal, Patal, Malin, Kambalam.*
2	*Rasarnava*	*Saktukam, Kaalakutam, Sitamusta, Shrungee, Krushnavisham (All are called Mahavisha)*
3	*Sushruta & Rasakamadhenu*	*Vairat, Paakalak, Kadarmaka, Kaalkuta, Mustaavish, Mahaavish, Vatsanabh, Haalahal Moolak, Sarshap, Shrungak, Market, Pundarik (all are Kandavisha and Tikshna visha)*
4	*Rasasara*	*Mayur, Kaalkut, Saktuk, Binduk Vatsanabh, Sunabham, Shankhnabham, Sumangalam, Shrungam, Karkotakam, Mustam Pushkaram, Shuklakandam, Raktashrungam, Haridram, Visham, Haalahalam, Bhraamar*
5	*Rasendra Chudamani*	*Kaalkuta, Mayur, Binduk, Saktuk, Vaaluk, Vatsanabh, Shankhnabh, Sumangal, Shrungi, Markat, Musta,*

		Kardam, Pushkar, Shikhi, Haridra, Harit, Chakra, Visha, Haalahal
67	*Ayurved Prakash & Rasatarangini*	*Haalahala, Kaalkuta, Shrungik, Pradipana, Saurashtik, Brahmputro, Haridra, Saktuk & Vatsanabh*

Out of above list ten poisonous namely *Kaalkuta, Meshshrungi, Dardur, Haalahal, Karkota, Granthi, Haaridra, Raktashrungi, Keshar and Yamadranshta* should be used for *Dhatuvada* (Alchemy) and not for medicinal purpose.[99-100] However out of nine poisonous described in *Rasatarangini Vatsanabh* is only recommended for *Rasayana*.[101]

Though, *Vatsanabh* (Aconite) is one of the important poisonous drugs still it has been used very scientifically with all precautions which have to be undertaken. It is very interesting to note that use of its antidote is also mentioned in Ayurveda classics, which shows the keenness of *Rasacharya* (ancient scientists) regarding thorough knowledge of the drug.[102-103]

Necessity to search for antidote indicated previous therapeutic utility of Aconite. It is not bound to the use of Aconite only but every drug is used very systematically and scientifically in Ayurveda whether it is poisonous or non poisonous. Every drug had been classified as per its therapeutic action. Every drug has synonyms as per its morphology and pharmacology. In this review attempt has been made to collect each and every minute information regarding this important poisonous drug.

Botanical Description, Morphology of *Vatsanabh* (Aconite): A shrub 30-60 cm high, leaves like the leaves of water melon, short and stalk with hairy structure. Flower stalk-straight external flower is blue and resembling the flower of pea.

Fruit: thorny like *Hurhur (Tilwan*), seeds – black and winged, Root-is 9 cm, in length, and 1.5 to3 cm in breadth. Externally it is brown in color but inside it is white, oily and shiny. In some texts, it is described as the plant which has leaves like *Sinduwar* i.e. *Nirgundi (Vitex nigundo*L).Shape is like umbilicus of calf. No plant grow near this plant. The plant is called *Bachanag*. Some text describe the rhizome of *Vatsanabh* as

resembling the udder of cow, not more than 5 fingers in length. In India24 types of *Vatsanabh*are available. **[104-106]**

According to *Rasatarangini* Tuber of *Vatsanabh* should be 5to 7 angula long, its thickness should be 1to2.5 angula. It should be tapering from *moola to shipha*. It is *pandur (*Yellowish white) in colour.**[107]**

(One angul =2.5 cm.)

Therapeutic Properties of *Vatsanabh* (Aconite)**:[108]**

Rasa: Madhura (sweet)

Veerya: Ushna (hot)

Vipaka: Madhur a(sweet)

Guna: Ruksha (dry)*, Tikshna (*penetrating)*, Laghu (lighta),*

Vyavayee (spread all over body without digestion)

Vikasi (It loosend the joints)

Action on Doshas*: Especially Kaphavaatshamak,,*

Ruksha, Tikshna Laghu so Kaphaghna (pacifies Kapha)

By *Madhura rasa Pitashamak (Pacifies Pitta)*

By *Ushnaveerya: Vatashamak (pacifies Vata) Vata Kapha strotogami*.

Microscopy:**[109]**

Root shows epidermis1-3layered,suberised, papilloseon outside, primary cortex consisting of 8-10 layers of oval to tangentially, elongated thin walled parenchymatous cells without or with a few intercellular spaces, a few rectangular, triangular stone cells in singles found scattered in this zone, primary cortex separated by distinct endodermis, inner bark, parenchymatous, consisting of round to oval cells, containing a few group of phloem strands, occupying more than half the radious, cambium having 6-10 angles, xylum vessels arranged almost in a ring, some scattered ,often forming v shaped ring enclosing xylem parenchyma in older portions, bundle compact often Wedge shaped having acute apex xylem exarch, metaxylum vessels met in center, starch grains simple measuring 6-18 micron in diameter, and compound grains consisting of 2-5

components with hilum in center, present in cortical cells Phloem parenchyma and xylem parenchyma.

Macroscopic:[110]

Roots paired , occasionally separated due to breakage ,ovoid, conical, small portions of stem sometimes attached tapering downwards to a paint, 2-4.5 cm rarely 5 cm long,0.4-1.8 cm thick, gradually decreased in thickness towards tapering ends, wrinkled longitudinally and transversely, rough due to root scars, dark brown to blackish brown, fracture, cartigenous, hard and white within the cambium ring and brownish outside cambium, dour indistinct, taste slightly bitter followed by a strong tingling sensation, poisonous.

Habitat of *Vatsanabh* (Aconite):[111-112]

Vatsanabh founds in Alpine regions, in ranges of Himalaya.

Uttapatti: Vatsanabhah parvat kshonishutpadyate| Ayu .Pra.

*Gadhapale ch kashmire nepaladau cha jayate|| R.T.*24/11

Vatsanabh obtained from mountain ranges.

In Himalayas from Sikkim to Garwhal at a height of 3000-5000meters [113]

Chemical Constituents of *Vatsanabh* (Aconite)**:**[114]

Aconitin (a toxin), picroeconine, benzoil, econine and homonepelin (gogte)

Types of of *Vatsanabh* (Aconite):

White and black are the two types of *Vatsanabh.* Pure *Vatsanabh* is actually white in color, it becomes black after conditioning. This conditioning is required to protect it from insects.[115]

According to *Rasakamdhenu* it is of two types *Shukla & Krushna* (white& black respectively) [116]

According to *Rasatarangini* 3 types of *Vatsanabha* are:

1) *Krushnabh*

2) Kapish

*3) Pandur(R.T.*24/15pg650)

These varieties became subsequently more superior than other**.**[117]

Races of *Vatsanabh* (Aconite)are of 4 types:[118-119]

Races	Colour	Karma
Brahman	*Pandur*	*For Roga* (Disease)
Kshatriya	*Raktavarna*	*Vishabhakshane* (to Treat Poisonous)
Vaishya	*Peetaprabh*	*Sarva Vyadhee* (All diseases)
Kshudra	*Krushnavarna*	*Sarpadrashta* (Snakebite)

Types of *Vatsanabh* (Aconite **) As per Botany & Pharmacognosy:**

Aconitum is genus belonging to the family Rannunculaceae. This is small herbaceous plants distributed mostly in the temperate region all over the world. Review of literature shows that there are more than hundred species of aconitum, of which only few are said to be growing in India. These include both toxic and non toxic species. Number of species available in India ranges between twenty seven to thirty three [120]

Datta and Mukherji (1950) revised the classification of the Aconite roots according to their botanical characters as well as on their physical and toxicological properties. According to them, there are five varieties of Aconite exist namely True Napellus, Indian Napellus, Ferox varieties, White Aconite, Non poisonous varieties[121]

In European commerce all the Indian forms of Aconite were classed as a form of *Aconitum ferox,* but critical assessment revealed that true *Aconitum ferox* Wall is not the most plentiful of all the Aconitum species growing in this country. It has been reported that the so called Aconite roots sold by the drug dealers in India is an indiscriminate mixture of the roots of *Aconitum ferox, Aconitum lycoctonum, Aconitum napellus and Aconitum palmatum* the latter predominating .According to Pandey and Ansari [122]*Visha* is *Aconitum ferox* (wall), *Ativisha* is *Aconitum heterophyllum (*Wall) *Prativisha* is *Aconitum palmatum* and *Shringivisha* is *Aconitum chasmathum* (Stapf). The drug dealers often sell the mixture of all the available varieties under name *Bachnaaga* so the commercial variety of Aconite in India is a mixture of the roots of several species of Aconitum other than above mentioned species too. *Ativisha* and *Prativisha* are considered non poisonous. In *Rasashastra Visha* or *Vatsanabh* (Aconite) are recommended for medicinal purpose only after *Shodhana* or the process of detoxificatoion. This *Visha* or *Vatsanabh* are represented by *Aconitum chasmathum* in

Ayurvedic Pharmacopoeia of India.[123] According to IUCN red listed medicinal plants of Siddha both of these species [*Aconitum ferox* & *Aconitum chasmathum*] come under critically endangered and endangered category[124]. Considering above situation Pharmacognosy study became essential before preparation of any formulation containing *Vatsanabh* as an ingredient to know its actual species.

Such study regarding authentication of *Vatsanabh* (Aconite species) is in process by author & result will be published on completion of work.

Vatsanabh* from *Bruhattrayee:

Charak Samhita, Charakcharya included it in *Sthavara visha*.[125]

Also it is one of the ingredient of *Aaindra Rasayana*.[126]

Also in *Charak Samhita adhyay* 3, *Vatsanabh* is used for *Dharanakarma (to wear)* to avoid *Vishamajvara*.[127]

In Ashtang Hrudaya,[128]*Visha/Vatsanabha* is recommended in the treatment of *kushtha A.hr.ci.19/83,Timir(A.hr.ut.13/33),*

Gandamala (A.hr.ut.30/19), Naadivrana & Apachi (A.hr.ut.30/23),

Akhuvisha (A.hr.ut.38/32),

Aconite is also classified in various *Nighantu* as follows:[129]

Sr No	**Name of *Nighantu***	***Varga***
1	*Ashtang Nighantu*	*Viprakira varga*
2	*Abhidhan Ratnamala/Shadrasanighantu*	*Kashayaskandha*
3	*Dhanvantari Nighantu*	*Mishrakadi varga*
4	*Paryayratnamala*	*Visha*
5	*Bhavprakash Nighantu*	*mishravarga*
6	*Mahaushadha Nighantu*	*Swarnadi varga*
7	*Kaidev Nighantu*	*Visha varga*
8	*Raaj Nighantu*	*Mishrak Varga*

***Grahya Lakshanas*[130]:**

According to classical text Rasataranini It should be plucked during winter or spring. It should be collected when it is fully matured, riped, developed (after fruiting), free from insects and other factors which destroy its therapeutic efficacy, it should be *Nava* (fresh), Guru(heavy), *Snigdha* (unctuous), and *Sthula* (of desired thickness).

***Shodhana* of Aconite:**

Importance of Aconite *shodhana:* *Vatsanabh*/Aconite has two important synonyms *Visha* (poison) and *Amrita* (Nectar).

Both are indicating the action of *Vatsanabh* before and after *Shodhana* (detoxification). If it is used in impure form it will lead to *Daha* (burning), *Moha* (inebriant state), *Hrudgatirodhana* (cardiac depressant & *Mrutyu* (death)[131]

Contrary if it is used after purification it will proved to be life saving like Nectar.

Effect of *Shodhana* of Aconite in cow urine and cow milk was studied by ***P.K Sarkar and etal,*** reveal that the duration of administration of raw *Vatsanabh* increased, the cardio toxic activity also increased. The major effect observed were increase in heart rate, prolongation of the duration of QRS complex& prolongation of the repolarization period between two QRS complexes. These three toxic changes were much less in group of purified *Vatsanabh* processed with cow milk and cow urine. This clearly shows that the cardio-toxicity producing principles observed in raw *Vatsanabh* is removed by *Shodhana* process. [132]

Variety of methods for the Vatsanabh Shodhana (processing of Aconite) is described by different author of *Rasashastra texts.*

Sr no	**Text book**	**Method of *Shodhana***
1	*Rasatarngini*[133]	1) *Vatsanabh* cut into small pieces and boiled in Cow milk for 3 to 6 hrs in *Dolayantra*. (Special device in which drugs are kept in pottali in suspended manner in liquid media recommended for *Shodhana*) 2) Instead of cow milk, goat milk should used for detoxication 3) Similar to *Ayurved Prakash* small pieces of *Vatsanabh* tied in cotton cloth pottali & kept in pot filled with cow urine subjected to *teevra Atapa* (scorching sun heat) for three days. Everyday fresh cow urine should be added with removal

		of previously added cow urine.
2	***Ayurved Prakash***[134]	1) As per *Yogatarangini*, pieces of *Vatsanabh* kept in cotton cloth pottali. This pottali is kept in container filled with cow urine. This container should kept in *teevra Atapa* (scorching sun heat) for 3 days. Everyday stale cow urine from container should be removed and fresh cow urine should be added. After 3 days This *Vatsanabh* used for medicinal purpose. consider to be *Shuddha Vatsanabh* and *2) Vatsanabh* pieces are kept in *Dolayantra* filled with goat milk and boiled for 1 yaam i.e. 3 hrs. In absence of goat milk, cow milk is recommended. 3) Small pieces of *Vatsanabh* should be kept in faeces of buffalo. Then heat should be given by *dhrudhmudra* /anaerobic incineration. After cooling itself *Vatsanabh* pieces should be removed and considered as *Shuddha Vatsanabh*.
3. Rasendra sarasangraha & other[135] Other than cow milk, cow urine & goat milk, *Triphla quath* is also recommended for *Shodhana* to fill *dolayantra*. **4. Other[**[136] Most of the classical texts described *Vatsanabh shodhana* in cow milk or cow urine.		

Therapeutic Action:[137-138]

• *Pranahar*: Fatal if use inappropriately. • *Vyavayee*: spread all over body without digestion • *Vikasi*: It loosend the joints • *Aagneya:* hot in potency • *Yogvaah*i: Increases the therapeutic potential of the drug to which it is accompanied	• *Panduhar*: Anaemia • *Jvarahar*: In fever/pyrexia • *Amavatahara*: Rheumatic conditions • *Vataghna:*used in diseases occurred due to vitiation of vata • *Timi*r:opthalmic condition • *Naishandhyahara*: night blindness • *Abhishyand*: conjunctivities • *Netrashoth*:opthalmic condition

• *Bruhan*: nourishes body • *Veeryavardhana*: Aphrodisiac. • *Kanthrukaghna: Throat diseases* • *Sannipataghna*: useful in diseases caused by vitiation of all three i.e. *Vata, pitta & kapha* • *Rasayana*: Rejuvenating and anti *aging* • *Deepan*: Improve digestive power • *Balavardhana: improves strength* • *Agnimandyahara:* Correcting *digestive disorders* • *Kasahara:*Releaves cough • *Gulmahara:* Inflammatory condition *in internal organs* • *Kushthara*: diseases of skin	• *Akhu, vrushchik, sarpavishahara:* Rodentbite, scorpiobite & snake bite • *Karnashoth*:Otitis media • *Shirashoolahara:*headache • *Ghrudhrasi*:sciatica • *Kativedna*:Lower backache • *Plihodar*: useful in splenic disorders • *Vataraktahara*: in gout • *Shwashara*: Respiratory diseases • *Gudamayahara:* Anorectal diseases • *Grahanihara*: metabolic diseases

Antidotes of *Vatsanabh*:

According to *Rasaratnasamucchaya*[139]Cow ghee is considered as one of the best antidotes for *Visha***.** *Tankan (*Borax) administered with ghee is main Antidote of Aconite. *Arjuna* (Terminalia Arjuna) bark along with honey/ghee & *Rajani (Curcuma longa* Linn*), Meghnad (Butea frondosa* Wild*), Sarpakshi (Ophirrhiza mungos* L*) churna* along with ghee is another antidote described in *Rasakamdhenu***.**[140] Double quantity of *Marich* (*Piper nigrum*) is also recommended as an antidote in absence of borax [141]

During consumption of Aconite rules regarding behavioral and dietary aspects are also explained in *Rasaratnasamucchaya.*[142]

For *Rasayana p*urpose *Vatsanabh* should be used after *Ghrutpana* (consumption of Ghee) & *Shodhana* (Detoxication) For *Sattva* predominant people it should be administered during winter and spring, conditionally it should be used during summer

for the treatment of diseases. It should not be used in rainy season and during cloudy weather.

Contraindicated in *Krodha*(anger), *Pitta* predominance, *bhrama (*giddiness), *Klaibya* (Impotence), King or Royal employees, when feeling hungry or thirsty, after walking long distance (tired), *Marmarogas*, pregnant ladies, kids, old age people, emaciated individuals. Diet rich in pungent, sour, salty tastes, oil, sleep during day time, exposure to fire and heat are also forbidden.

Dose as per different Ayurvedic texts:

Rasasanket Kalika[143]

Uttam= 8 yava

Madhyam= 6 yava

Hina= 4 yava

According *to Ayu Prakash*.[144]

Staring from dose equal to til seed [seasom] to 8 *yava*. It cures all disease of human being.

According to *Rasatarangini*[145]

1/*16th Ratti* to1/8th*Ratti(1Ratti=125mg)*

Fatal Dose as per toxicology:[146]

1gm root,2-5mg of Aconitine, Extract-250mg, Tincture 2-30ml

Fatal period 2-6 hrs **(**According to API dose 15-30mg powder form.[147]

Researches on Aconite:

Comparative Physico-chemical profile *of Vatsanabh (Aconitum ferox, Ranunculaceae) mula* processed through cow urine and cow milk concluded that *shodhan a* (purification) alters the physicochemical parameters of *Vatsanabhmula* and also Rf value of the sample in HPTLC less numbers of spot were detected under both 254 and 365 nm after shodhana with Gomutra (Cow urine) indicating denaturation of some component after *shodhana.* This study concluded that for purification of Vatsanabh Mula, Gomutra (Cow urine) is better extraction media than *Godugdha (cow milk)* as far as toxic alkaloid are concerned.[148]

Another study was conducted to evaluate likely impact of *Shodhana* treatment on cardiac activity, antipyretic activity, and neuromuscular activity of Aconite[149]. Pharmacological activities of raw Aconite (RV), treated Aconite in cow's urine (SM) and treated aconite in cow's milk (SD) were evaluated and compared. Aconites are found to have potent antipyretic effect, the aconite group of alkaloids has the body temperature lowering effect The result of antipyretic study also supports this activity of Raw *Vatsanabh (*RV). A significant body temperature lowering effect was found in Raw *Vatsanabh* (RV) treated group, even the antipyretic activity of Raw *Vatsanab*h (RV) was comparable to that of paracetamol. The body temperature lowering activity was found to be less in aconite in cow's urine (SM) and aconite in cow's milk (SD) treated group in comparison to Raw *Vatsanabh (*RV) treated group. In this respect aconite in cow's milk (SD) treated group was found to be better antipyretic agent than aconite in cow's urine (SM) group.

After *shodhana* treatment total alkaloid content was reduced to 80%. Aconitine content of aconite in cow's milk (SD) treated group was found to be reduced to70%and no trace of aconitine is found in Aconite in cow's urine (SM). These facts may be considered as the cause behind low antipyretic activity exhibited by SM & SD and better result of SD as antipyretic agent than SM.

This study concluded for the efficacy of the *Shodhana* process in removing toxic principles from raw aconite, which are mainly cardiac and neuro-muscular toxic aconitine related alkaloids & it is also proved that *shodhana* process though leads to some loss of antipyretic activity will not remove the benefit in drastic manner. *Vatsanabh* is one of the important poisonous drug used in *Rasashastra* after proper processing. Since years together it is used to cure number of disease conditions. Considering variation in its species it become essential to undertake study, cultivation, research project to treasure this miraculous drug so it should not be included in rare and endangered species in future.

3. AIM AND OBJECTIVES

Aim : Assessment of Antipyretic and Analgesic activity of *Hinguleshvara Rasa* in experimental animal.

Primary Objective :

- Preparation of *Hinguleshvara Rasa.*
- Physicochemical analysis and characterization of *Hinguleshvara Rasa.*
- Assessment of antipyretic and analgesic activity of *Hinguleshvara Rasa.*

Secondary Objective :

- Assessment of acute toxicity study of *Shuddha Hingula*
- Assessment of acute toxicity study of *Shuddha Vatsanabh*
- Assessment of acute toxicity study of *Hinguleshvara Rasa*
- Assessment of antipyretic study of *Shuddha Hingula*
- Assessment of antipyretic study of Shuddha Vatsanabh
- Assessment of antipyretic study of *Pippali*
- Assessment of Analgesic activity of *Shuddha Hingula*
- Assessment of Analgesic activity of *Shuddha Vatsanabh*
- Assessment of Analgesic activity of *Pippali*

4. MATERIALS AND METHODS

Materials and Methods:

This study was conducted in three stages:

A] Preparation of *Hinguleshvara Rasa*

B] Analytical Study

C] Experimental Study

Hinguleshvara Rasa was prepared in accordance with the formulation mentioned in the classical text *Bhaishajya Ratnavali* **(BR 5/483)**

Preparation of *Hinguleshvara Rasa* includes following steps:

Collection& authentication of raw materials: .

Prior to actual preparation of formulation '*Hinguleshvara Rasa'* its ingredients *Hingula* (Red cinnabar: HgS) *Vatsanabh (Aconitum balfourii* Stapf) and *Pippali (Piper longum* Linn*)* were procured from reliable source and then authenticated in laboratory.

Collection of *Hingula* (Red Cinnabar:HgS)*:*

Hingula (Red Cinnabar:HgS) is one of the ingredients of *Hinguleshvara Rasa*. It was collected from local market.

On the basis of organoleptic characters described in classical texts, the collected material was authenticated as *Hingula* (Red Cinnabar: HgS). *The* collected sample was then subjected to Physico-chemical analysis. Percentage of Mercury (Hg) and Sulphur (S) was estimated as part of authentication. On completion of physicochemical analysis sample was subjected to its *Shodhana* process.

Collection *of Pippali (Piper longum* Linn*)*

Fruits of *Pippali (Piper longum* Linn.), the second ingredient of *Hinguleshvara Rasa* were directly collected from the site of cultivation from the farmers. Total 7 samples of *Pippali (Piper longum* Linn.) were collected from the same cultivation site for six consecutive years (winter 2014 to winter 2019) &in winter 2021 at the time of the plucking of fruits of *Pippali (Piper longum Linn*.). All the six samples were washed with water, dried and kept in airtight glass containers named as A, B, C, D, E, F and new *Pippali*. The samples were then subjected to examination for their authentication

in recognized laboratories. Physicochemical, pharmacognostic and phytochemical analysis of all the samples was then carried out. As per standards given in Ayurvedic Pharmacopoeia following physico-chemical test were conducted. Determination of Foreign matter content, determination of Loss on drying, determination of Total Ash and Acid-insoluble Ash, determination of Alcohol soluble extractive and Water soluble extractive. Pharmacognostic study was performed. In phytochemical analysis total alkaloid content & piperine % of each sample was carried out. On the basis of results obtained from all analysis, out of these 6 samples of fruits of *Pippali* (*Piper longum* Linn.) sample 'C' (plucked in the winter 2016) was used in the present preparation of *Hinguleshvara Rasa.*

Collection of Roots of *Vatsanabha* (Aconite:*Aconitum balfourii* Stapf)

Roots of *Vatsanabh* (Aconite:***Aconitum balfourii*** **Stapf)** are the third and the last ingredient of *Hinguleshvara Rasa.* There are number of species of *Aconite* available. All species are available in alpine area (ranges of Himalaya) of India. According to Ayurvedic Pharmacopoeia of India *Aconitum chasmanthum* Stapf species is considered as *Vatsanabh*. But *Aconitum ferox* Wall *as well as Aconitum chasmanthum* Stapf are scarcely available.

In the name of *Aconitum ferox* Wall *or Aconitum Chasmanthum* Stapf mixture of more than two or three species are sold in the market. Initially it was decided to take chasmathum species as per Ayurvedic Pharmacopoeia of India, for the preparation of *Hinguleshvara Rasa.*

Efforts were made to procure *chasmanthum species of Aconite.* But being endangered and unavailable in required quantity, it could not be procured. Attempt to procure *Aconitumferox* Wall also failed.

In an attempt to procure appropriate species for the present study, 8 sample of *Aconite* from different sources were collected & submitted to authenticate in two different institutes. Pharmacognosy study of all the samples was carried out. On the basis of this study and being unadulterated and available in required quantity, it was decided to use ***Aconitum balfourii*** **Stapf** for the present study for preparation of *Hinguleshvara Rasa.*

The collected sample of ***Aconitum balfourii* Stapf** was then subjected to Physico-chemical analysis. As per standards given in Ayurvedic Pharmacopoeia of India, following Physico-chemical test were conducted on the samples of roots *Aconitum balfourii* Stapf (Before and after *shodhana):* namely determination of Foreign matter content, determination of Loss on drying, determination of Total Ash, determination of Acid insoluble Ash, determination of Alcohol soluble extractive and determination of Water soluble extractive. This sample was then subjected to *shodhan*a process in three batches namely A, B & C. In phytochemical analysis total alkaloid content & Aconitine % of each batch was estimated.

Physicochemical analysis of *Hinguleshvara Rasa* and its ingredients:

Analytical study of Hinguleshvara *Rasa* and its ingredients namely *Hingula* (Red cinnabar: HgS), *Vatsanabha (Aconitum balfourii* Stapf), *Pippali (Piper longum* Linn) was performed as per *protocol* for testing Ayurvedic, Siddha & Unani medicines Government of India Department of Ayush, ministry of health & family welfare, pharmacopoeial laboratory for Indian medicines Ghaziabad. Details of test procedure implemented are as follows:

Physico-chemical analysis of *Hingula* (Red cinnabar: HgS) **before and after shodhana and *Hinguleshvara Rasa***

4.1Test performed For *Hingula* (Red cinnabar: HgS)

4.1a Determination of Moisture Content (Loss on Drying)[150]

Equipments: Evaporating dish, oven, weighing balance

Ingredients: test sample 10 gms of *Hingula* (Red cinnabar: HgS)

About 10 g *of Hingula* (Red cinnabar: HgS) was placed without preliminary drying) in a tared evaporating dish. After placing in the tared evaporating dish dried at 105º for 5 hours, and weighed. The drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent was continued. Constant weight was reached when two consecutive weightings after drying for 30 minutes and cooling for 30 minutes in a desiccators, showed not more than 0.01 g difference. Same procedure is applied for *Hingula* (Red cinnabar: HgS)
(before and after *shodhana)* & ***Hinguleshvara Rasa***

4.1bDetermination of Total Ash content)[151]:

Equipments: Silica dish, oven, weighing balance, ash less filter paper

Ingredients: test sample 2Gms ***Hinguleshvara Rasa***

Of about 2g accurately weighed, of the grounded ***Hinguleshvara Rasa*** in a tared platinum or silica dish was incinerated at a temperature not exceeding 450º until free from carbon, allowed to cool and weighed. If a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water; the residue was collected on an ash less filter paper. The residue and filter paper was incinerated, the filtrate was added and evaporated to dryness, and was ignited at a temperature not exceeding 450º. The percentage of ash was calculated with reference to the air-dried ***Hinguleshvara Rasa***.

4.1c Determination of Acid insoluble Ash)[152]:

Equipments: Silica dish, oven, weighing balance, ash less filter paper

Ingredients: sample ash, hot water

Chemicals : Dilute hydrochloric acid (2N),The ash obtained as above was boiled for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter in a Gooch crucible or on an ash less filter paper was collected, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air dried ***Hinguleshvara Rasa*.**

4.1d Determination of Loss on ignition *Hinguleshvara Rasa*)[153]:

Equipments: weighing machine, crucible, spirit lamp

Weight of ***Hinguleshvara Rasa*** before heating minus the weight after heating is initial loss on ignition. % of loss on ignition determined by dividing initial loss by initial weight of ***Hinguleshvara Rasa*** and multiplied by 100 (in house method used by Bhide foundation)

(W2-w3/w2-w1) x100

Where w1=weight of empty crucible.

W2=initial weight of crucible and *Hinguleshvara Rasa*.

W3 =final weight of crucible and *Hinguleshvara Rasa.*

Estimation of Mercury (Hg) and Sulphur(S) content: Same procedure is applied for *Hingula* (Red cinnabar: HgS) (before and after shodhana). **For estimation of Sulphur(S) from** *Hingula* (Red cinnabar: HgS) (before and after *shodhana*)

4.1e Analysis of Sulphur)[154]

Method used:

Using about 60mg of precipitated Sulphur, accurately weighed, proceed as directed under Oxygen Flask Combustion (471), using a 1000ml flask and using a mixture of 10 ml of water and 5.0 ml of hydrogen peroxide TS as the absorbing liquid. When the combustion is complete, fill the lip of the flask with water, loosen the stopper, then rinse the stopper, the sample holder, and the sides of the flask with water, and remove the stopper assembly. Heat the contents of the flask to boil, and boil for about 2 minutes. Cool to room temperature, add phenolphthalein TS, and titrate with 0.1N sodium hydroxide VS. Perform a blank determination, and make any necessary correction. Each ml of 0.1N Sodium hydroxide is equivalent to 1.603mg of S

Estimation of Mercury (Hg) from *Hingula* (Red cinnabar: HgS) (before and after ***shodhana***) by Atomic Absorption Spectrophotometry (AAS) and Estimation of Mercury (Hg) from *Hinguleshvara Rasa was* performed by Induced couple plasma method respectively.

1 Reagents:

Preparation of reducing reagents:

Sodium boro hydratere agent (NaBH4):

Weight accurately1.0g Sodium borohydrate in 100 mL beaker.

Add little distilled water to dissolve it.

Weight accurately 1.25g Sodiumhydroxide in another100mL beaker.

Add little distilled water to dissolve it.

Mix both the beaker solution in 250mLvolumetric flask.

Makeup the volume 250mLwith distilled water.

5% Hydrochloric acid (HCl):

Take 146 mL distilled water in 250 mL volumetric flask. Slowly add 104 mL conc. HCl in it. Mix well the solution.

5% Potassiumiodide (KI) solution:

Accurately weight1.25g KI in 25mL volumetric flask.

Add distilled water to dissolve it properly.

Makeup the volume up to 25 mL with the distilled water.

5% Ascorbic acid (Vitamin C) solution:

Accurately weight1.25g Ascorbic acid in 25 mL volumetric flask.

Add distilled water to dissolve it properly.

Makeup the volume up to 25 mL with the distilled water.

2 Procedure:

Preparation of Standard solutions:

Determination of Mercury(Hg) content)[155]

1000 ppb Hg (Working standard): Accurately pipette out 0.1mL standard solution of Mercury (1000 ppm) in 100mL volumetric flask and make up the volume with distilled water.

20ppb Hg: Accurately pipette out 1.0mLworking standard (1000ppb) in 50mL volumetric flask and makeup the volume with distilled water.

40ppb Hg: Accurately pipette out 2.0mLworking standard (1000ppb) in 50 mL volumetric flask and makeup the volume with distilled water.

60ppb Hg: Accurately pipette out 3.0mLworking standard (1000ppb) in 50 mL volumetric flask and makeup the volume with distilled water.

80ppb Hg: Accurately pipette out 4.0mLworking standard (1000ppb) in 50mL volumetric flask and makeup the volume with distilled water.

Preparation of test solution:

Sample preparation (Applicable for Capsule, Tablet, Cream, Syrup, Oil and all raw materials)**:**

Accurately weigh 0.5g or 0.5mL sample in PFA (Perfluoroalkoxy) Teflon vessels. Add 8mL 69% Nitric acid (HNO_3) along the sides of the vessel so as to remove the adhering matter. If effervescence is formed, then keep vessel open for 30 minutes in fumigation chamber.

Close the vessels tightly and keep on the turner. Set the method parameters as follows:

Vessel No.	Powr		Ramp (Min)	°C (control)	Holdtime (min)
	MAX	%			
8	800 W	100	30	180	10
16	800 W	100	30	180	10

After digestion procedure is complete, take out the vessels and keep it in fumigation chamber for 10 minutes. Open the vessels slowly to release the pressure from the vessel. Leave the vessel undisturbed till the fumes rereleased completely.

Transfer the solution in 50mL volumetric flask using funnel. Make up the volume with distilled water. Shake well and filter using what man filter paperNo.1.

(Note: To avoid any loss of sample and for accurate reading 2 – 3 times rinse the vessel with distilled water.)

Use this solution as attest solution. Prepare the blank solution simultaneously.

Sample preparation (Applicable for Inorganic materials)**:**

Accurately weigh 0.25g sample in PFA (Perfluoroalkoxy) Teflon vessels. Add4.8mL 69% Nitric acid (HNO_3) and 1.2mL Conc. HCl along the sides of the vessel so as to remove the adhering matter. If effervescence is formed, then keep vessel open for30 minutes in fumigation chamber.

Close the vessels tightly and keep on the turner. Set the method parameters are as follows:

Vessel no.	Power		Ramp (Min)	°C (control)	Holdtime (min)
	MAX	%			
8	800 W	80	30	180	10
16	1600 W	80	30	180	10

After digestion procedure is complete, take out the vessels and keep it in fumigation chamber for 10 minutes. Open the vessels slowly to release the pressure from the vessel. Leave the vessel undisturbed till the fumes are released completely. Again add 3mL Conc. Hydrochloric acid (HCl), 0.5mL Hydrofluoric acid (HF),0.5mL Hydrogen peroxide (H_2O_2) in the vessel. Keep it for pre-digestion (till effervescence stops).

Close the vessels tightly and keep for the digestion by setting method parameters
After digestion procedure is complete, take out the vessels and keep it in fumigation chamber for 10 minutes. Open the vessels slowly to release the pressure from the vessel. Leave the vessel undisturbed till the fumes are released completely.

Transfer the solution in 50ml volumetric flask using funnel. Makeup the volume with distilled water. Shake well and filter using what man filter paper No.1. (**Note:** To avoid any loss of sample and for accurate reading 2–3 times rinse the vessel with distilled water.)

Use this solution as a test solution. Prepare the blank solution simultaneously. **(Note:** Vessel scan be used either 8 or 16 . If 8 vessels are used, then keep them alternatively on turner. Don't remove the turner from the digestive system till the temperature is high.)

Detection:
Plot a Calibration curve on Atomic Absorption spectrophotometer, detect the metals in samples using calibration curve. (SOP No. VRC/QA/IN/029/2) and (SOP No. VRC/QA/IN/030/2).

4.2For Ardrak Swarasa (Juice of *Zingiber officinale* Rosc)

4.2a pH Ardrak swarasa[156]

Determination of pH: The term "pH" refers to the measurement of hydrogen ion activity in the solution. Since the direct measurement of the pH is very difficult, specific electrodes are needed for quick and accurate pH determination. pH is measured on a scale of 0 to 14, with lower values indicating high H+ (more acidic) and higher values indicating low H+ ion activity (less acidic). A pH of 7 is considered as neutral.

Every whole unit in pH represents a ten-fold increase in or decrease in hydrogen ion concentration. pH is measured using pH meter, which comprises a detecting unit consisting of a glass electrode, reference electrode, usually a calomel electrode connected by KCl Bridge to the pH sensitive glass electrode and an indicating unit which indicates the pH corresponding to the electromotive force is then detected. Before measurement, pH meter should be calibrated by using at least two buffers.

Equipment and material required:-

1. pH meter
2. pH electrode filled with KCL solution
3. Buffer solutions of pH4 and pH 7
4. Clean beakers
5. Tissue papers
6. Distilled water
7. Thermometer
8. Ginger (*Zingiber officinale* Rosc)

Procedure:-

- Plug in the pH meter to power source and let it warm up for 5 to 10 minutes
- Wash the glass electrode with distilled water and clean slowly with a soft tissue.
- Note the temperature of water and set the same on the pH meter
- Place the electrode in pH 7 buffer solution and set the value of 7 on the pH meter turning the Calibrate knob on the meter.
- Take out the electrode, wash with DW and clean.
- Dip the electrode in the pH 4 buffer solution. Adjust the value on the pH read out meter by the Slope switch. Repeat with pH 7 and pH4 buffers till a correct and stable reading is displaced.
- While moving and cleaning the electrode, put the selector switch on standby mode. Turn to pH mode for recording the pH.
- Now place the electrode in the sample whose pH is to be determined.
- You can take a number of simultaneous readings for different samples until the power is on pH Meter
- Same procedure was used to determine pH of Gomutra (cow urine).

4.2b Determination of Moisture Content @110$^{0C)}$[157]

Equipments: Evaporating dish, oven, weighing balance

Ingredients: test sample 10 gms of Ardrak juice (Juice of *Zingiber officinale* Rosc)

About 10 g *of* Ardrak juice (Juice of *Zingiber officinale* Rosc) was placed without preliminary drying) in a tared evaporating dish. After placing in the tared evaporating dish dried at 105º for 5 hours, and weighed. The drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent was continued. Constant weight was reached when two consecutive weightings after drying for 30 minutes and cooling for 30 minutes in a desiccators, showed not more than 0.01 g difference.

4.2cDetermination of Total Ash content)[158]

Equipments: Silica dish, oven, weighing balance, ash less filter paper

Ingredients: test sample 2Gms Ardrak juice (Juice of *Zingiber officinale* Rosc) of About 2g accurately weighed, grounded Ardrak juice (Juice of *Zingiber officinale* Rosc) was taken in a tared platinum or silica dish and was incinerated at a temperature not exceeding 450º until free from carbon, allowed to cool and weighed. If a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water; the residue was collected on an ash less filter paper. The residue and filter paper was incinerated, the filtrate was added and evaporates to dryness, and was ignited at a temperature not exceeding 450º. The percentage of ash was calculated with reference to the air-dried Ardrak juice (Juice of *Zingiber officinale* Roscoe)

4.2d Determination of Acid insoluble Ash:)[159]

Equipments: Silica dish, oven, weighing balance, ash less filter paper

Ingredients: sample ash, hot water

Chemicals : Dilute hydrochloric acid (2N)

The ash obtained as above was boiled for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter in a Gooch crucible or on an ash less filter paper was collected, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air dried **(**Juice of *Zingiber officinale* Roscoe

4.2e Specific Gravity of Ardrak juice (Juice of *Zingiber officinale* Rosc))[160]

Method given in Indian pharmacopoeia was used for determination of specific gravity.

Def: Specific gravity of a liquid is the weight of a given volume of the liquid at 25°C unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air.

Principle:

Weight of the **Ardrak juice (Juice of *Zingiber officinale* Roscoe)** contained in pycnometer

$$\text{Specific Gravity} = \frac{\text{Weight of the Ardrak juice (Juice of } \textit{Zingiber officinale} \text{ Roscoe) contained in pycnometer}}{\text{Weight of the water contained in pycnometer}}$$

(Both determined at 25°unless otherwise specified)

$$\text{Specific Gravity} = \frac{W1-W}{W2-W}$$

Where W_1=Weight of specific gravity bottle (pycnometer) completely filled with Ardrak juice (Juice of *Zingiber officinale* Rosc

W_2 = Weight of specific gravity bottle completely filled with water.

W=Weight of empty pycnometer.

All weights were taken in air at constant temperature.

Three reading were taken & mean of these readings was considered as specific gravity of Ardrak juice (Juice of *Zingiber officinale* Rosc)

4.2f TLC: of Ardrak juice (Juice of *Zingiber officinale* Rosc) [161]

Extraction: Adrak Swarasa

Adsorbent used: Silica gel G_{60} F_{254}

Mobile phase :- Toluene : Ethyl Acetate (6 : 4: 1**)**

Thin layer chromatography was carried out on precoated silica gel $60F_{254}$plate.

Mobile phase :- Toluene : Ethyl Acetate (6 : 4: 1)

Test Solution: In 1.0 gm sample of Adrak Swarasa (Juice of *Zingiber officinale* Roscoe about 5 ml of Toluene was added Then this mixture was heated on water bath for 5 minutes and filtered.

Procedure: 10 ul each of test solution was applied on TLC plate as bands of 10mm developed the plate to a distance of 8 cm from the line of application. The plate was dried in air spray with solution of Dragendorff reagent. The plate was heated at 110^0 C for 5 min. or till the bands were clearly visible. Bands were observed and Rf value was calculated.

4.2g Detection of Heavy metals like Cadmium, Arsenic, Mercury &Lead from Ardrak juice (Juice of *Zingiber officinale* Rosc)**[162]**

Principle-

Limit test of heavy metals is based on the reaction of metallic impurities with hydrogen sulphide in acidic medium to form brownish colour solution. Metals that response to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum. The metallic impurities in substances are expressed as parts of lead per million Parts of the substance. The usual limit as per Indian Pharmacopoeia is 20 ppm

Procedure:

The Indian Pharmacopoeia has adopted three methods for the limit test of heavy metals.

Method II: Use for the substance which do not give clear color less solution under the specific condition.

Test sample	**Standard compound**
Weigh specific quantity of Ardrak Juice (Juice of *Zingiber officinale* Rosc, moisten with sulphuric acid and ignite on a low flame till completely charred Add few drops of nitric acid and heat to 500 °C Allow to cool and add 4 ml of hydrochloric acid and evaporate to dryness Moisten the residue with 10 ml of hydrochloric acid and digest for two minutes Neutralize with ammonia solution and make just acid with acetic acid	Take 2 ml of standard lead solution and dilute to 25 ml with water
Adjust the pH between 3 to 4 and filter if necessary	Adjust the pH between 3 to 4 by adding dilute acetic acid 'Sp' or dilute ammonia solution 'Sp'
Dilute with water to 35 ml	Dilute with water to 35 ml
Add freshly prepared 10 ml of hydrogen sulphide solution	Add freshly prepared 10 ml of hydrogen sulphide solution
Dilute with water to 50 ml	Dilute with water to 50 ml
Allow to stand for five minutes	Allow to stand for five minutes
View downwards over a white surface	View downwards over a white surface

Observation:

The color produce in sample solution should not be greater than standard solution. If color produces in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa. Lead is a most undesirable impurity in medical compounds and comes through use of sulphuric acid, lead lined apparatus and glass bottles use for storage of chemicals.

Principle:

Limit test of lead is based on the reaction of lead and diphenylthiocabazone (dithizone) in alkaline solution to form lead dithizone complex which is read in color. Dithizone is green in color in chloroform and lead-dithizone complex is violet in color,

so the resulting color at the end of process is red.

Procedure: **Test sample**	**Standard compound**
A known quantity of (Juice of *Zingiber officinale* Rosc solution is transferred in a separating funnel	A standard lead solution is prepared equivalent to the amount of lead permitted in the sample under examination
Add 6ml of ammonium citrate	Add 6ml of ammonium citrate
Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride	Add 2 ml of potassium cyanide and 2 ml of hydroxylamine hydrochloride
Add 2 drops of phenol red	Add 2 drops of phenol red
Make solution alkaline by adding ammonia solution.	Make solution alkaline by adding ammonia solution.
Extract with 5 ml of dithizone until it becomes green	Extract with 5 ml of dithizone until it becomes green
Combine dithizone extracts are shaken for 30 mins with 30 ml of nitric acid and the chloroform layer is discarded	Combine dithizone extracts are shaken for 30 mins with 30 ml of nitric acid and the chloroform layer is discarded
To the acid solution add 5 ml of standard dithizone solution	To the acid solution add 5 ml of standard dithizone solution
Add 4 ml of ammonium cyanide	Add 4 ml of ammonium cyanide
Shake for 30 mins	Shake for 30 mins
Observe the color	Observe the color

Observation:

The intensity of the color of complex, is depends on the amount of lead in the solution. The color produce in sample solution should not be greater than standard solution. If color produces in sample solution is less than the standard solution, the sample will pass the limit test of lead and vice versa.

Reasons:

Ammonium citrate, potassium cyanide, hydroxylamine hydrochloride is used to make pH optimum so interference and influence of other impurities have been eliminated. Phenol red is used as indicator to develop the color at the end of process

Lead present as an impurities in the substance, gets separated by extracting an alkaline solution with a dithizone extraction solution.

4.3 Characterization of *Hingula* (Red cinnabar: HgS) **(Before and after shodhana) and *Hinguleshvara Rasa* by SEM, particle size analyzer, FTIR & X-ray Diffraction technique.**

4.3a Field Emmission Scanning Electron Microscopy (FESEM)-:

Instrument Detail-FE-SEM Model no. Nova Nano450 was used for SEM Testing.

In vial a pinch of *Ashuddha Hingula* (Red cinnabar: HgS) + 1ml of Isopropyl Alcohol were mixed and a solution was prepared. It was then kept for10 min sonication and subjected for analysis.

Similarly sample of *Shuddha Hingula* (Red cinnabar: HgS) *and Hinguleshvara Rasa* were prepared and subjected for analysis.

4.3b Particle size analyzer (P.S.A_): P.S.A.biovis instrument model was used to detect particle size. No pre sample treatment was required. Direct analysis on P.S.A. biovis give reports of particle size of all samples of *Shuddha and Ashuddha Hingula* (Red cinnabar :Hgs before and after processing with juice *of Zinziber officinale* Rosc) *Hinguleshvara Rasa* by ball mill and *Hinguleshvara Rasa* by manual grinding.

4.3c Fourier Transferred Infrared Spectroscopy (F.T.I.R):

Fourier Transferred Infrared Spectroscopy (F.T.I.R) helps to give functional group. It detects absorbance. No pre sample treatment is required. F.T.I.R Agilent carry 600 model was used for F.T.I.R of all samples of *Shuddha and Ashuddha Hingula* (Red Cinnabar :Hgs before and after processing with juice *of Zinziber officinale* Rosc) *Hinguleshvara Rasa* by ball mill and *Hinguleshvara Rasa* by manual grinding F.T.I.R of Ardrak swarasa (Ginger Juice, *Zinziber officinale* Rosc juice) was also performed. Particle size analysis (P.S.A)

And Fourier Transferred Infrared Spectroscopy (F.T.I.R) were performed at Dr Sabnis'Jeevanrekha Analytical services.

4.3d X-ray Diffraction:

XRD Instrument: X-ray Diffraction (XRD) recorded on a PAN alytical X'pert PRO powder X-ray diffractometer with Cu Kα radiation (λ = 1.54

Å, 30 mA, 40 kV) was used for analysis. The test was performed in the Department of characterization of elements, National chemical laboratory Pune).

4.4Physico-chemical Analysis of samples of *Vatsanabh (Aconitum balfourii* Stapf*)) obtained before and after *shodhana*

4.4aPharmacognosy of *Vatsanabh (Aconitum)*

Authentication of *Aconitum balfourii* Stapf was carried out from two different institutes. Methods used for pharmacognosy study is described as below.

Methodology:

Different varieties of *Aconite* from different places like Srinagar, Shahajahanpura, Mumbai, Delhi, cultivated species from Himachal Pradesh were collected.

4.4.a-1.Preservation of samples)[163]

Few pieces of collected *Aconite* root samples were preserved in glass jar containing AAF solution70 % Ethyl alcohol: Glacial acetic acid: Formalin) in the ratio of 90:5:5 (v/v/v) to utilize them for microscopic studies whenever needed and also for future research work.

4.4.a-2.Preparation of powder

Collected *Aconite* roots were cleaned properly to remove soil or other filthy materials and then dried in shade. Dried material was cut into small pieces, subjected for powdering individually in mechanical mixer grinder and sieved through 80# and 120# individually for further study. Then the powder of different samples was individually stored in airtight glass containers.

4.4.a-3.Macroscopic evaluation)[164-165]

Macroscopic characters like shape, size, color etc. of root, of *Aconite* samples were studied systematically as per visual observation, following standard procedures of

taxonomy and verified with existing floras, taxonomy, botany textbook and Pharmacognosy books.

4.4.a-4.Organoleptic characters) [166-167]

Color, odor, taste and texture of powders of different root samples of *Aconite* were recorded separately through visual and sensory observations.

4.4.a-5.Microscopic evaluation [168-169]: Free hand transverse section of root, were taken, cleared with chloral hydrate, if necessary. The sections were first observed in distilled water under compound microscope (QUASMO, India). For retaining the sections for longer period under the cover slip, glycerin was added

For microscopic evaluation of the powder, a small pinch powder was placed on glass slide, and then 2-4 drops of water were added and observed under compound microscope) [170]

4.4.a-6.Micrometric evaluation[171]

Microscopic measurement like dimension of cells, fibers, trichomes, stone cells and cellular contents like starch grains, oil globules, brown content, crystals etc. were performed using micrometer with the help of compound microscope

4.4.a-7.Histochemical evaluation.[172-173]

Thin transverse sections as well as powder of roots of Aconite were stained with different reagents like phloroglucinol along with hydrochloric acid, iodine solution and ferric chloride solution to find lignified elements, starch grains and tannin respectively. Isolation of tissue was done by Schultz's maceration process. small fragments of the root were taken in a test tube and 5ml of 50%nitric acid (HNO3) was added, then the test tube was heated till the pieces become very soft. The tube was removed from the flame and pinch by pinch the powder of potassium chlorate (KCLO3) was added in the hot HNO3 solution till the effervescence was subsided gently. The soften pieces of the root were removed in another test tube and was thoroughly washed with fresh water and then was shaken vigorously by adding few drops of water to get all the separated lignified elements. The element were allowed to settled down and taken on glass slides and observed under microscope at different magnifications.)[174]

4.4b TLC Method of *Vatsanabh (Aconite Root)* [175]

Thin layer chromatography was carried out on precoated silica gel $60F_{254}$ plate.

Chloroform: Methanol :(1:1v/v) was used as solvent system.

Test Solution: In 1.0 gm sample of root of *Aconite*, about 5 ml of Methanol was added Then this mixture was heated on water bath for 5 minutes and filtered.

Procedure: 10 ul each of test solution was applied on TLC plate as bands of 10mm developed the plate to a distance of 8 cm from the line of application. The plate was dried in air spray with solution of Dragendorff reagent. The plate was heated at 110^0 C for 5 min. or till the bands were clearly visible. Bands were observed and Rf value was calculated.

Physicochemical tests as per Ayurvedic pharmacopoeia of India are as follows:

4.4cDetermination of Foreign Matter)[176]

Equipments: Weighing machine, Microscope.

Ingredients: test sample 10 Gms

100g of the *Vatsanabh (Aconitum balfourii* Stapf*)* to be examined was weighed and spreaded in a thin layer. The foreign matter was detected by inspection with the unaided eye or by the use of a lens (6xs). The foreign matter was separated and weighed. The percentage of present foreign matter content was calculated. For each sample of *Vatsanabh (Aconitum balfourii* Stapf*) same* procedure was applied.

4.4d Determination of Moisture Content (Loss on Drying) [177]

Equipments: Evaporating dish, oven, weighing balance

Ingredients: test sample 10 gms

About 10 g *of Vatsanabh (Aconitum balfourii* Stapf*)* was placed without preliminary drying) in a tared evaporating dish. After placing in the tared evaporating dish dried at 105º for 5 hours, and weighed. The drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent was continued. Constant weight was reached when two consecutive weightings after drying for 30 minutes and cooling for 30 minutes in a desiccators, showed not more

than 0.01 g difference. For each sample of *Vatsanabh (Aconitum balfourii Stapf)* same procedure was applied.

4.4e Determination of Total Ash) [178]

Equipments: Silica dish, oven, weighing balance, ash less filter paper

Ingredients: test sample 2Gms

About 2g accurately weighed, of the grounded *Vatsanabh (Aconitum balfourii* Stapf*)* in a tared platinum or silica dish was incinerated at a temperature not exceeding 450º until free from carbon, allowed to cool and weighed. If a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water; the residue was collected on an ash less filter paper. The residue and filter paper was incinerated, the filtrate was added and evaporates to dryness, and was ignited at a temperature not exceeding 450º. The percentage of ash was calculated with reference to the air-dried *Vatsanabh (Aconitum balfourii* Stapf*)*. For each sample of *Vatsanabh (Aconitum balfourii* Stapf*)* same procedure is applied.

4.4f Determination of Acid Insoluble Ash) [179]

Equipments: Silica dish, oven, weighing balance, ash less filter paper

Ingredients: sample ash, hot water

Chemicals: Dilute hydrochloric acid(2N) ,

The ash obtained as above was boiled for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter in a Gooch crucible or on an ash less filter paper was collected, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air dried *Vatsanabh (Aconitum balfourii* Stapf).

For each sample of *Vatsanabh (Aconitum balfourii* Stapf) same procedure is applied.

4.4g Determination of Alcohol Soluble Extractive) [180]

Equipments: closed flask, filter paper, flat bottom shallow dish, oven

Ingredients: 5Gm powdered sample of *(Aconitum balfourii* Stapf,)

Chemicals : Ethyl Alcohol

5 g of the air dried *Vatsanabh (Aconitum balfourii* Stapf*) was macerated*, coarsely powdered, with 100 ml of alcohol of the specified strength in a closed flask for twenty-

four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105º, to constant weight and weighed. The percentage *Vatsanabh (Aconitum balfourii* Stapf), of alcohol-soluble extractive was calculated with reference to the air-dried dried *Vatsanabh (Aconitum balfourii* Stapf*)).* For each sample of *Vatsanabh (Aconitum balfourii* Stapf) same procedure was applied.

4.4h Determination of Water Soluble Extractive) [181]

Equipments: closed flask, filter paper, flat bottom shallow dish, oven,
Weighing machine

Ingredients: 5Gm powdered sample of *(Aconitum balfourii* Stapf,) hot water

Chemicals : Chloroform water -2.5 ml of chloroform added to purified water 1000ml and shaking frequently until a clear solution is formed. 5 g of the air dried *Vatsanabh (Aconitum balfourii Stapf) was macerated,* coarsely powdered, with 100 ml of chloroform water of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105º, to constant weight and was weighed. The percentage of water -soluble extractive was calculated with reference to the air-dried *Vatsanabh (Aconitum balfourii* Stapf*)* For each sample of *Vatsanabh (Aconitum balfouriiI* Stapf *)* same procedure was applied.

Physico-chemical Analysis of *Pippali (Piper longum* Linn was conducted:

Authentication of *Piper longum Linn* was carried out from recognized institutes.
Methods used for Pharmacognosy study is described as below.

4.5.Pharmacognosy of *Piper longum Fruits*:

4.5a Materials and methods

Sample of *Piper longum* Linn fruits were collected from Agricultural College district Akola state Maharashtra. Six samples of six successive years during plucking period i.e. in winter 2014, 2015,2016,2017,2018 and2019were collected. Out of which *Pippali*

/Piper longum Linn plucked in winter 2016 was used in the preparation of *Hinguleshvara Rasa* in the present study.

4.5.a-1.Preservation of sample[182]

Fruits of *Pipper longum* Linn during plucking period i. e. winter were collected. Dried in sunlight. Kept in airtight container/glass bottle and subjected to authentication, physicochemical, Pharmacognosy and phytochemical studies.

4.5.a-2.Preparation of powder)

Fruits of *Piper Longum* Linn cleaned to remove soil and other filthy material and dried. Dried material was cut into small pieces, subjected for powdering individually in mechanical mixer grinder and sieved through 100# individually for further study. Then the powder of fruits was stored in airtight glass containers.

4.5.a-3.Macroscopic evaluation [183-184]

Macroscopic characters like shape, size, color etc. of fruit of *Piper longum* Linn were studied systematically as per visual observation, following standard procedures of taxonomy and verified with existing floras, taxonomy, botany textbook and pharmacognosy books.

4.5.a-4.Organoleptic characters [185-186]

Color, odor, taste and texture of powders of fruits of *Piper longum* Linn. were recorded separately through visual and sensory observations

4.5.a-5.Microscopic evaluation[187,188,189]

Free hand transverse sections of fruit were taken, cleared with chloral hydrate, if necessary. The sections were first observed in distilled water under compound microscope (QUASMO, India). For retaining the sections for longer period under the cover slip, glycerin was added for microscopic evaluation of the powder, a small pinch powder was placed on glass slide, then 2-4 drops of water were added and observed under compound microscope

4.5.a-6.Micrometric evaluation[190]

Microscopic measurement like dimension of cells, fibers, trichomes, stone cells and cellular contents like starch grains, oil globules, brown content, crystals etc. were performed using micrometer with the help of compound microscope.

4.5.a-7.Histochemical evaluation[191]

Thin transverse sections as well as powder of fruits of *Piper longum* were stained with different reagents like phloroglucinol along with hydrochloric acid, iodine solution and ferric chloride solution to find lignified elements, starch grains and tannin respectively

4.5b TLC Method for *Piper longum Linn*[192]

Thin layer chromatography was carried out on precoated silica gel $60F_{254}$ plate.
Chloroform: Methanol : (1:1v/v) was used as solvent system.

Test Solution: In 1.0 gm sample of fruits of ***Piper longum Linn***, about 5 ml of Methanol was added Then this mixture was heated on water bath for 5 minutes and filtered.

Procedure: 10 ul each of test solution was applied on TLC plate as bands of 10mm developed the plate to a distance of 8 cm from the line of application. The plate was dried in air spray with solution of Dragendorff reagent. The plate was heated at 110^0 C for 5 min. or till the bands were clearly visible. Bands were observed and Rf value was calculated.

Physicochemical tests as per Ayurvedic pharmacopoeia of India are as follows:

4.5.c Determination of Foreign Matter *of Piper longum Linn* [193}

Equipments: Weighing machine, Microscope.
Ingredients: test sample 10 Gms
100g of fruits of the ***Piper longum Linn*** to be examined was weighed and spreaded in a thin layer. The foreign matter was detected by inspection with the unaided eye or by the use of a lens (6xs). The foreign matter was separated and weighed. The percentage of

present foreign matter content was calculated. For each sample of fruits of ***Piper longum Linn)*** same procedure was applied.

4.5.d Determination of Moisture Content of *Piper longum Linn fruits* [194]

Equipments: Evaporating dish, oven, weighing balance

Ingredients: test sample 10 gms

About 10 g *of fruit of* ***Piper longum Linn)*** was placed without preliminary drying) in a tared evaporating dish. After placing in the tared evaporating dish dried at 105º for 5 hours, and weighed. The drying and weighing at one-hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent was continued. Constant weight was reached when two consecutive weightings after drying for 30 minutes and cooling for 30 minutes in a desiccators, showed not more than 0.01 g difference. For each sample of ***Piper longum Linn)*** same procedure was applied.

4.5.e Determination of Total Ash of *Piper longum Linn fruits* [195]

Equipments: Silica dish, oven, weighing balance, ash less filter paper

Ingredients: test sample 2Gms

About 2g accurately weighed, of the grounded ***Piper longum* Linn)** in a tared platinum or silica dish was incinerated at a temperature not exceeding 450º until free from carbon, allowed to cool and weighed. If a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water, the residue was collected on an ash less filter paper. The residue and filter paper was incinerated, the filtrate was added and evaporate to dryness, and was ignited at a temperature not exceeding 450º. The percentage of ash was calculated with reference to the air-dried ***fruits of Piper longum* Linn**. For each sample of ***Piper longum* Linn** same procedure is applied.

4.5.f Determination of Acid Insoluble Ash of *Piper longum Linn fruits* [[196]

Equipments: Silica dish, oven, weighing balance, ash less filter paper

Ingredients: sample ash, hot water

Chemicals: Dilute hydrochloric acid(2N) ,

The ash obtained as above was boiled for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter in a Gooch crucible or on an ash less filter paper was

collected, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air dried ***Piper longum* Linn**
For each sample of fruits of ***Piper longum* Linn** same procedure was applied.

4.5.g Determination of Alcohol Soluble Extractive of *Piper longum Linn fruits* [197]:

Equipments: closed flask, filter paper, flat bottom shallow dish, oven
Ingredients: 5Gm powdered sample of *(**Piper longum* **Linn**,)
Chemicals : Ethyl Alcohol

5 g of the air dried ***Piper longum Linn)*** *was macerated,* coarsely powdered, with 100 ml of alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105º, to constant weight and weighed. The percentage ***Piper longum* Linn**), of alcohol-soluble extractive was calculated with reference to the air-dried dried ***Piper longum* Linn** For each sample ***Piper longum* Linn**) same procedure was applied.

4.5.h Determination of Water Soluble Extractive of *Piper longum Linn fruits* [198]

Equipments: closed flask, filter paper, flat bottom shallow dish, oven,
Weighing machine
Ingredients: 5Gm powdered sample of *(**Piper longum* **Linn**,) hot water
Chemicals : Chloroform water-2.5 ml of chloroform added to purified water 1000 ml and shaking frequently until a clear solution is formed.
5 g of the air dried ***Piper longum* Linn)** *was macerated,* coarsely powdered, with 100 ml of chloroform water of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried at 105º, to constant weight and was weighed. The percentage of water -soluble extractive was calculated with reference to the air-dried fruits of ***Piper longum* Linn**) For each sample of fruits of ***Piper longum* Linn**) same procedure was applied.

4.5.i Estimation of Microbial load /viable count of *Piper longum Linn fruits* [**:[199-200]**

Viable count for bacteria and fungi is counted in *Pippali* ***Piper longum*** **Linn**)

Total viable count of bacteria and total yeasts and mould were determined using plate count method. This method is based on the principle that when material containing bacteria is cultured every viable bacterium develop into a visible colony on a nutrient agar medium.

In this process a small measured volume or weight was mixed with a large volume of sterile water or saline called diluents.
Dilutions were made usually in multiples of ten.
Dilution=volume of the sample/Total volume of the sample & the diluents
Serial dilutions were prepared by transferring a known volume of the dilution to second dilution blank and so on once diluted, the specified volume of the dilution sample (1ml/0.1ml) from various dilution was added to sterile plate to which molten and cooled agar was added. The colonies were counted. The number of organisms developed on the plate after an incubation period of 24-48 hrs for bacteria and 2-3 days for fungi. (Room Temperature) The number of colonies were calculated as number of cells per ml=No of colonies/Amount plated into dilution.

Requirements:

1) Sample
2) 9ml dilution blank (7)
3) Sterile petriplates (12)
4) Sterile 1 ml pipette
5) Nutrient Agar
6) Potato dextrose Agar.
7) Colony counters

Procedure:

1) The blank was labeled as 10-^{1},10^{-2},….10^{-7}
2) Initial dilution was prepared by adding 1ml per 1 gm in 1^{st} tube and serially diluted it further upto10^{-7}

3) 1 ml or 0.1 ml of suspension was transferred with sterile pipette to sterile petriplates.
4) Approximately 15 ml of nutrient media was added- melted, cooled to 45^0C to each petriplates containing diluted sample
5) It was then mixed and rotated and allowed to solidify.
6) Then it was subjected to incubate at 37^0C for 24-48 hrs for bacteria and at room temperature for 48-72 hrs for fungi.

Observations: The plate was observed and the colonies were counted in the range of 30-300.

Result:

Organism per gm of samples=no of colonies/Amount plated*dilution factor.

Viable count for bacteria and fungi was counted in *Shuddha Hingula* (Red cinnabar: HgS) (*Gomutra (Cow urine) Shuddha Vatsanabh (Aconitum balfourii* Stapf*), and Hinguleshvara Rasa)* by same procedure.

4.5.j.Estimation of Total solid content from Gomutra[201]

Total solid content is expressed in ratio of weights obtained before and after the drying process. The test protocol consist of placing a sample (25ml) in a beaker in an oven at a temperature of 105^0 Cuntil a steady mass is obtained

Dry solid content=M2x100/M1%

M1=weight of the initial sample

M2=weight after drying

4.6 Phytochemical Analysis:

It includes High performance liquid chromatography for

1 Estimation of Piperine content and total alkaloid content from fruits of *Piper longum* Linn

2 Estimation of Aconitine content and total alkaloid content from *Ashuddha* and and all batches of *Shuddha Vatsanabh (Aconitum balfourii Stapfii* Stapf)

3 Estimation of total alkaloid,and estimation of Aconitine and Piperine content of *Hinguleshvara Rasa.*

4.6.a) Following methods applied for estimation of Piperine content:

Preparation of Standard Solutions(S): Accurately weighed 10.0mg of Standard Piperine in a 100mL Volumetric flask, add Methanol HPLC Grade to dissolve it upto mark. Sonicate for 5 minutes. Filter it using 0.22 microns syringe filter and it for HPLC analysis.		
Preparation of Sample Solutions(T): Accurately weighed 2.0g of sample Pippali powder in an iodine flask and reflux on water bath for 15minutes.Transfer it100 mL Volumetric flask and add Methanol HPLC Grade to make volume upto mark. Sonicate for 5 minutes. Filter it using 0.22 microns syringe filter and it for HPLC analysis.		
Buffer Preparation: Weigh accurately 68 mg Potassium dihydrogen Orthophosphate in 250 mL Volumetric flask and dissolve in water up to mark. Adjust its pH to 2.5 with dilute Orthophosphoric acid solution. Filter With 0.22 micron vacuum filter paper and use for further analysis.		
Chromatographic Conditions:		
HPLC System		Shimadzu UFLC Modular
Stationary Phase		C18
Column Temperature		40^{o}C
Filtering System		0. syringefilter
Runtime		30 minutes
Sample Application Volume		20 µL
Mobile Phase(MP)		Acetonitrile: Phosphatebuffer
Flow Rate		1.0 mL/min
Wavelength		345 nm
Mode of Separation		Gradient
Sample	**Piperine Standard**	**Pippali (*Piper longum* Linn)**

4.6.b) Following method is applied for estimation of Aconitine content:

Preparation of Standard Solutions (S): Accurately weighed 5 mg of Standard Aconitine in a 2.5 mL Volumetric flask, add Methanol HPLC Grade to dissolve it up to mark. Sonicate for 5 minutes. Dilute 1mLfrom it in 2 mL volumetric flask and make volume up to mark. Filter it using 0.22 microns syringe filter and it for HPLC analysis.

Preparation of Sample Solutions (T) : Accurately weighed 5.0g each sample of Sample A, Sample B, Sample C and Ashuddha Vatsanabh powder in a 250mL iodine flask, add 50mL Methanol HPLC Grade and reflux for 2 hours, filter by Whatman Filter paper No. 1. Concentrate up to 5 mL and add 10 % Sulphuric Acid solution to it. Stir it and filter it. Make it Alkaline with strong ammonia. Extract with Chloroform 10 mL for 3 times. Collect all Chloroform layer and evaporate to dryness. Dissolve it in methanol and transfer to 10mL Volumetric Flask and make volume with methanol. Filter using 0.22 microns syringe filter and it for HPL Canalysis.	
Chromatographic Conditions:	
HPLC System	Shimadzu UFLC Modular
Stationary Phase	C18
Column Temperature	40 ^{o}C
Filtering System	0.22µsyringefilter
Runtime	60 Minutes
Sample Application Volume	20 µL
Mobile Phase(MP)	Acetonitrile: Tetrahydrofuran: Triethanolamine
Flow Rate	1.0mL/min
Wavelength	238 nm
Mode of Separation	Gradient

4.6.c) Estimation of total alkaloid[202]

Assay of Total alkaloid by Gravimetry

Reagents:

1N H_2SO_4:

Take about 25 mL of distilled water in100mL volumetric flask.

Add 2.8mL of Conc. Sulphuric acid into it and make up the volume to 100mL with distilled water.

Procedure:

Assay of Total Alkaloids:

Take about 2.0 g accurately weighed sample or test substance in 150mL conical flask.

Add 5mL of10% liquorammonia to the sample (if required add more drop-wiseto ensure that the material is completely soaked) and shake for 5 min.
Now add 100 mL of mixture of solvent ether : methanol [75:25]. Mix the content properly and then place it on shaker for one hour.
After shaking, filter the content through filter paper. Give additional washing to the residue in the same flask with mixture of ether : methanol [75:25] and filter with filter paper. Repeat twice.

Pool both the clear filtrates into a separating funnel.
Add 25mL of 1 Nsulphuric acid into it, shake well and collect the acidl ayer in separate beaker. Repeat twice.

Then add mixture of a 25mL of 1Nsulphuric acid, 55mL of distilled water and 20mL of methanol into the solution, shake well and collect the acid layer in separate beaker.
Collect the all acid layers in suitable size of separating funnel and wash it with 25mL chloroform.

Collect the acid layer and make it alkaline with the addition of 10% liquor ammonia (Make sure by checking red litmus turns blue).

Take this alkaline solution in separating funnel and add 25mL chloroform. Collect the chloroform layer in separate beaker. Repeat 4 times.
Pool all the chloroform layers and give wash with water (10mL) and discard the water layer.
Filter the chloroform layer in a previously weighed beaker and evaporate to dryness

$$\%\text{of Alkaloid} = \frac{\text{Weight of residue}}{\text{Wt. of substance taken}} \times 100$$

4.7 Shodhana of Ingredients of *Hinguleshvara Rasa*

Shodhana (processing of detoxiction) of *Vatsanabh (Aconitum balfourii* Stapf), *Shodhana* of *Hingula* (Red cinnabar: HgS)

4.7.Shodhana of *Hingula* (Red cinnabar: HgS)[203]

सप्तकृत्वा ऽऽर्द्रकद्रावैर्ल कुचस्याम्बुनाऽ थवा ।

शोषितो भावयित्वाऽथ निर्दोषो जायते खलु ।।136।।

R.R.S.3/136

Shodhana of *Hingula* (Red cinnabar: HgS) was performed according to description *in Rasa Ratna* Samuccaya 3/152. By this method 7 times trituration of *Hingula* (Red cinnabar: HgS) with *Ardrak swaras* (Fresh juice of Ginger: *Zinzibar officinalis*) was performed.

Details of *Hingula* (Red cinnabar: HgS) ***shodhana* are as follows:**

Initially 690 gms of *Hingula* (Red cinnabar: HgS)*)* was taken, out of which 60 gms was kept aside for analysis. Thus 630 gms of *Hingula* (Red cinnabar: HgS) was subjected to first *bhavana* of *Ardrak swarasa* (Ginger Juice).

Preparation of *Ardrakswarasa*/ Ginger Juice (Juice of Zinziber officinalis .250 gms of *Ardrak* (Ginger)+50 ml water was added and grounded in mixture and filter to obtain Juice =210 ml.For each batch same method was applied to prepare juice.

630 gm of *Hingula* (Red cinnabar: HgS)) + 100 ml of *Ardrakswarasa* was added in wet grinder and triturated at the rate of 115 revolutions /min. Again 110 ml of *Ardrakswarasa* was added. Total 210 ml swarasa was added for first *bhavana*. Trituration in wet grinder performed for 8hrs daily. (9am to 5pm) and kept for drying whole night.

After completion of 1st*bhvana,* 620gms of *Hingula* (Red cinnabar: HgS) was obtained out of which 50 Gms of *Hingula* (Red cinnabar: HgS) was kept aside and remaining 570gms of *Hingula* (Red cinnabar: HgS)was subjected to 2nd*bhavana.* Same procedure was repeated for 2nd*bhavana*.After completion of 2nd*bhavana* same 570 gms of *Hingula* (Red cinnabar: HgS) was obtained which was then subjected to 3rd*bhavana.*After completion of 3rdbhavana 560gms of *Hingula* (Red cinnabar: HgS)was obtained which was subjected to 4th *bhavana.* Preparation of *Ardrak swarasa* was kept constant (as given in first bhavana) for all seven bhavanas. On completion of 4th *bhavana* 542 gms of *Hingula* (Red cinnabar: HgS) *was* obtained. After completion of 4th *bhavana* 50 gms

of *Hingula* (Red cinnabar: HgS) was kept aside.542-50=492 gms of *Hingula*(Red cinnabar: HgS) was then subjected to5th *bhavana.* On completion of 5th*bhavana* there was increase in weight of *Hingula* (Red cinnabar: HgS) observed. 508 gms of *Hingula* (Red cinnabar: HgS)*)* was obtained after 5th*bhavana,* which was subjected to 6th*bhavana.*

With the same *bhavana* procedure, 6th bhavana was completed to obtain 532 gms of *Hingula* (Red cinnabar: HgS)) which was then subjected to 7th *bhavana.*

532 gms of *Hingula* (Red cinnabar: HgS))+210 ml of *Ardrak swarasa* was added and 7th *bhavana* was completed. On completion of 7th*bhavana* 547gms of *Shuddha Hingula* (Red cinnabar: HgS) was obtained

During the whole procedure of *shodhana* it was observed that weight of *Hingula* (Red cinnabar: HgS) was reduced during first 4 *bhavanas* and on 5th,6th and 7th *bhavana* there was weight gain in *Hingula (Red Cinnabar)* Out of 547gms 50 gms of *Shuddha Hingula* (Red cinnabar: HgS) was kept aside for physic-chemical analysis .Remaining was used for *Hinguleshvara Rasa.*

4.8 *Shodhana* of *Vatsanabh (Aconitum balfourii* Stapf) [204]

विषं तु खण्डशः कृत्वा वस्त्रखण्डेन बन्धयेत् ॥ ५४ ॥
गोमूत्रेऽथ विनिक्षिप्य स्थापयेदातपे त्र्यहम् । गोमूत्रं तु प्रदातव्यं
प्रत्यहं नूतनं बुधैः ॥ ५५ ॥
त्र्यहेऽतीते तदुद्धृत्य शोषयेन्मृदु पेषयेत् । शुध्यत्येवं विषं सेवायोग्यं भवति रोगजित् ।। Ayu. Pra.6/54-56

For s*hodhana* of *Vatsanabh (Aconitum balfourii* Stapf) cultivated and authenticated species of *Vatsanabh (Aconitum balfourii* Stapf) was used. *Shodhana* of *Vatsanabh (Aconitum balfourii* Stapf) was performed according to method described in *Ayurveda prakash.*

By this method small pieces of *Vatsanabh (Aconitum balfourii* Stapf)) were kept in bundle of cotton cloth in pottali form . These pottali were kept suspended in container

full of *Gomutra* (cow urine).The containers containing cow urine and Aconite pottali were exposed to scorching Sun heat (*Atapa*) for three days. Every day *Gomutra* (cow urine) was changed with fresh cow urine. On 4th day *Aconite* from pottalies were taken out, washed with lukewarm water and subjected to drying. This dried *Vatsanabh (Aconitum balfourii* Stapf) subjected to grinding to produce powder of *Shuddha Vatsanabh (Aconitum balfourii* Stapf). This powder was used in the preparation of *Hinguleshvara Rasa.*

Details of *Shodhana* procedure are as follows:

Raw *Vatsanabh (Aconitum balfourii* Stapf) was pounded with stainless steel *Udukhalyantra* (mortar and pestle). Each root of *Vatsanabh (Aconitum balfourii* Stapf) was pounded in such a way that each root was broken into 4-6 pieces. Pounding of *Aconitum* root in powder form was avoided to prevent its loss in cow urine during s*hodhan*a. So small pieces of *Aconitum root* (and not powder) were prepared. These small pieces were equally distributed in three batches namely A B & C as described above. Each batch contained 1200 gms of *Ashuddha Vatsanabh* (impure Aconite/ *Aconitum balfourii* Stapf)

Preparation of pottalies

Three pottalies containing were stitched from white cotton cloth. Each pottali filled with 1200 gm of *Ashuddha Vatsanabh (Aconitum balfourii* Stapf)). Pottalies were properly tied with cotton thread. These pottali were named as A, B, C respectively.

Vessels used *for Vatsanabh (**Aconitum balfourii*** Stapf) ***Shodhana:***

3 buckets made up of stainless steel were used for *Vatsanabh (Aconitum balfourii* Stapf) *shodhana*. They were labeled as A, B, C respectively. Three pottalies of *Ashuddha Vatsanabh (Aconitum balfourii* Stapf) (impure *Aconite*) namely A, B, C were kept in these buckets respectively.

Collection of Cow urine

Cow urine was collected from cow of '*Gir'* species. Three cows were selected and named as A, B, C. Fresh cow urine from these three cows were separately collected in three stainless steel container labeled as A, B C respectively. Sample of 200 ml of each cow urine was kept aside in glass bottle. These samples were covered with silver foil

and subjected to laboratory for Analysis. After getting reports of microbial content of cow urine, *shodhana* of *Vatsanabh (Aconitum balfourii* Stapf) started. Cow urine of cow A and C were used for *shodhana* purpose as microbial load was found within limits as per standards in these batches.

Everyday 2000 ml of fresh cow urine was added in each bucket namely A, B and C. Care was taken to maintain the level of *Gomutra* above pottali so that pottali was properly dipped in cow urine. These three batches were kept in sunlight from 9am to 4pm daily for 3 days. Every day before addition of fresh cow urine, previously added cow urine was taken out. The same procedure was repeated for three batches A, B, C for three days respectively.

On fourth day residual of cow urine from all three batches were taken out.
Pottalies of *Aconite* were opened. *Aconite* was taken out. These *Aconitum* batches A, B, C were washed with lukewarm water and subjected to drying.
These three batches were considered as *Shuddha Vatsanabh (Aconitum balfourii* Stapf) namely, A, B and C.

These three batches of *Shuddha Vatsanabh (Aconitum balfourii* Stapf) were subjected to physicochemical, pharmacognostic and phytochemical analysis.

4.9 Preparation of *Hinguleshvara Rasa*)[205]

Materials and methods of preparation of *Hinguleshvara Rasa* is as follows: Preparation of *Hinguleshvara Rasa* in accordance with the reference of classical text *Bhaishajya Ratnavali Jvarachikitsa:*

तुल्यांश मर्दयेत् खल्ले पिप्पलीं हिङ्गुलं विषम् । गुञ्जाऽर्द्धं मधुना देयं वातज्वरनिवृत्तये ।। ४८३।।

B.R 5/483

Hinguleshvara Rasa was prepared according to description in *Bhaishajya Ratnavali Jvara Chikitsaadhyaya* 5/483 In this formulation equal quantities of *Shuddha Hingula* (Red Cinnabar*), Pippali* (Piper longum Linn), and *Vatsanabh* (roots of Aconitum balfourii Stapf) were grounded together to produce *Hinguleshvara Rasa.*

Sr. No.	INGREDIENTS	QUANTITY	EQUIPMENTS
1	*Shodhit Vatsanabh* (Aconite processed with Cow urine)	250 gms	*Khalwayantra* (Mortar pestle)
2	*Shodhit Hingula* (Red cinnabar: HgS)	250gms	*Khalwayantra* (Mortar pestle)
3	*Pippali* (*Piper longum Linn*)	250gms	*Khalwayantra* (Mortar pestle)

Powder of *Shuddha* ***Hingula*** **(Red cinnabar: HgS)** passed through sieves having Mesh Size 200, Powder of *Pippali/Piper longum* Linn and *Vatsanabh* (roots of *Aconitum balfourii* Stapf*)* each passed through sieves having mesh size 100 were mixed together. All three ingredients were subjected to mix in Ball mill machine.

Total 750 Gms of material was kept in drum of ball mill machine having 20 porcelene balls. Mixing was performed 8hrs daily (9am to 5pm.) for 3 days.

On fourth day uniform mixture of *Hinguleshvara Rasa* was obtained. This mixture was subjected to trituration in *Khalvayantra* (mortar pestle) for 7 days.7hrs (9am to4pm) trituration performed daily for 12 days. One day =12 hrs i.e. from sunrise to sunset, so for7 days trituration =84 hrs. So total 84 hrs trituration performed to get *Hinguleshvara Rasa.* This *Hinguleshvara Rasa was* then subjected to characterization by SEM, Particle size analyzer, FTIR and XRD, and physicochemical, phytochemical analysis.

Experimental study

This study was comprised of three parts:

- 4.10 Study of Acute oral toxicity of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfouri* Stapf*), & Shuddha Hingula (*Red Cinnabar:HgS) by following OECD 423 guidelines
- 4.11 Study of antipyretic activity of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*), & Shuddha Hingula (*Red Cinnabar:HgS) *& Pippali (Piper longum* Linn*)*

- 4.12 Study of Analgesic activity of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfouri* Stapf*), & Shuddha Hingula (*Red Cinnabar:HgS) *& Pippali (Piper longum* Linn*)*

4.10 Study design adopted for determination of acute oral toxicity was as follows:

MATERIALS AND METHODS

Acute oral toxicity of *Hinguleshvara Rasa*, *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf), & *Shuddha Hingula* (Red Cinnabar:HgS) by following OECD 423 guideline

4.10.a TEST Material

1) **Test Material : "*Hinguleshvara Rasa*"**
   Physical State : Solid
2) **Test Material : "*Shuddha Hingula (*Red Cinnabar) HgS"**
   Physical State : Solid
3) **Test Material : "*Shuddha Vatsanabh (Aconitum balfourii* Stapf*)*"**
   Physical State : Solid

4.10.b DOSE SELECTION AND PREPARATION:

Sample was stored at room temperature.

The dose of each test material selected for the study was 300 mg/kg at step 1 and repetition of 300 mg/kg at step 2 for test material *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*),& Shuddha Hingula (*Red Cinnabar:HgS)

The doses of each test material selected for the study were 2000 mg/kg at step 3 for the test material *Hinguleshvara Rasa*, *Shuddha Vatsanabh (Aconitum balfouri* Stapf*), & **Shuddha Hingula (*Red Cinnabar:HgS)** The Formulation of the test material was prepared shortly before dosing. The vehicle used for dose preparation was corn oil.

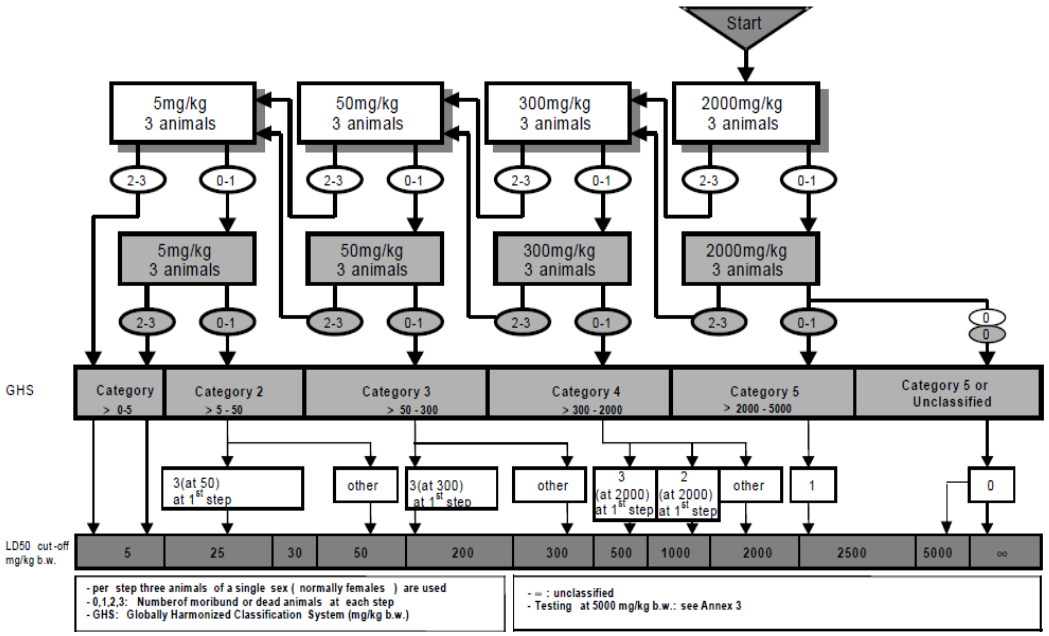
4.10.c TEST SYSTEM AND MANAGEMENT

1. Species	:	Mice
2. Strain	:	Swiss Albino
3. Source	:	APT Testing and Research Pvt Ltd, Pune
4. Age and Sex	:	Female, 06-08 weeks

5. Body weight range	:	25-30 gm
6. Identification	:	By unique identification number marked by writing on cage tag and by corresponding colour body markings.
7. No. of animals	:	Three animals were tested in per group.
8. Acclimatization	:	The mice were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
Husbandry		
9. Environmental Conditions	:	Room temperature maintained between 22±3^{0}C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark.
10. Accommodation	:	Three mice per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet	:	Pelleted feed supplied by Supplier.
12. Water	:	Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

OECD/OCDE 423

ANNEX 2d: TEST PROCEDURE WITH A STARTING DOSE OF 2000 MG/KG BODY WEIGHT



According to OECD Annex 2c, if the dose of a substance is unknown we can start the dose at 300 mg/kg if it shows mortality after 4-6hrs that dose will be considered fatal dose otherwise lower or higher dose have to be studied.

The study was performed in a stepwise manner.

In step 1 study, 3 female mice of each group were administered orally using oral feeding needle with the respective test materials **"***Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*), & Shuddha Hingula (*Red Cinnabar:HgS*)"* of concentration of 300mg/kg dose. The mice were deprived of feed 3-4 hours before and 2 hours after dosing. Water was allowed *ad libitum*. All the animals were observed for 14 days after dosing.

Then in step 2 repetition of dose was given to 3 female mice of each group, where the mice were administered with respective test materials *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*), & Shuddha Hingula (*Red Cinnabar:HgS at the above 300 mg/kg doses. All the animals were observed for 14 days after dosing.

In step 3 study, another 3 female mice of each group were administered with the respective test materials *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*) & Shuddha Hingula (*Red Cinnabar:HgS) of concentration of 2000 mg/kg. The mice were deprived of feed 3-4 hours before and 2 hours after dosing. Water was allowed *ad libitum*. All the animals were observed for 14 days after dosing.

In step 4 i.e repetition of dose was given to 3 female mice of group ***Hinguleshvara Rasa and Shuddha Hingula (*Red Cinnabar:HgS)** where the mice were administered with respective test materials at 2000mg /kg doses. All the animals were observed for 14 days after dosing.

In step 4 the repeat dose of 2000mg/kg for *Shuddha Vatsanabh (Aconitum balfourii* Stapf*)* was skipped, as the animals were found dead at dose of 2000 mg/kg,

4.10.d Study Design and Dose Progression

Step	Test material	Test Dose (mg/10 ml)	Test Concentration (mg/ml)
1	*a) Hinguleshvara Rasa*	300	30.0
	b) Shuddha Hingula (Red Cinnabar HgS)		
	c) Shuddha Vatsanabh (Aconitum balfourii Stapf*)*		
2	*a) Hinguleshvara Rasa*	300	30.0
	b) Shuddha Hingula (Red Cinnabar: HgS)		
	c) Shuddha Vatsanabh (Aconitum balfourii Stapf*)*		
3	*a) Hinguleshvara Rasa*	2000	200.00
	b) Shuddha Hingula (Red Cinnabar: HgS)		
	c) Shuddha Vatsanabh (Aconitum balfourii Stapf*)*		
4	*a) Hinguleshvara Rasa*	2000	200.00
	b) Shuddha Hingula (Red Cinnabar:HgS)		

4.10.E.ADMINISTRATION OF TEST MATERIAL

Each of the test materials, at the concentration described above in Table 1, was administered to each mouse by a single oral gavage. The animals were dosed using a stainless-steel intubation needle fitted onto a suitably graduated syringe. The dosage volume administered to individual mouse was as tabulated above, and was adjusted according to its most recently recorded body weight.

4.10.f.Pre- Terminal Deaths and Toxic Signs:

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the

first 4 hours and daily thereafter, for a total of 14 days. However, the duration of observation was not being fixed rigidly. All observations were systematically recorded with individual records being maintained for each animal in the daily observation record format.

4.10.g.Body Weight

Individual weights of animals were determined shortly before the test substance was administered, (Pre-administration fasting weight) and at least weekly there after i.e. on Day 7^{th} and Day 14^{th} post treatment or at death (At the end of the test surviving animals are weighed and humanely killed). Weight changes were calculated and recorded.

4.10.h.Clinical Signs and Symptoms

All animals were observed for the following-

Changes in skin and fur, eyes and mucous membranes. Also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity, behavior pattern. Attention was directed to the observations of tremors, convulsions, salivation, diarrhoea, lethargy.

4.10.i.Necropsy

All the animals were euthanized by CO_2 Asphyxia. Then the test animals were subjected to gross necropsy. All gross pathological changes were recorded for each animal.

4.11 EXPERIMENT FOR ANTIPYRETIC ACTIVITY:-

MATERIALS AND METHODS

Antipyretic activity of Hinguleshvara Rasa, *Shuddha Vatsanabh* (Aconitum balfourii *Stapf*), Pippali (*Piper longum* Linn*), Shuddha Hingula* (Red cinnabar: HgS) was assessed by Brewer's yeast induced pyrexia model.

4.11.a MATERIALS AND METHODS

a. TEST MATERIAL

Test Material	**:**	***Hinguleshvara Rasa***
Physical State	**:**	**Solid**

Quantity	**:**	**100 gm**
Color	**:**	**Orange**

b. TEST MATERIAL

Test Material	**:**	***Shuddha Hingula* (Red cinnabar:HgS)**
Physical State	**:**	**Solid**
Quantity	**:**	**100 gm**
Color	**:**	**Orange**

c. TEST MATERIAL

Test Material	**:**	***Shuddha Vatsanabha***
Aconitum balfourii Stapf		
Physical State	**:**	**Solid**
Quantity	**:**	**100 gm**
Color	**:**	**Light brown**

d. TEST MATERIAL

Test Material	**:**	**Pippali (*Piper longum* Linn)**
Physical State	**:**	**Solid**
Quantity	**:**	**100 gm**
Color	**:**	**Light brown**

4.11.b DOSE SELECTION AND PREPARATION:

Sample was stored at room temperature.

The doses selected for the study was as mentioned in the study design for test material *Hinguleshvara Rasa* (Classical drug).

The Formulation of the test material was prepared 93.6mg/ Kg (93.6 mg Test drug in 10 ml water).

Dose Calculation[206]: Human dose of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*), Pippali (Piper longum* Linn), *Shuddha Hingula* (Red cinnabar: HgS) was converted to animal dose in accordance to Paget G E and Barnes, J.M.' Toxicity tests' In:" Evaluation of drug activities pharmacometrics" (Laurene, D.R. and Bacharach, A.L eds)Vol 1,Academic press London 1964 pp -135-165

Classical doses of Pippali 1 masha=750mg (Sha.Sa) [[207]

As per *Sharangdharasamhita* Dose of *Pippali (Piper longum* Linn),churna is 1 masha1 ratti =125 mg

6ratti=1 masha[208]

Vatsanabh1/8 Ratti=15.6mg(RT)[209]

Hingula 1 Ratti=125mg (Ayu.Pra)[210]

Hinguleshvara Rasa Half gunja =62.5mg (Bhaishajya Ratnavali)[211]

Human to animal dose were converted by using Paget and Barner's scale as in given table:

Drugs		*Pippali*	*Hingula*
Human Dose		750mg	125mg
Animal Dose	Mice 20 gm	750*0.0026= 1.95mg Dose/kg bdwt= 1.95*1000/20 =97.5mg	125*0.0026= 0.325 mg Dose/kg bdwt= 0.325*1000/20= 16.25mg
	Rat 200 gm	750*0.018= 13.5mg Dose/kg bdwt= 13.5*1000/200 =67.5	125*0.018=2.25 mg Dose/kg bdwt= 2.25*1000/200=11.25 mg

Drugs		*Vatsanabh*	*Hinguleshvara Rasa*
Human Dose		15.6mg	62.5mg
Animal Dose	Mice 20 gm	15.6mg*0.0026= 0.04056mg Dose/kg bdwt= 0.04056mg*1000/20 =2.028mg	62.5*0.0026= 0.1625mg Dose/kg bdwt= 0.1625*1000/20= 8.125mg
	Rat 200 gm	15.6mg *0.018= 0.2808mg Dose/kg bdwt=	62.5*0.018=1.125 mg Dose/kg bdwt= 1.125*1000/200=5.625mg

		0.2808mg *1000/200 =1.404mg	

These doses of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*), Pippali (Piper longum* Linn), *Shuddha Hingula* (Red cinnabar: HgS) were used for both in antipyretic study as well as analgesic study model.

Animal Dose of Paracetamol in Rat is 150mg/kg body wt for Antipyretic study as standard is taken.[212-214]

EXPERIMENT FOR ANTIPYRETIC ACTIVITY:-

4.11.c ADMINISTRATION OF TEST MATERIAL

Test materials were administeredorally.

1. Species	:	Rat
2. Strain	:	Wistar Rats
3. Source	:	APT Testing and Research Pvt Ltd, Pune
4. Sex	:	Both male and female
5. Body weight range	:	200-250g
6. Identification	:	By unique identification number marked by writing on cage tag and by corresponding colour body markings.
7. No. of animals	:	42 animals
8. Acclimatization	:	The rats were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
Husbandry		
9. Environmental Conditions	:	Room temperature maintained between 22 ± 3^0C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark.
10. Accommodation	:	One rat per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water

		bottle, and bedding of clean paddy husk.
11. Diet	:	Pelleted feed supplied by Supplier.
12. Water	:	Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

STUDY DESIGN:

The antipyretic activity was conducted on Wistar Rats both male and female (weight range 200-250g gms) in 7 groups. Each group was comprised of 6 animals. (3M + 3F)

EXPERIMENT FOR ANTIPYRETIC ACTIVITY:-

Assessment of anti-pyretic activity in rats[212-214]:

Purpose: Subcutaneous injection of Brewer's yeast suspension is known to produce fever in rats. A decrease in temperature can be achieved by administration of compound with antipyretic activity. Antipyretic test in rats can be regarded as a classical method in pharmacology.

Brewer's yeast induced pyrexia model

Before inducing pyrexia the initial rectal temperature of the animals was monitored. Animals fasted overnight was used. Pyrexia was induced by injecting 20% v/v Brewer's yeast suspension (10mg/kg S.C) into the animal dorsum region. 18 hrs after the injection, the rectal temperature of each rat was measured using digital thermometer. Only rats which show an increase in temperature at least 0.7^0 C was used for experiments. The treatment with *Hinguleshvara Rasa, Shuddha Hingula* (Red cinnabar: HgS), *Shuddha Vatsanabh (Aconitum Balfourii* Stapf*) & Pippali (Piper longum* Linn) was administrated orally at suitable dose. After 30 mins of drug administration the rectal temperature was monitored at 30min, 60 min, 2hrs, 4 hrs, 6 hrs, 8 hrs, 24 hrs and 48 hrs**.**

4.11.d groups of animals for antipyretic activity:-

S. No.	Groups	No of rats	Drug	Route
1	Normal Control	6	---	Oral
2	Disease control	6	Brewer's Yeast (10 mg/Kg, s.c)	parenteral
3	Standard	6	Brewer's Yeast+ Paracetamol (150 mg/kg)	parenteral
4	Test(T1)	6	Brewer's Yeast+ *Hinguleshwara Rasa* (5.63 mg/Kg)	Oral
5	Test(T2)	6	Brewer's Yeast+ *Shodhita Hingula* (Red Cinnabar:HgS (11.25 mg/Kg)	Oral
6	Test(T3)	6	Brewer's Yeast+ *Shodhita Vatsanabh Aconitum balfourii* Stapf) (1.40 mg/Kg)	Oral
7	Test(T4)	6	Brewer's Yeast+ *Pippali* (*Piper longum* Linn) (67.5 mg/Kg)	Oral

4.12 EXPERIMENT FOR ANALGESIC ACTIVITY:-

4.12.a MATERIALS AND METHODS

a. Test Material : *Hinguleshvara Rasa*
Physical State : Solid
Quantity : 100 gm
Color : Orange

b. Test Material : *Shuddha Hingula* (Red cinnabar:HgS)
Physical State : Solid
Quantity : 100 gm
Color : Orange

c. Test Material : *Shuddha Vatsanabha*
***Aconitum balfourii* Stapf**

	Physical State	**:**	**Solid**
	Quantity	**:**	**100 gm**
	Color	**:**	**Light brown**
d.	**Test Material**	**:**	***Pippal i*(*Piper longum* Linn)**
	Physical State	**:**	**Solid**
	Quantity	**:**	**100 gm**
	Color	**:**	**Light brown**

4.12.b. DOSE SELECTION AND PREPARATION:

Sample provided was stored at room temperature.

The doses selected for the study was as mentioned in the study design for test material *Hinguleshvara Rasa* (Classical drug).

EXPERIMENT FOR ANALGESIC ACTIVITY:-

4.12.c.ADMINISTRATION OF TEST MATERIAL

4.12.c Test materials were administered orally.

1. Species	:	Mice
2. Strain	:	Swiss albino mice
3. Source	:	APT Testing and Research Pvt Ltd, Pune
4. Age and Sex	:	Female, 06-08 weeks
5. Body weight range	:	18-22
6. Identification	:	By unique identification number marked by writing on cage tag and by corresponding colour body markings.
7. No. of animals	:	Six animals were tested in per group.
8. Acclimatization	:	The mice were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
Husbandry		

9. Environmental Conditions	:	Room temperature maintained between 22±3^{0}C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark.
10. Accommodation	:	Three mice per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet	:	Pelleted feed supplied by Supplier.
12. Water	:	Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.

Part B: EXPERIMENT FOR ANALGESIC ACTIVITY:-

The analgesic activity was conducted on Swiss Albino female mice (weight range 18-22 gms) in 6 groups. Each group was comprise of 6 animals.

4.12.d The groups are as follows:

S. No.	Groups	No of Mice	Drug	Route
1	Diseased Control	6	1) Writhing- 0.1 ml of 1% acetic acid solution, i.p 2) Formalin test- 20 µl of 1% formalin solution under right hind paw 3) Hot plate- No drug treatment	Parenteral
2	Standard	6	For- 1) Writhing and Formaline- Diclofenac (10mg/Kg) 2) Hot Plat- Pentazocine- 1.4 mg/kg	Parenteral
3	Test (T1)	6	*Hinguleshvara Rasa* *(8.125ma /kg)*	Oral
4	Test (T2)	6	*Shodhita Hingula* (Red Cinnabar:HgS) (16.25mg /kg)	Oral
5	Test (T3)	6	*Pippali* (*Piper longum* Linn) *(97.5 mg / kg)*	Oral
6	Test (T4)	6	*Shodhita Vatsanabh* *Aconitum balfourii* Stapf) *2.028mh / kg*	Oral

Following tests were conducted on (Swiss Albino female mice) for analgesic activity.

4.12.d-1Formalin test

4.12.d-2Writhing assay

4.12.d.3Hot plate test

4.12.d-1 Formalin test

Purpose: The formalin test in mice has been proposed as chronic pain model which is sensitive to centrally active analgesic agents by Dubuiss on and Dennis(1977)

After 30 minutes of drug administration, 20 µl of 1% formalin solution was injected just under the skin of the dorsal surface of the right hind paw using a micro syringe with a 26-gauge needle. The mouse was then put back into the chamber. Licking of the injected paw was recorded after the injection of a small nociceptive amount of formalin (20 µl, 1%). The results showed that the response to formalin was biphasic with an early (0–5 min) and a late (20–30 min) phase of high licking activity.[215-216]

4.12.d-2Acetic acid induced writhing method[217-218]:

Purpose : Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristics stretching behavior which is called writhing. The test is suitable to detect analgesic activity although some psychoactive agents also show activity. An irritant agent such as acetic acid is injected intraperitoneal to mice and the stretching reaction is evaluated. The reaction is not specific for irritant.

After 30 minutes of drug administration, 0.1 ml of 1% acetic acid solution was injected i.p. Mice were placed individually into glass chamber and five minutes were allowed to elapse. They were then observed for a period of ten minutes and the numbers of writhes were recorded in each animal. For scoring purpose, a writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

Percentage inhibition was calculated using the following formula:

% inhibition= { $(W_c - W_t) \times 100$ } / Wc

Where, W_c = No. of writhes in control group, W_t = No. of writhes in test group

Compounds with less than 70% inhibition were considered to have minimal analgesic activity.

4.12.d-3 Hot plate test[219-222]

Purpose : The paws of mice and rats are very sensitive to heat at temperature which are not damaging the skin. The responses are jumping, with drawl of paws, and licking of paws. The time until these responses occurred is prolonged after administration of centrally acting analgesic whereas peripheral analgesics do not generally affect these responses.

Evaluation of analgesic activity of the test drugs was also carried out using hot plate method . The mice were placed on a hot plate maintained at 55°C. The reaction time (in seconds) or latency period was determined as the time taken for the mice to react to the thermal pain by licking their paws or jumping. The reaction time was recorded at 30, 60, 90 and 120 min after the administration of the treatments. The maximum reaction time was fixed at 15 sec to prevent any injury to the tissues of the paws. If the reading exceeds 15 sec, it would be considered as maximum analgesia.

RESULTS

Analytical Results of *Hingula* (Red Cinnabar: HgS)

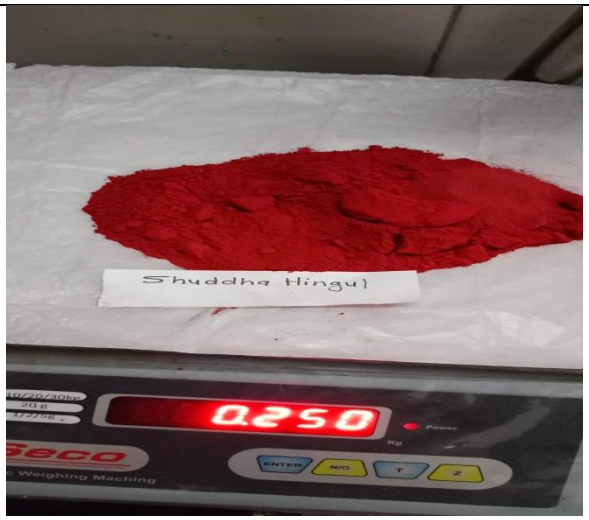
5.1 Organoleptic Characters of *Hingula* (Red Cinnabar: HgS)

Sr No	Test/Description	Before *Shodhana*	After *Shodhana*
1	Colour	Brick Red	Blakish Red
2	Odour	Not specific	Ginger Like
3	Texture	Rough	Smooth
4	Appearance	Lusture present	Lusture absent

5.1A.Photos of *Shuddha and Ashuddha Hingula* (Red Cinnabar: HgS)



***Ashuddha Hingula* (Red Cinnabar: HgS)**



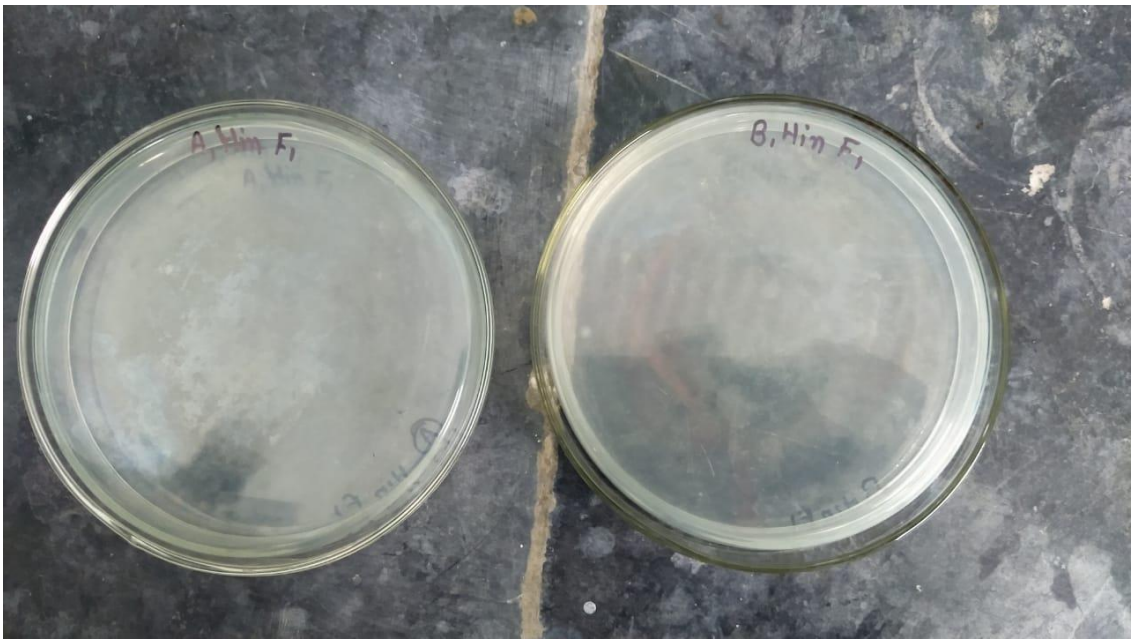
***Shuddha Hingula* (Red Cinnabar:HgS)**

5.2 Physico chemical Analysis of *Hingula* (Red Cinnabar: HgS) Sample of *Hingula* (Red Cinnabar: HgS) Before and after shodhana (5.2) are analyzed to determine % of Hg, S etc.

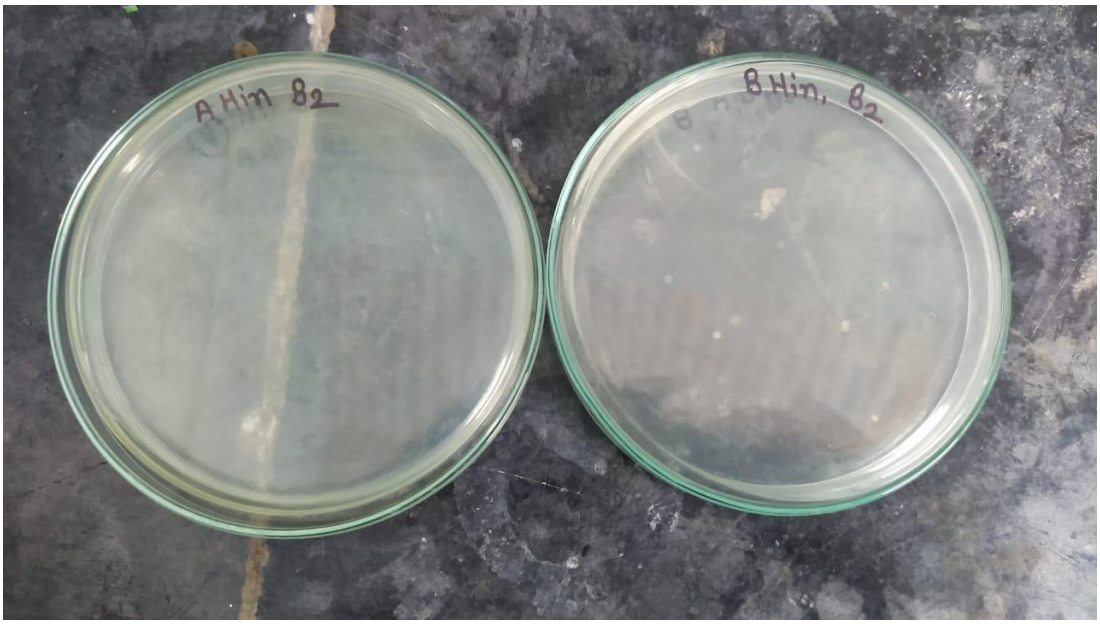
No.	**Test Name**	**Result Obtained %**	
		Ashodhit Hingula	***Shodhit Hingula***
1	Moisture Content @110 ^{o}C (As per 4.1a)	< 0.1 %	0.72 %
2	Mercury Content (As per 4.1e)	80.92 %	77.08 %
3	Sulfur Content (As per 4.1e)	12.72 %	12.70 %

5.3 Microbial load of *Hingula* (Red Cinnabar: HgS) before and after *Shodhana*

Sr No	**Sample**	**Total viable bacterial Count per ml**	**Total fungal count per ml**
1	*Ashuddha Hingula* A	Nil	Nil
2	*Shuddha Hingula* B	Nil	Nil



5.3.A. *Ashuddha Hingula* , *Shuddha Hingula* (Fungal Load) Fig for 5.3



5.3.B. *Ashuddha Hingula, Shuddha Hingula* (Bacterial Load) Fig for 5.3

5.4 Physicochemical Results of *Ardrak Swarasa* (Juice of *Zinziber officinale* Rosc)

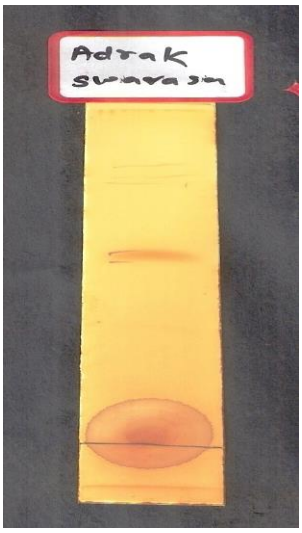
No.	**Test Name**	**Result Obtained %** *(Adrak Swarasa)*
1	pH(As per 4.2a)	6.94
2	Moisture content @110^{o}C(As per 4.2b)	45.77 %
3	Specific Gravity (As per 4.2e	1.0296 gm / ml
4	Cadmium (As per 4.2g)	Negative
5	Arsenic (As per 4.2g)	Negative
6	Mercury (As per 4.2g)	Negative
7	Lead (As per 4.2g)	Positive

5.5 TLC Report of analysis of the *Ardrak Swarasa* (Juice of *Zinziber officinale* Rosc):

Sr.no.	Name of Sample	Result Obtained
1	***Adrak Swarasa***	Done
TLC: : (As per 4.2f) Extraction: *Adrak Swarasa* Adsorbent used: Silica gel G_{60} F_{254} Mobile phase :- Toluene : Ethyl Acetate (6 : 4: 1)		

UV 365nm: 2 Spots were observed.		
Spot	RF Value	Colour
1 st	0.89	Yellow
2 nd	0.76	Violet
Iodine chamber: 1 Spots were observed.		
Spot	RF Value	Colour
1 st	0.76	Yellow

TLC of *Ardrak Swarasa*



TLC of *Ardrak Swarasa*

5.6 Reports of *Pippali (Piper longum* Linn)

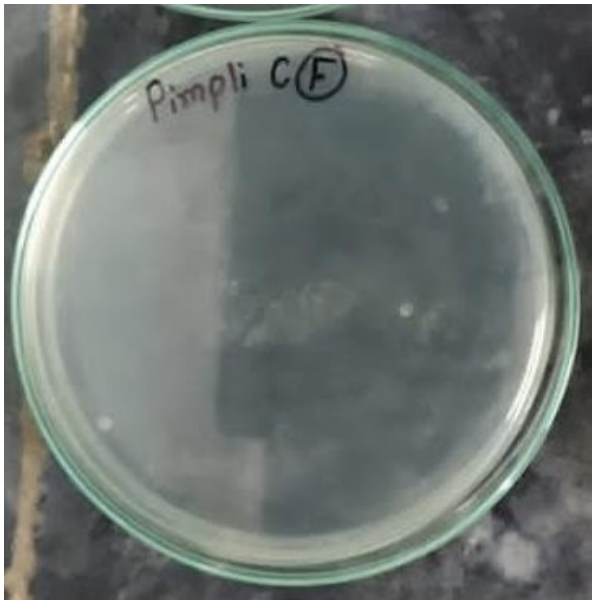
Authenticated *Pippali* sample 'C' is used for preparation of *Hinguleshvara Rasa* in the present study.



Pippali (piper longum Linn)

5.6 Microbial Load of *Pippali (piper longum* Linn)are as per method (4.5i)

Sr No	Sample	Total viable count per ml	Total fungal count Per ml
1	C	Nil	Nil



***5.6APhoto of Pippali* C Fungal Load (Fig for 5.6)**



***5.6BPhoto of Pippali* C Bacterial load (Fig for 5.6)**

5.7 Results of Physicochemical Analysis of *Pippali (piper longum* Linn)are as follows:

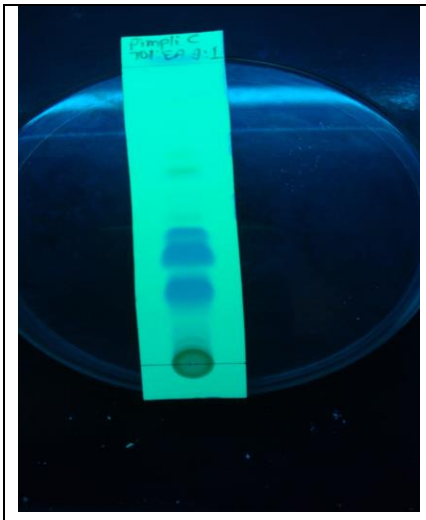
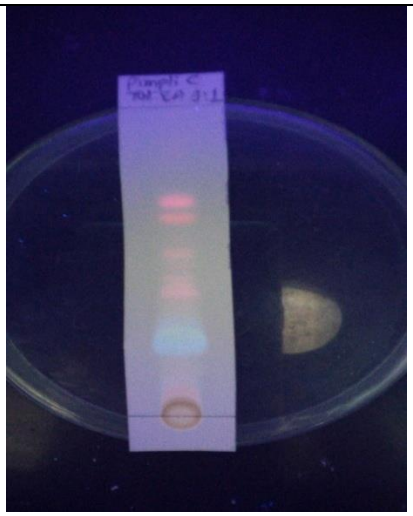
Sr . No	Name of analytical test	observation
1	Foreign matter(As per 4.5c)	0.7%
2	Loss on Drying(As per 4.5d)	11.09%
3	Total Ash(As per 4.5e)	2.53%
4	Acid insoluble Ash(As per 4.5f)	0.43%
5	Alcohol soluble Extractive(As per 4.5g)	19.97%
6	Water soluble extractive(As per 4.5h)	40.30%

5.8 TLC of *Pippali* (*Piper longum* Linn))[247]:

TLC of dried sample of 'C' of *Pippali* (*Piper longum* Linn.) fruit was conducted,as per methods given in chapter materials and methods(4.5b).Reports are as follows:

***Pippali* Sample**	**In UV at 254nm**	**In UV at 365nm**	**After Derivatization with Dragendorff reagent**
C	0.10,0.13,0.26,0.40,0.65,0.71 (All Yellow)	0.36,0.6,0.50 (All Blue)	0.26,0.40 (All orange)

5.9 Photos of TLC of *Pippali* (*Piper l ongum* Linn)

		
Pippali 'C'@254	Pippali 'C'@365	Pippali 'C' Iodine

5.10 Results of Phytochemical Analysis of *Piper longum* Linn are as follows:

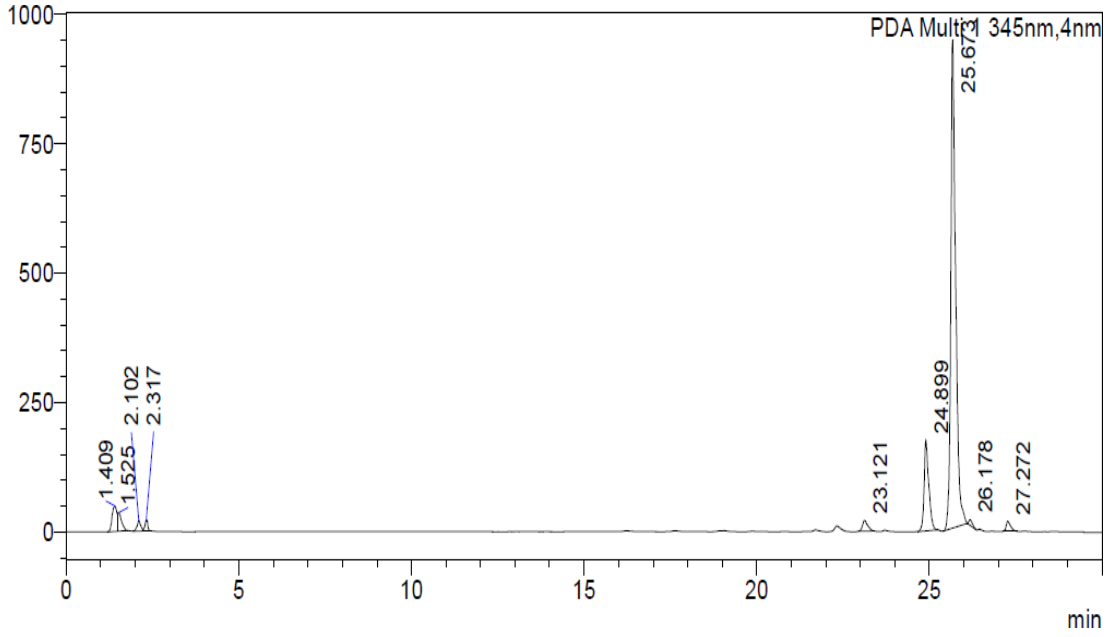
5.10.A.Total Alkaloid of C sample of ***Pippali (Piper longum* Linn)**

Pippali (Sample-C)	0.48 %

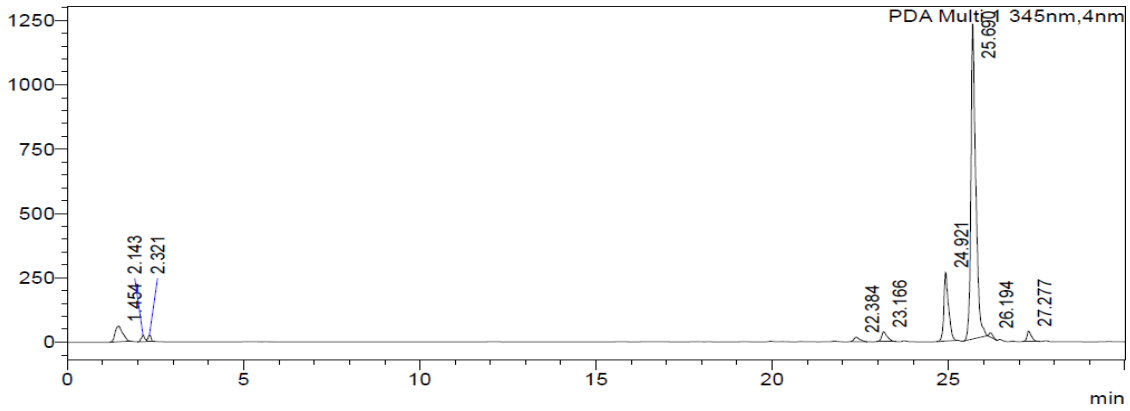
5.10.B.% Piperine content of C sample of ***Pippali (Piper longum* Linn)**

Sample	**Piperine Standard**	***Pippali* (Sample C)**
Rt	25.817	25.690
Area	8817543	11727114
% Piperine	-	0.72 %
% Total Alkaloids (By Gravimetry)	0.48 %	

5.11.A.HPLC chromatogram of Standard Piperine



5.11.B. HPLC chromatogram of *Pippali (Piper longum* Linn) (Sample-C)



5.12 Pharmacognosy of fruits of *Pippali (Piper longum* Linn) 'C' :

PIPPALI

5.12. a. Macroscopic study of fruits of *Pippali (Piper longum* Linn) 'C'

Fruit greenish-black to black, cylindrical, 2.5 to 5 cm long and 0.4 to 1 cm thick, consisting of minute sessile fruits, arranged around an axis; surface rough and composite; broken surface shows a central axis and 6 to 12 fruitlets arranged around an axis; taste, pungent producing numbness on the tongue; aromatic odour.

5.12.b. Microscopic fruits of *Pippali (Piper longum* Linn) 'C'

Diagrammatic section of the *Pippali* (plucked in winter 2016) shows that Outer epicarp, middle mesocarp central endocarp along with vascular bundles

Detailed T.S. shows that Bracts well defined containing parenchyma cells and oil globules in moderate quantity. Catkin shows 6 to 8 fruits, arranged in circle on a central axis, each having an outer epidermis. Outermost epicarp consists of thin layer of cuticle with polygonal cells followed by hypoderm made off 4-7 tangentially elongated parenchyma cells with numerous stone cells.

Mesocarp is broad zone mainly consists parenchyma cells which are loaded by oil globules and rarely prismatic crystals. Mesocarp followed by oil zone which orange- brown colored rounded to oval shaped parenchyma cells loaded by oleoresin and oil content.

Endocarp and seed coat fused to form a deep zone, outer layer of this zone composed of thin-walled cells and colorless, inner layer composed of tangentially elongated cells, having reddish-brown content; most of endocarp filled with, round to oval shaped starch grains. Well developed vascular bundles consists xylem and phloem in this region. Spermoderm made up off well developed ovule attached with endosperm.

5.12.c. Powder microscopy fruits of *Pippali (Piper longum* Linn) 'C':

Diagnostic powder microscopic characters of powder shows that deep moss green, shows fragments of parenchyma, black debris, oval to elongated stone cells, fibres, prismatic crystals, oil globules and round to oval starch grains, oleoresin content, epidermal and epicarp cells, annular and spiral vessels etc. depicted in the table

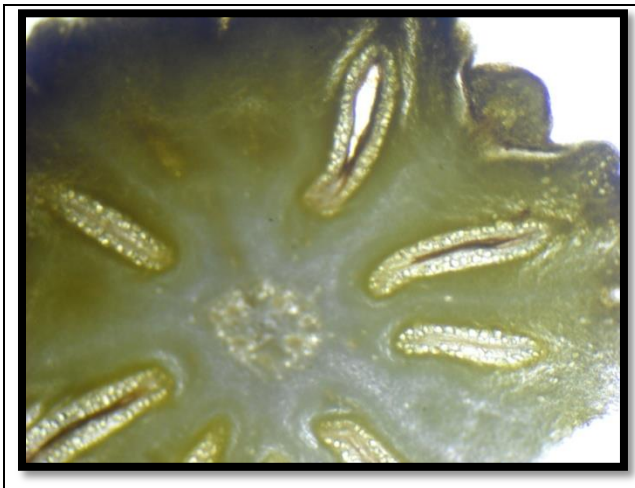
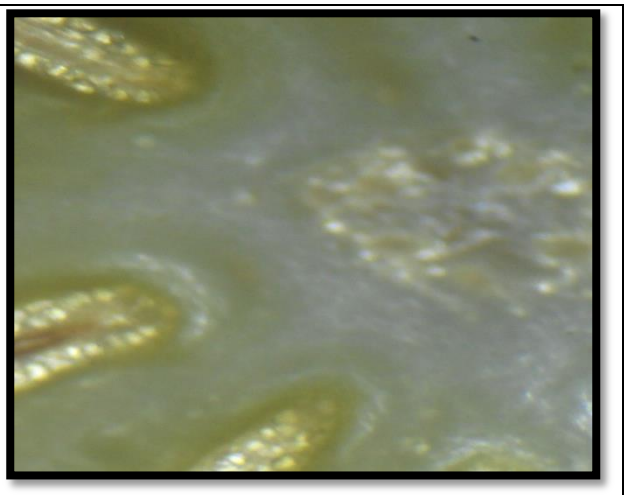
Characters	2016
Stone cells	+++
Fibres	++
Oil globules	+++
Starch grains	+++
Oleoresin content	++
Annular and spiral vessels	+++
Epidermal cells	+++
Epicarp cells with brown content	++

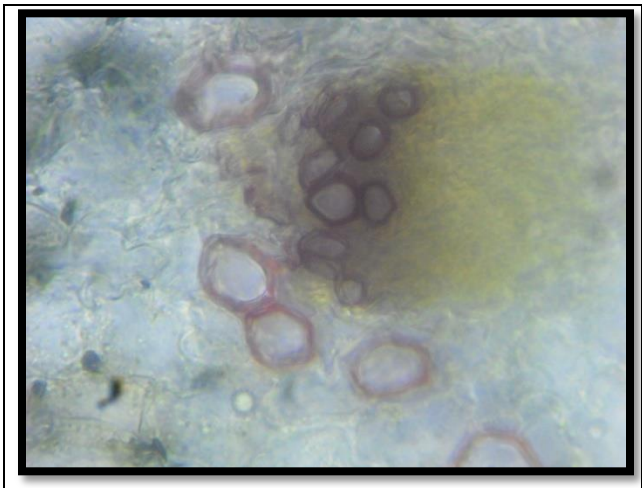
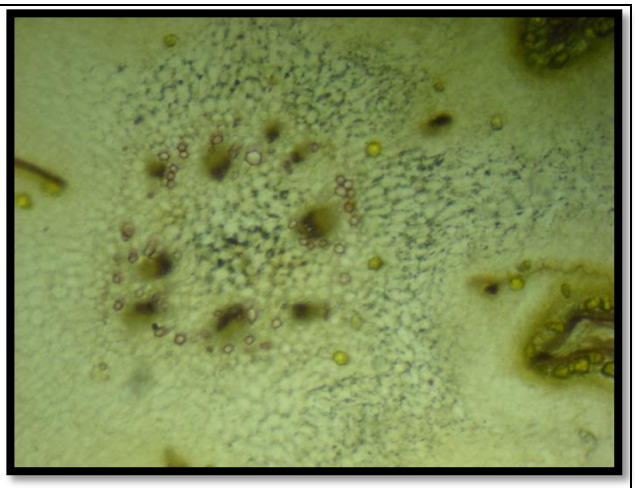
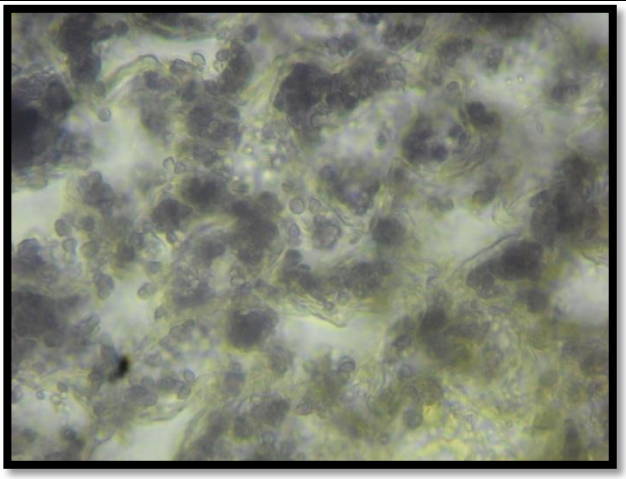
++ = Moderate +++ = More

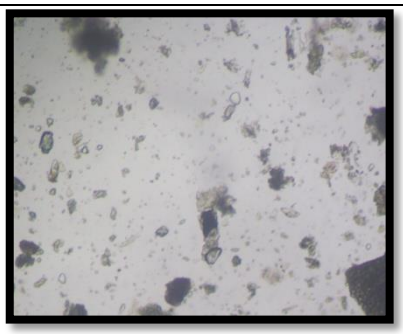
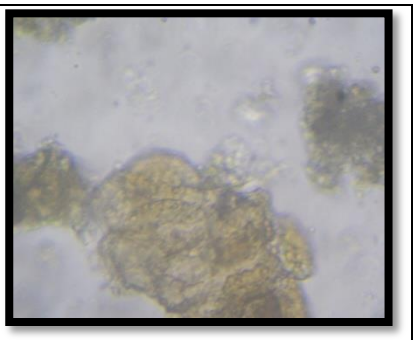
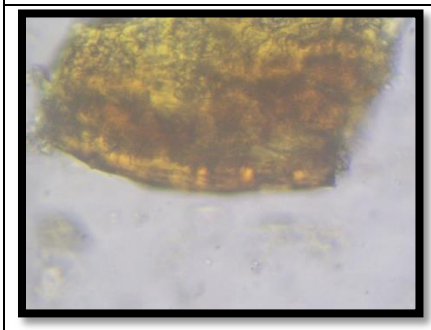
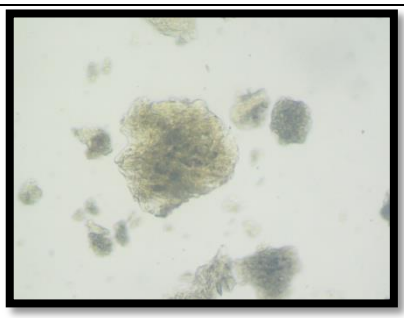
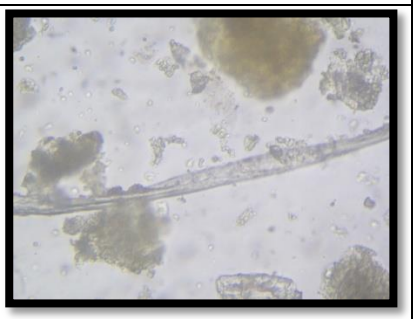
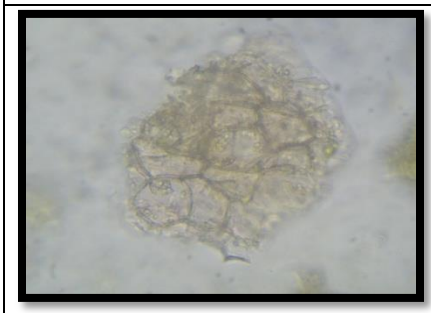
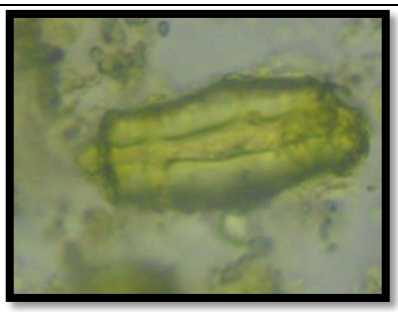
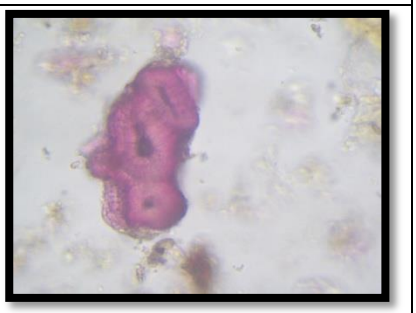
5.12.d .Histochemical study of fruits of *Pippali (Piper longum* Linn) 'C'

Characters	2016
Lignin	++
Starch	++
Oil	+++
Oleoresine	+++
Calcium oxalate	--

Photos for 5.12 Pharmacognosy of fruits of *Pippali (Piper longum* Linn)are as follows:

	
Diagrammatic section with epicarp, bracts, central axis mesocarp and endocarp	**Central axis with vascular bundle**

	
Phloem and Linfied xyle	**Mesocarp with oil globules endocarp with endosperm**
	
Endocarp with endospem and olioresin, oil globules	**Iodine stained section with starch grains**

		
Powder as such	**Starch grains**	**Epidermal cells**
		
Epicarp cells with olioresine cantant	**Epicarp cells with olioresine cantant**	**Simple fibres**
		
Endosperm cells	**Stone cell**	**Lignified stone cell**

5.13 Organoleptic characters of fruits of *Pippali (Piper longum* Linn)are as follows:

Organoleptic characters ie. color, odor, taste and touch are recorded and depicted in the following table.

Characters	**2016 'C'**
Color	Black
Odor	Pungent
Taste	Strong tingling
Touch	Coarse fine

Reports of *Gomutra (*Cow urine):

For *Vatsanabh Shodhana (*processing of ***Acinitum Balfourii* Stapf*),*** *Gomutra* (Cow urine) was used.

5.14 Analytical Findings of Gomutra (cow urine) are as follows:

Physicochemical as well as microbial results of cow urine are as follows:

Sr	**Name of Test**	**Sample 1**	**Sample 2**	**Sample 3**
1	Description	Yellowish Liquid	Yellowish Liquid	Yellowish Liquid
2	pH(4.2a method same as ardrak swaras)	7.66	7.99	7.58
3	Specific gravity(4.2e method same as ardrak swaras)	1.034	1.018	1.035
4	Total Solid (4.5j)	9.08	10.29	10.72
5	Microbial Load(4.5i)			
	Total Viable count	Nil	$9*10^2$	Nil

	Cfu/ml			
	Total Fungal count Cfu/ml	5	$3*10^2$	15

5.14APhotos of Microbial count of Cow urine viable and fungal:

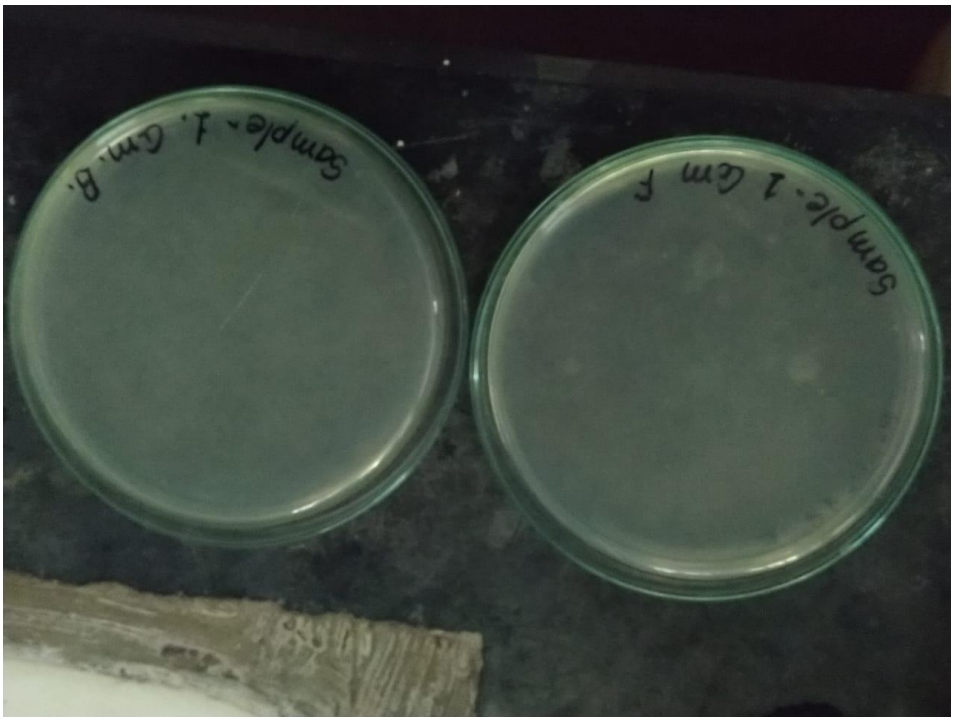


Fig 5.14A1 Cow urine sample 1 Viable and fungal count

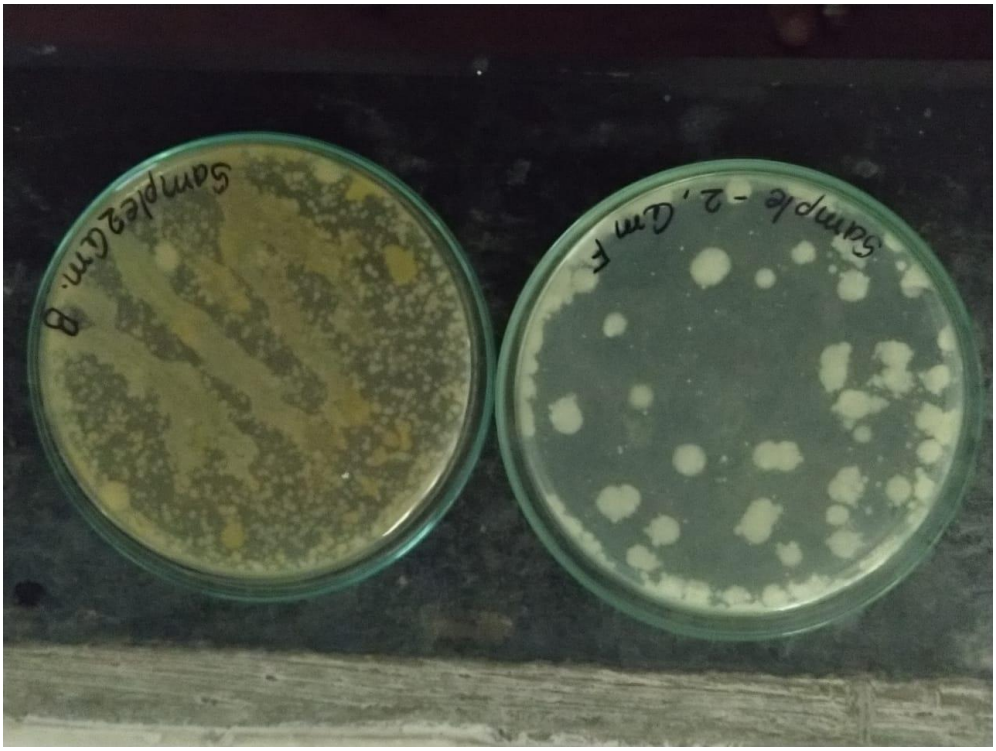


Fig5.14A2 Cow urine sample 2 viable and fungal count



Fig5.14A3cow urine sample 3 viable and fungal count

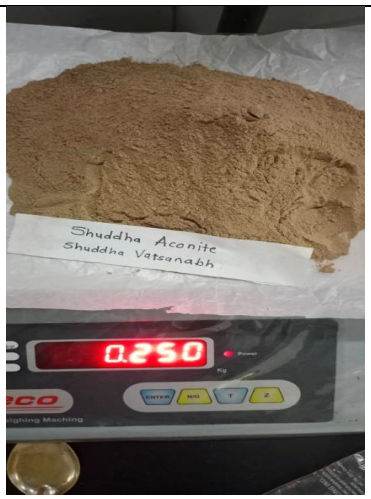
5.15 *Vatsanabh/Aconite(Aconitum balfourii* Stapf):

5.15. A *Shodhana* of *Vatsanabh* (*Aconitum balfourii* Stapf) as per method (4.8):

To remove the variability induced due to manual processing, *Shodhana* process carried out in three batches namely A,B and C. *Vatsanbh shodhana* was performed as per chapter materials and methods(4.8),with the reference of *Ayurveda prakash* (2/72).[250] *Vatsanabh shodhana in* cow urine was performed in three batches and each batch contain 1200 gms of *Vatsanabh* before *shodhana*. **After s*hodhan*a approximately 800 gms of *Vatsanabh* was obtained from(786,784 and 802 gms) each batch.**

After *shodhana* product obtained from all three batches namely A, B, C were combined and grounded to prepare powder of *Shuddha Vatsanabh (Composite A+B+C)* ***(Acinitum Balfourii* Stapf processed in cow urine)** which was subjected to experimental study.

5.15.B. *Shodhana* of *Vatsanabh* (*Aconitum balfourii* Stapf) :photos are as follows

		
***Ashuddha Vatsanabh (Aconitum Balfourii* Stapf)**	***Pieces of Vatsanabh dipped* in cow urine**	***Shuddha Vatsanabh (Aconitum Balfourii* Stapf)**

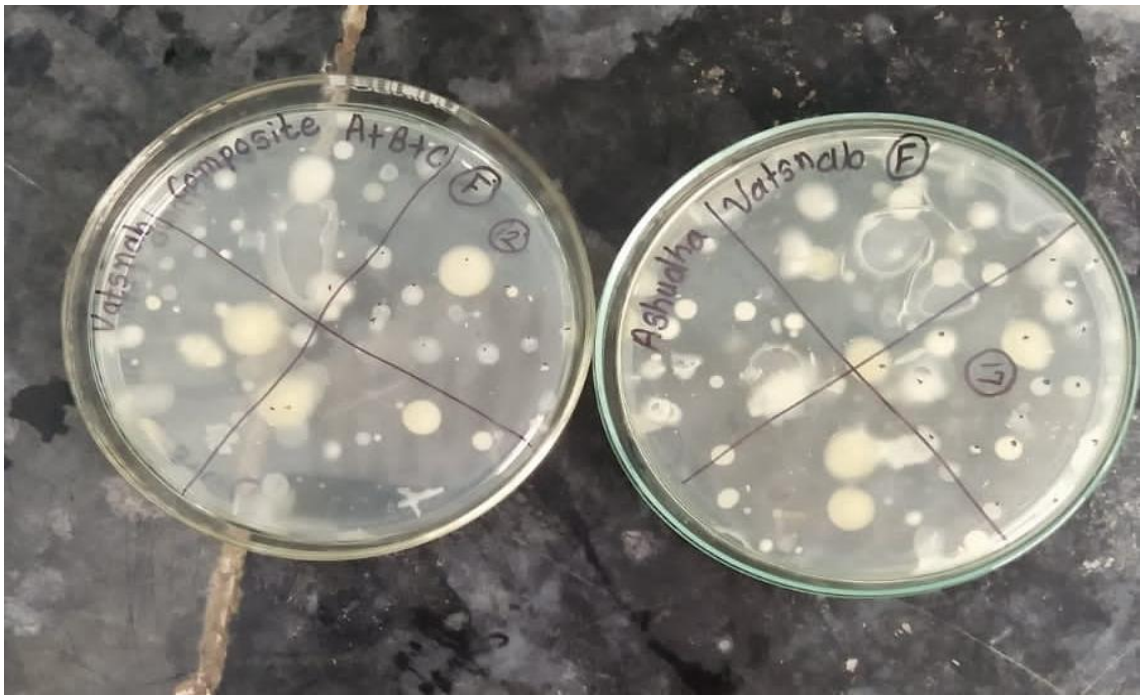
5.16 Results of physicochemical analysis (methods applied as per 4.4) of *Aconitum balfourii* Stapf (before and after *shodhana*) are as follows:

Sr. No	Name of analytical test	Observation				
		Sample A	**Sample B**	**Sample C**	**Sample Composite**	**Sample Ashuddha**
1	**Foreign matter(As per 4.4c)**	Nil	Nil	Nil	Nil	Nil
2	**Loss on Drying(As per 4.4d)**	12.41	12.38	12.12	12.33	11.48
3	**Total Ash(As per 4.4e)**	2.20	2.96	1.99	1.98	1.53
4	**Acid insoluble Ash(As per 4.4f)**	0.48	0.47	0.46	0.47	0.48
5	**Alcohol soluble Extractive(As per 4.4g)**	11.04	10.40	9.46	11.91	6.31
6	**Water soluble extractive(As per 4.4h)**	65.61	67.84	67.03	67.41	62.33

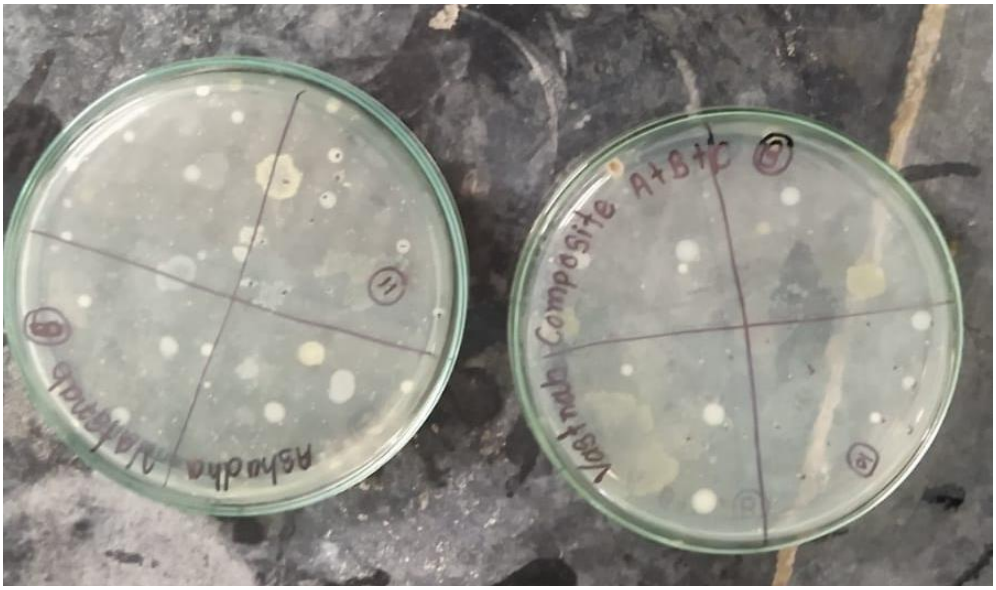
5.17 Microbial load of *Vatsanabh* was performed as described in chapter (4.5i)

Reports of Estimation of Microbial load[251]

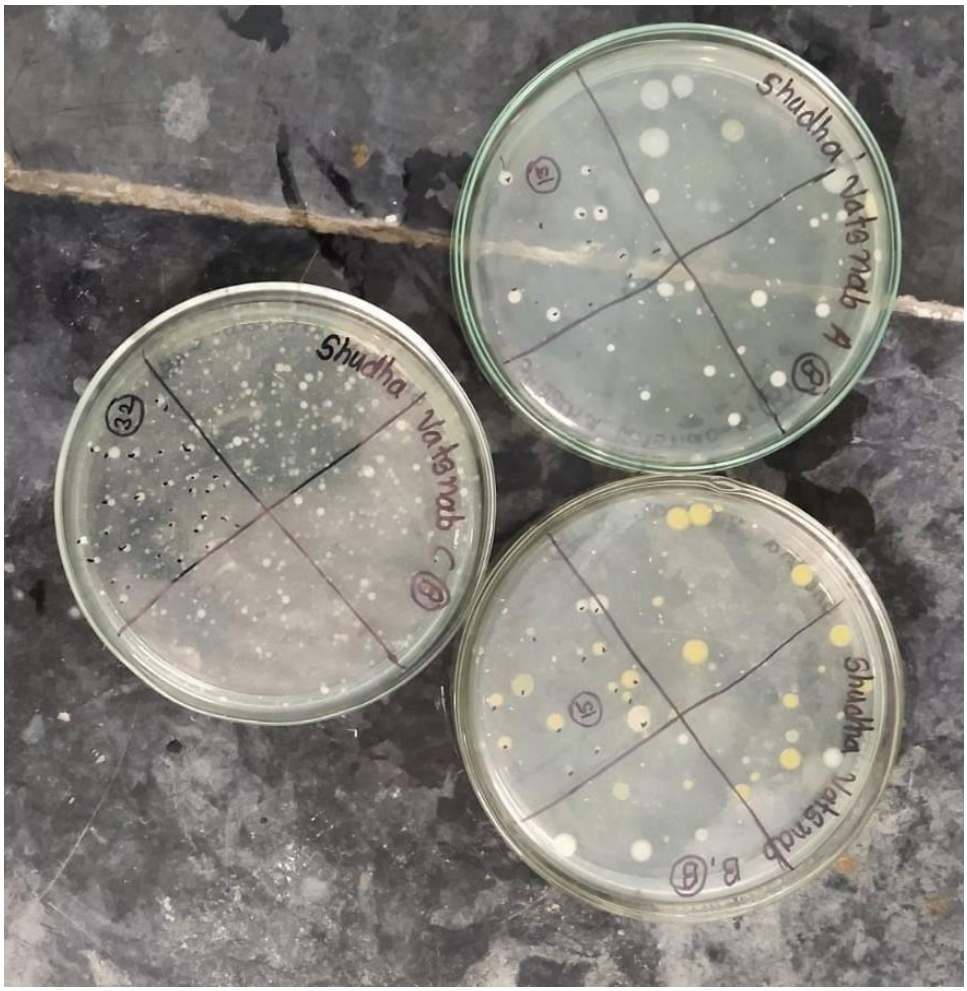
Sr No	Sample	Total viable count Per ml	Total Fungal count per ml
1	Shuddha Vatsanabh –A	52	7
2	Shuddha Vatsanabh –B	60	9
3	Shuddha Vatsanabh –C	128	250
4	Vatsanabh Composite A+B+C	80	50
5	Ashuddha Vatsanabh	44	68



5.17.A. Photos of *Ashuddha* and *Shuddha* Composite (A+B+C) *Vatsanabh* (*Aconitum balfourii* Stapf) Fungal count



5.17.B Photos of *Ashuddha* and *Shuddha* Composite (A+B+C) Vatsanabh (*Aconitum balfourii* Stapf) viable count:



5.17.C.Photos of *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) A,B,C Viable count

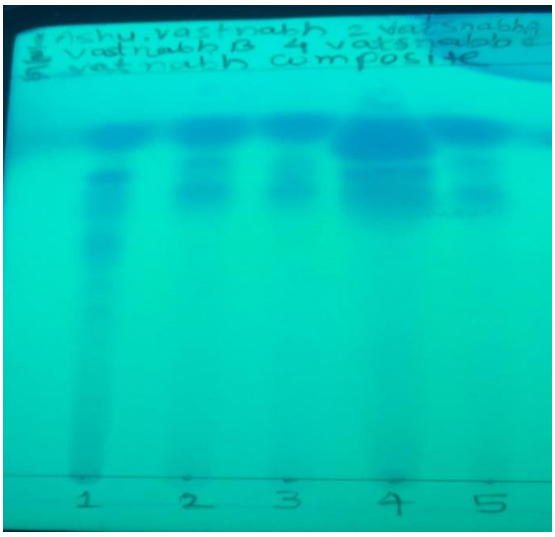
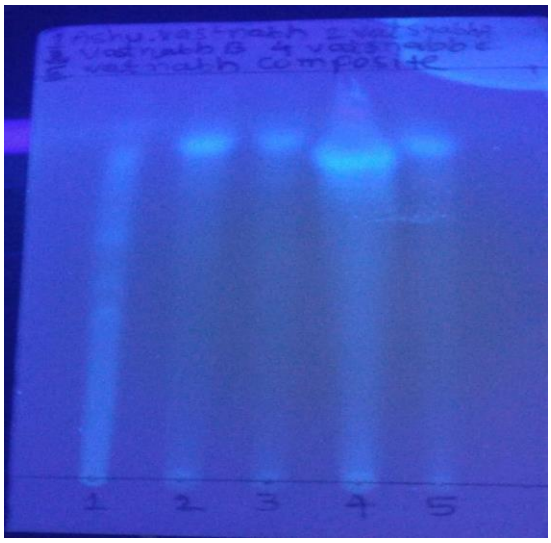
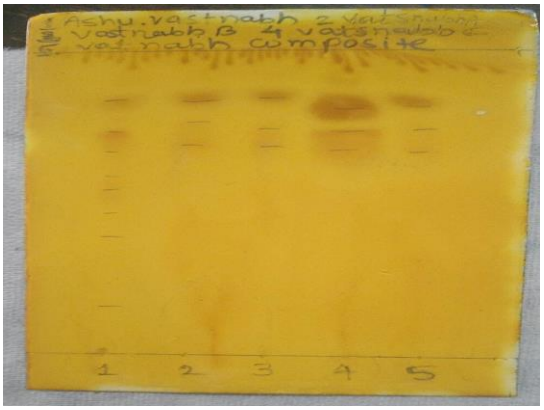


5.17.D. Shuddha Vatsanabh (*Aconitum balfourii* Stapf) A,B,C fungal count

5.18 TLC of *Vatsanabh (Aconitum balfourii* Stapf*)* methods performed as per4.4b

Aconite Sample	**In UV at 254nm**	**In UV at 365nm**	**After Derivatization with Dragendorff reagent**
Sample A Shuddha ***Vatsanabh***	0.68,0.76,0.83 (All Violet)	0.68,0.76,0.83 (All Blue)	0.68,0.76, 0.83 (All orange)
Sample B Shuddha ***Vatsanabh***	0.68,0.76,0.83 (All Violet)	0.68,0.76,0.83 (All Blue)	0.68,0.76, 0.83 (All orange)
Sample C Shuddha ***Vatsanabh***	0.68,0.76,0.83 (All Violet)	0.68,0.76,0.83 (All Blue)	0.68,0.76 ,0.83 (All orange)
Sample Composite Shuddha ***Vatsanabh*** A+B+C	0. 68,0.76,0.83 (All Violet)	0.96 (All Blue)	0.94 (All orange)

Ashuddha ***Vatsanabh***	0.16,0.38,0.46,0.55,0.58,0.66 0.72,0.83 (All Violet)	0.16,0.38,0.46, 0.55,0.58,0.66 0.72,0.83 (All Blue)	.16,0.38,0.46, 0.55,0.58, 0.66,0.72, 0.83 (All orange)

	
Aconite @254	**Aconite @365**
	
Aconite @dragondroff reagent	

5.19 photos of TLC of *Vatsanabh (Aconitum balfouri* Stapf) as per4.4b

5.20 Organoleptic characters of *Vatsanabh (Aconitum balfouri* Stapf) :

Organoleptic characters ie. color, odor, taste and touch are recorded and depicted in the table below:

Characters	Raw Aconite	A	B	C
Color	Dark Brown	Light Brown	Light Brown	Light Brown
Odor	Strong	Cow urine's smell	Cow urine's smell	Cow urine's smell
Touch	Rough	smooth	smooth	smooth

5.21 Reports of Phytochemical Analysis of *Vatsanabh (Aconitum balfourii* Stapf) includes estimation of total alkaloid as well as estimation of Aconitine from roots of *Vatsanabh (Aconitum balfourii* Stapf),methods applied as per 4.6b and 4.6c.

5.21.1. Estimation of Aconitine contents:

HPLC QUANTIFICATION REPORT

Sample	Aconitine Std.	Ashuddha *Vatsanabh*	*Vatsanabh* (Sample-A)	*Vatsanabh* (Sample-B)	*Vatsanabh* (Sample-C)
Rt	29.309	29.866	29.328	30.201	29.556
AUC	16248504	2229871	110278	452496	295888
% Aconitine	-	0.027%	0.00133%	0.00547%	0.00358%

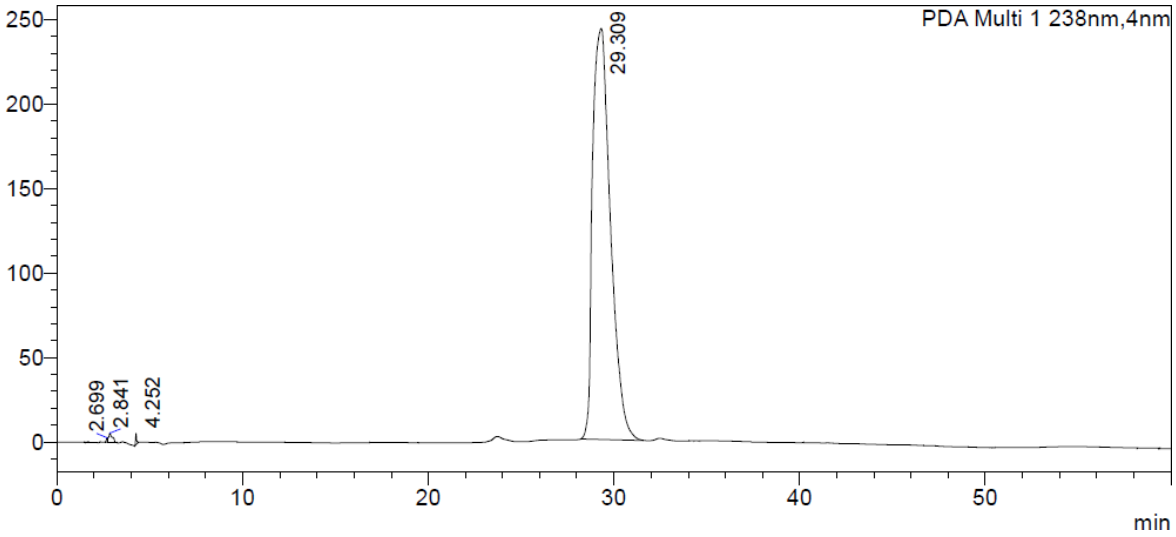
Sample	Aconitine Std.	Shuddha Composite A+B+C
Rt	31.853	31.977
AUC	951872	182481
% Aconitine	-	0.00363 %

5.21.2.Estimation of Total Alkaloid contents:

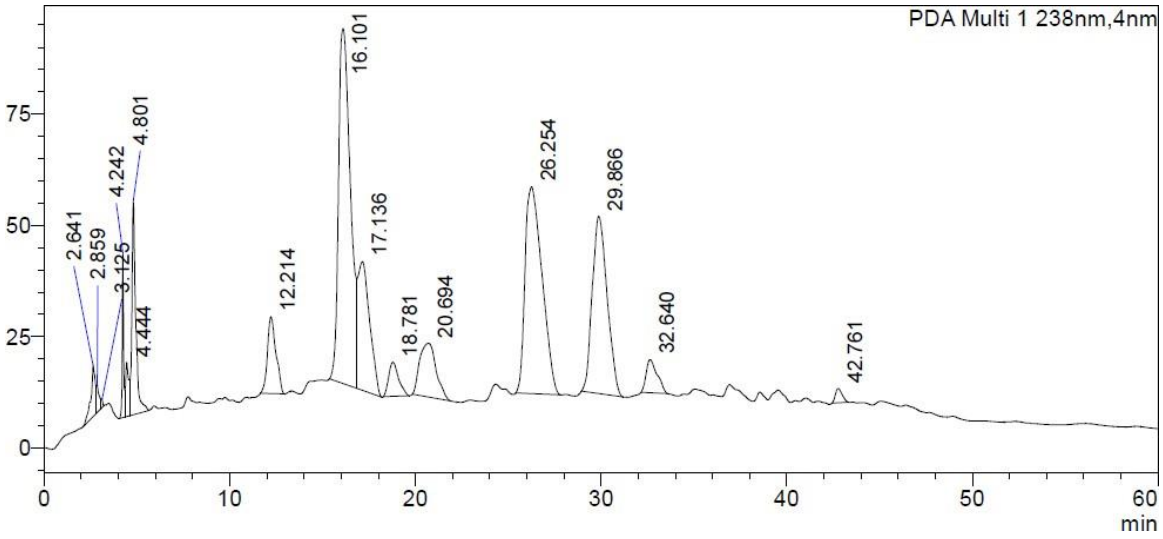
Sr No	**Parameters**	***Ashuddha Vatsanabh***	***Vatsanabh* (Sample-A)**	***Vatsanabh* (Sample-B)**	***Vatsanabh* (Sample-C)**	**Mixture (A, B & C)**
	Total Alkaloidsby Gravimetry(as per 4.6c)	0.34 %	0.63 %	0.34 %	0.41 %	0.46 %

5.22 HPLC Chromatograph of *Vatsanbh* (*Aconitum balfourii* Linn) included :

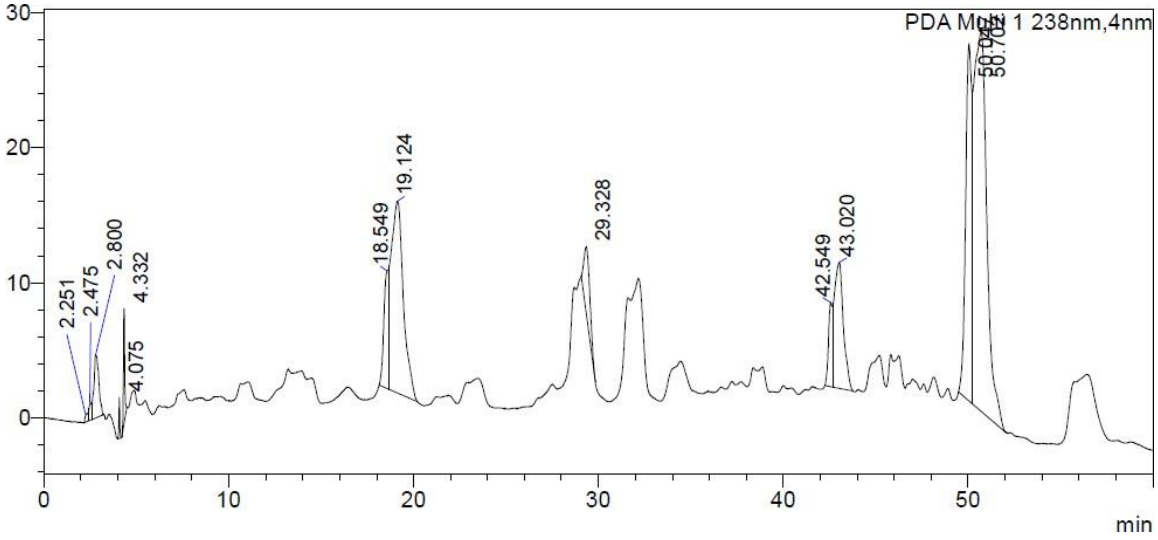
5.22.1 HPLC chromatogram of Standard Aconitine



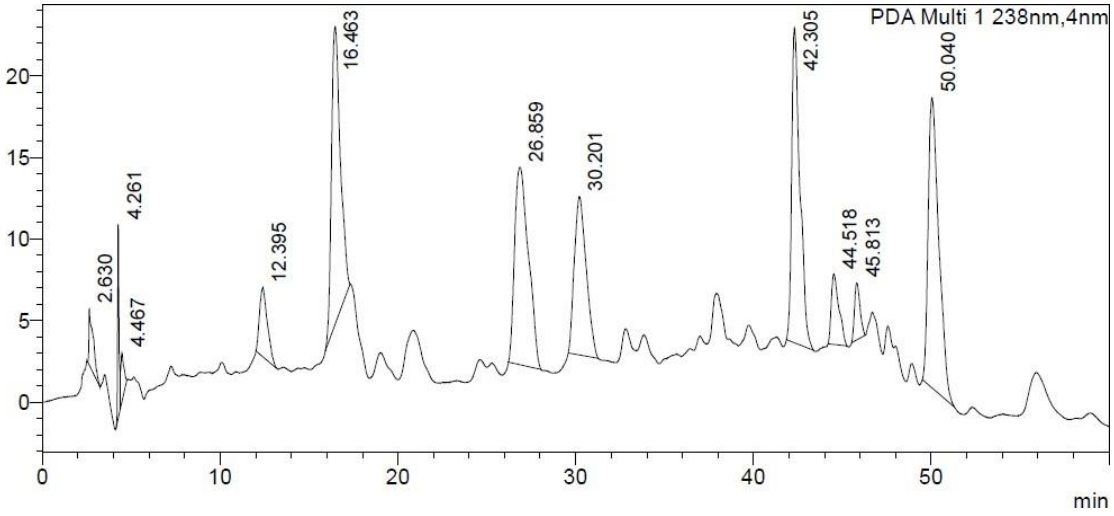
5.22.2 HPLC chromatogram of *Ashuddha Vatsanabh* Powder



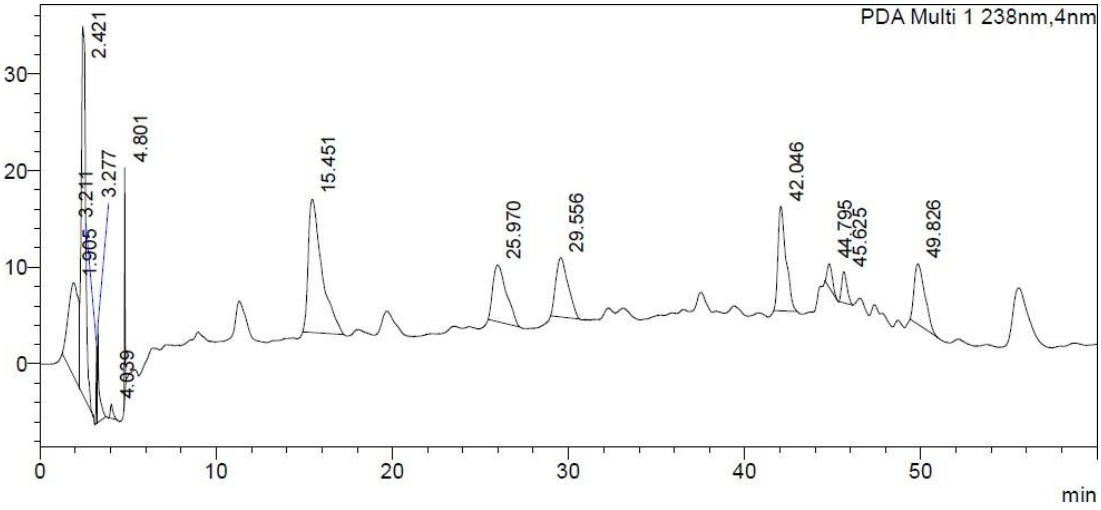
5.22.3 HPLC chromatogram of *Shuddha Vatsanabh* Powder (Sample-A)



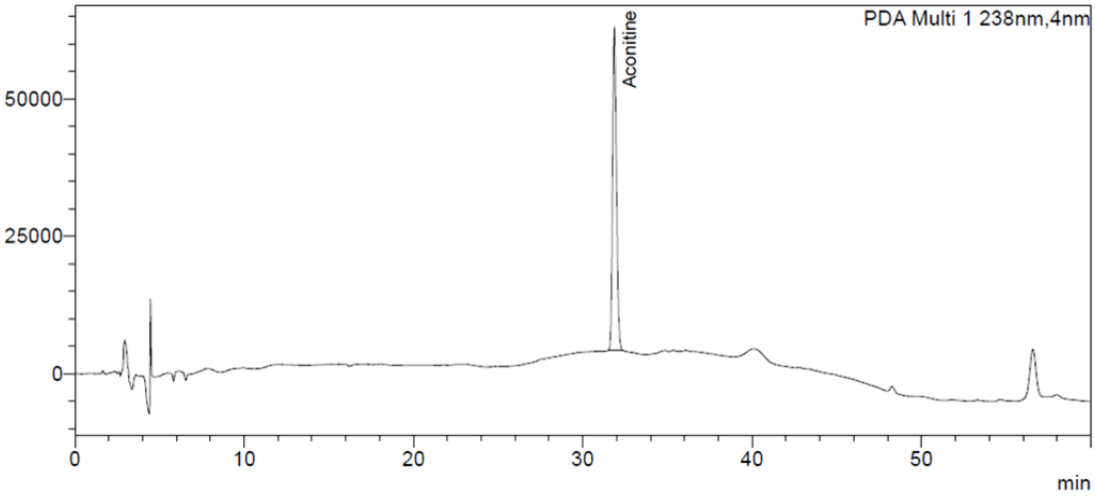
5.22.4 HPLC chromatogram of *Shuddha Vatsanabh* Powder (Sample-B)



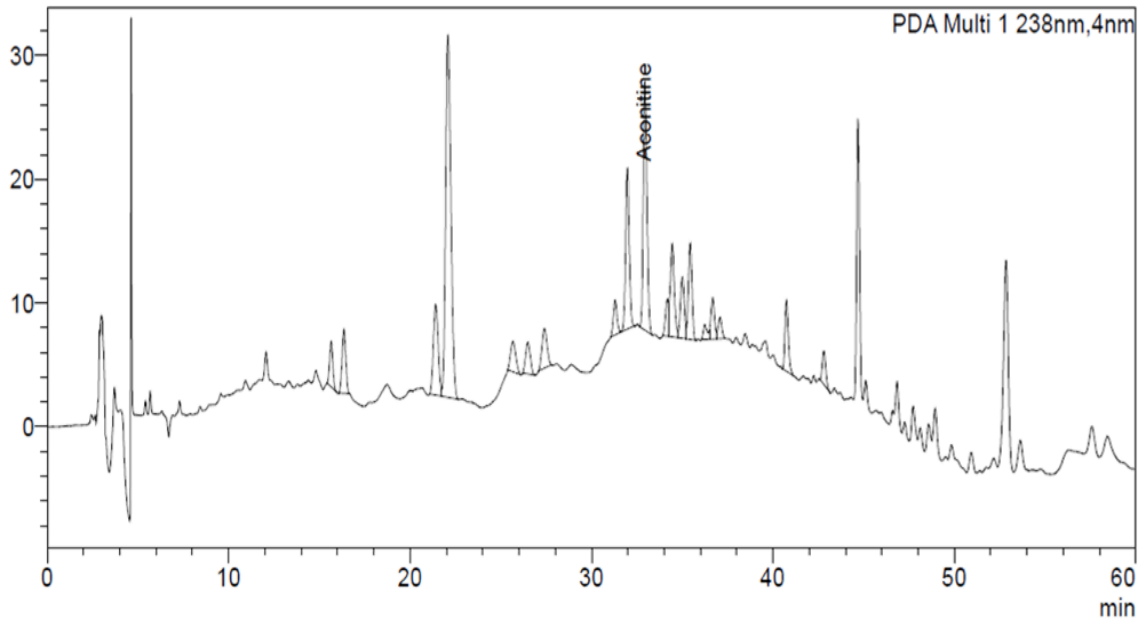
5.22.5 HPLC chromatogram of *Shuddha Vatsanabh* Powder(Sample-C)



5.22.1 HPLC chromatogram of Standard Aconitine



5.22.6 HPLC chromatogram of *Shuddha Vatsanabh* Powder (Composite - A, B& C)



5.23 Pharmacognosy of *Vatsanabh* (*Aconitum balfourii* Stapf) methods applied as per4.4a

5.23.AContinued T.S. *Vatsanabh* of *Aconitum balforii* Stapf as per method (4.4a)

Diagrammatic of T.S. *Vatsanabh Aconitum balfori* shows that outer exoderm followed by cortex and central stellar region.

Detailed T.S root tubers of *Aconite* showed that. Outer exoderm made up off collapsed parenchyma like cells with brown content and are also not lignified followed by large cortex. The tuber has abruptly distributed "vascular strands" embedded in the corticalzone. Each exarch vascular strand has 2-4 radiating xylem strands, the large gaps between which are filled with parenchyma cells. Phloem occurs in patches just outside the xylem strands in a more or less discontinuous ring. The parenchyma cells of vascular strands continue into the ground tissue through spaces between the phloem patches. The ample amount of simple and compound, round to oval starch grains embedded in cortical cells. The parenchyma cells are in very regular radiating files (cambiform), There is a typical single layered endodermis extending throughout the entire perimeter, the inner tangential walls and radial wall of whose cells are thickened with suberin. Outside endodermis are a few layers of cells divided into an outer zone of 2 to 3 cork layers and an inner zone of 2 to 3 layers of thin-walled cells. The central stellar region occupied by the radially arranged more xylem vessels up to the centre with xylem fibres and parenchyma cells. Centrally situated pith narrow and made up of radially elongated to isodiametric parenchymatous cells which abundantly contain starch.

Powder study

5.23.B.Powder microscopic study of *Vatsanabh* /*Aconitum balfourii* Stapf : Diagnostic powder microscopic characters of powder shows that fragments of parenchyma cells, Stone cells, pitted stone cells, scleroids, pitted scleroids, browncontent, fibers, starch grains with hilum, oil globules and annular and spiral vessels etc. depicted in the table below:

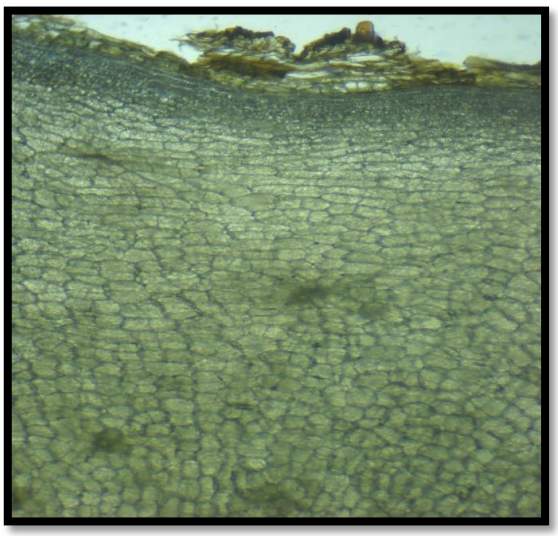
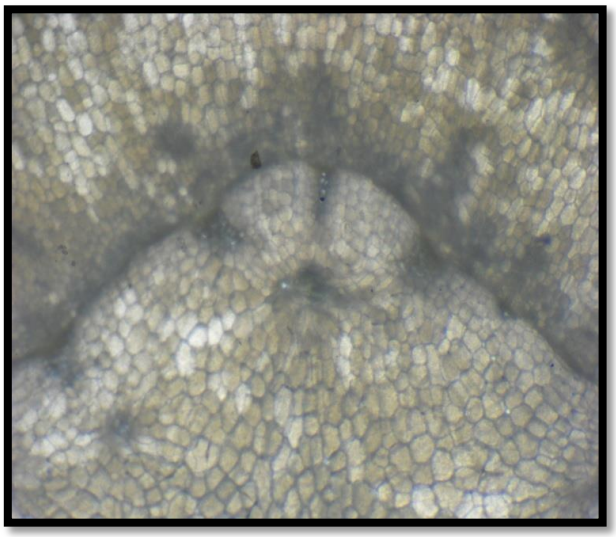
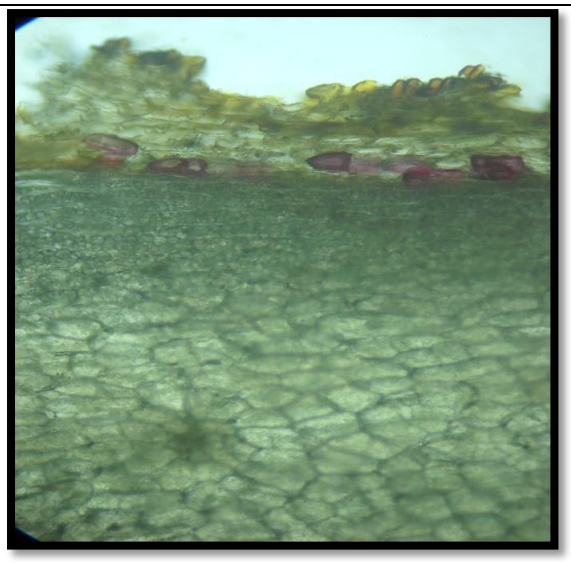
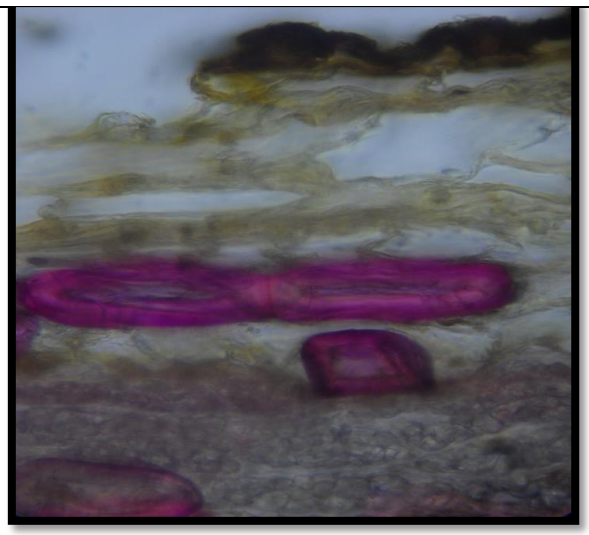
Characters	Raw Aconite	A	B	B
Fragments of parenchyma	+	++	++	++
Stone cells	+++	++	++	++
Fibres	+++	++	++	++
Brown cantant	+++	++	++	++
Starch grains	+++	++	++	++
Annular and spiral vessels	++	++	++	++

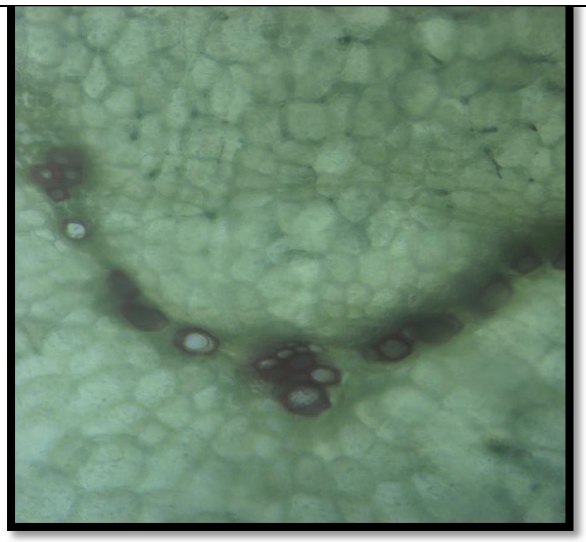
5.23.C.Histochemical study of *Vatsanabh /Aconitum balfourii* Stapf

Characters	Normal	A	B	C
Lignin	++	++	++	++
Starch	++	++	++	++
Oil	++	++	++	++
Oleoresine	++	++	++	++
Calcium oxalate	--	--	--	--

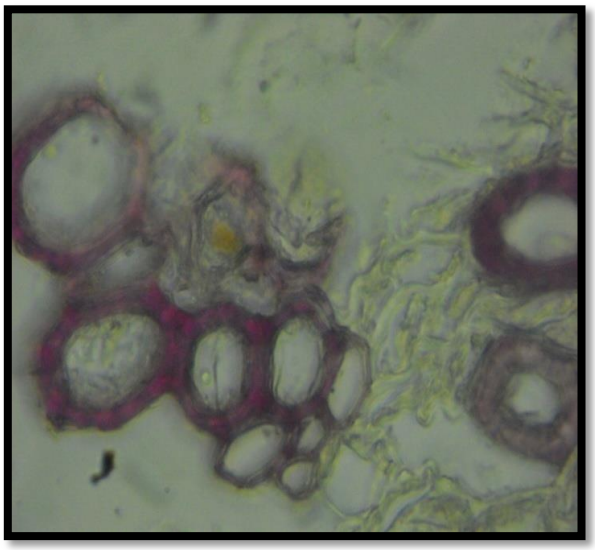
5.24 Images of Pharmacognosy of *Vatsanabh (Aconitum balfourii* Stapf*)*

5.24.A Images of Pharmacognosy of *Ashuddha Vatsanabh (Aconitum balfourii* Stapf*)*

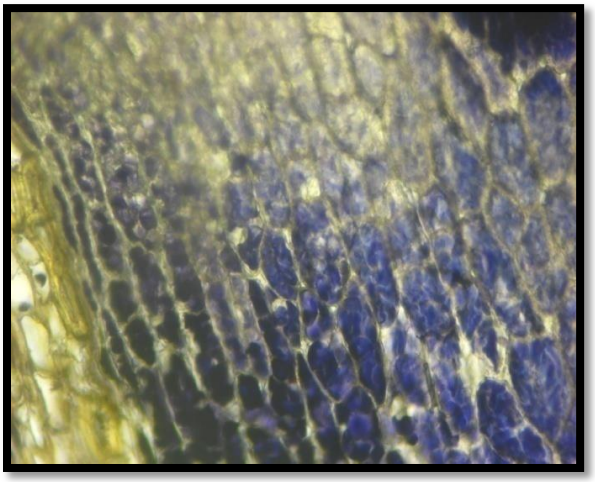
 **T.S. with exoderm and large cortical zone**	 **V-Shaped vascular bundle along with pith region**
 **Exoderm, cortical zone along withlignified elements**	 **Stone cells and scleroids in the exodermal region**



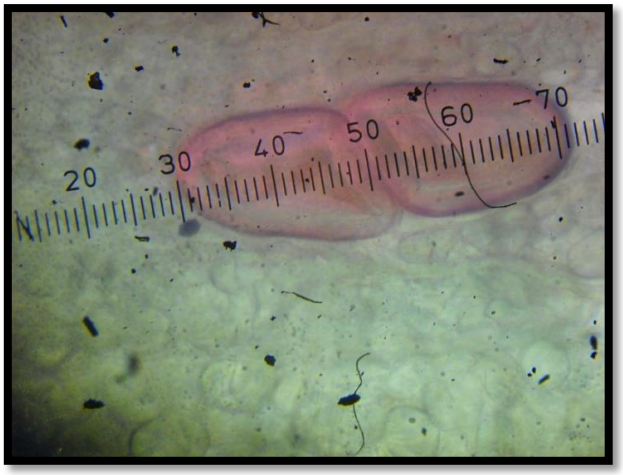
V- Shaped vascular bundle



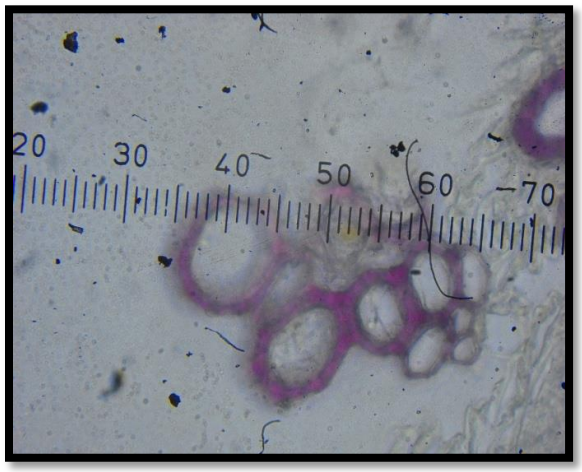
Vascular bundle with Pholem and xylem vessels

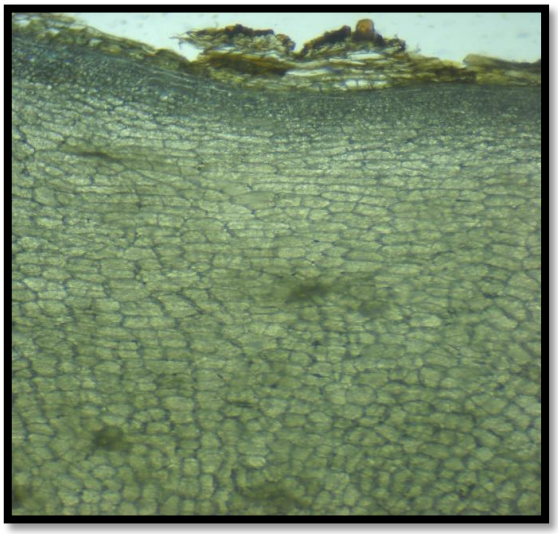


Iodine stained cortical zone consists starch grains

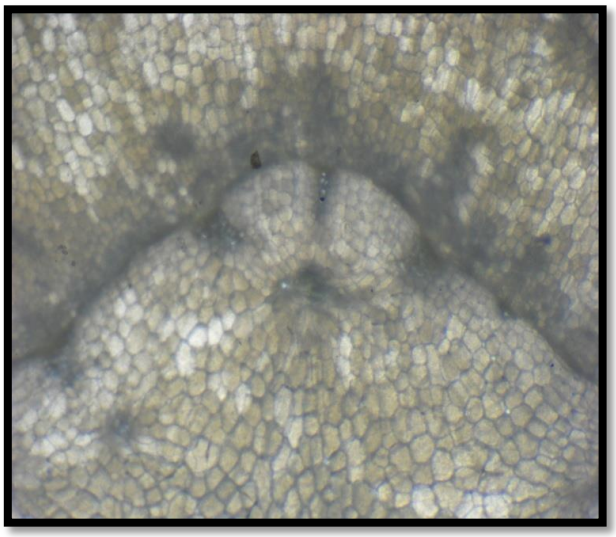


Stone cells with micromeasurment

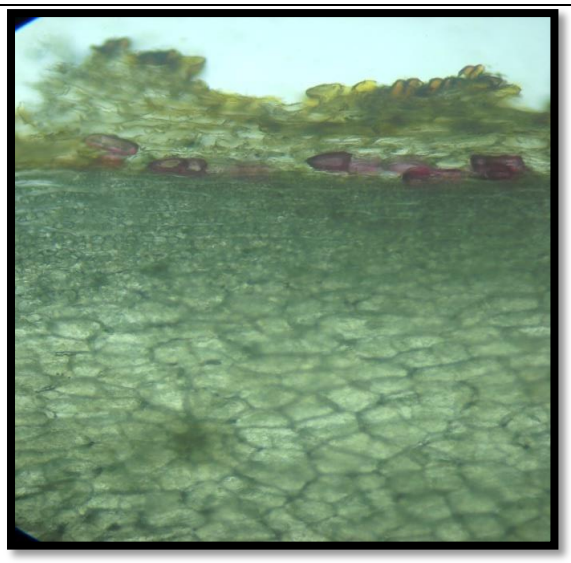
	
Lignified xylem with micromeasurment	**Lignified stone cells and scleroids with micromeasurment**



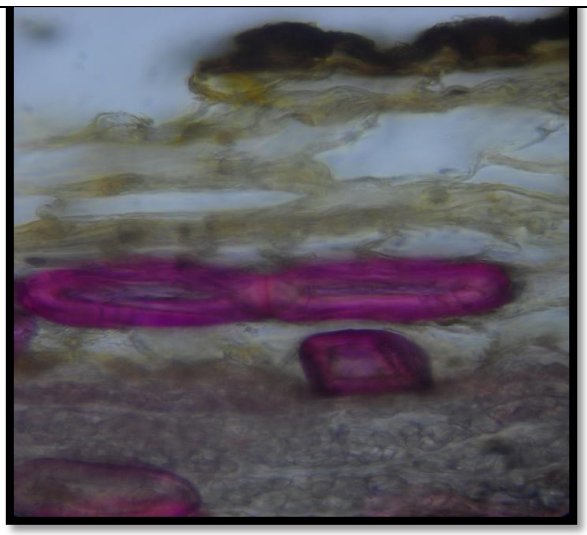
T.S. with exoderm and large cortical zone



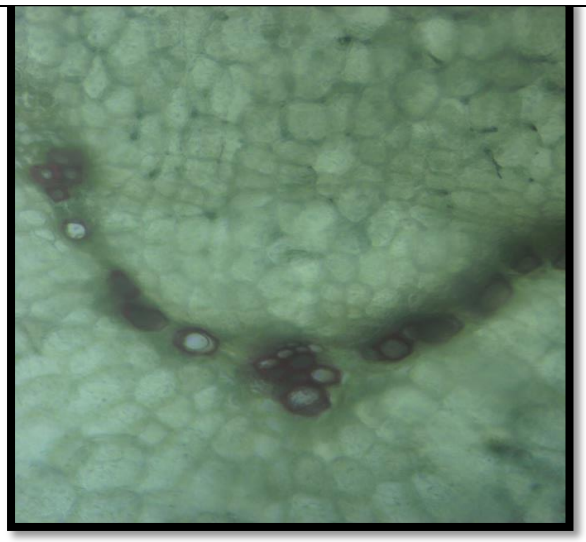
V-Shaped vascular bundle along with pith region



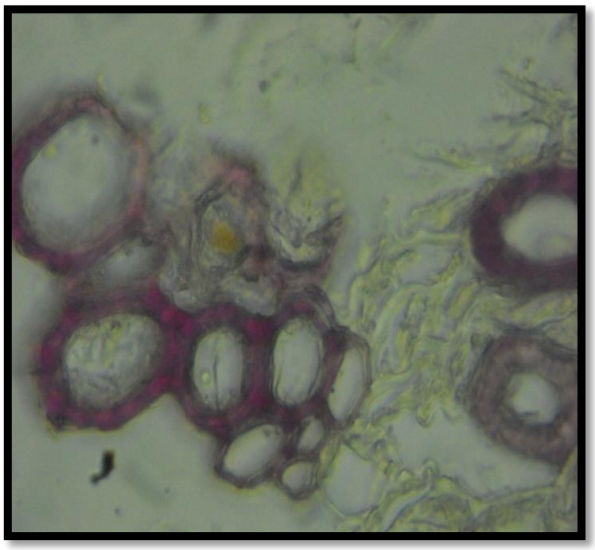
Exoderm, cortical zone along withlignified elements



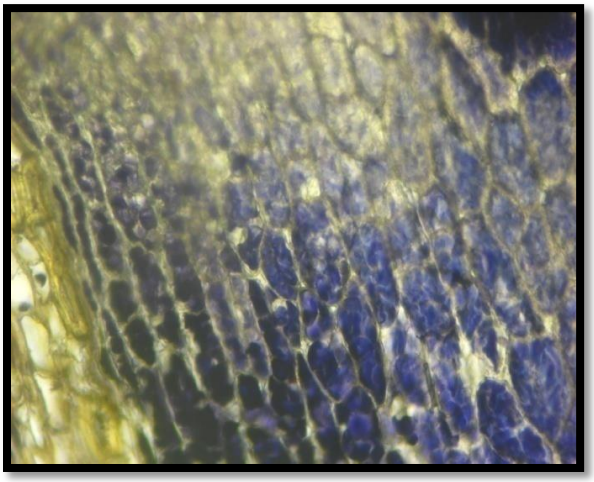
Stone cells and scleroids in the exodermal region



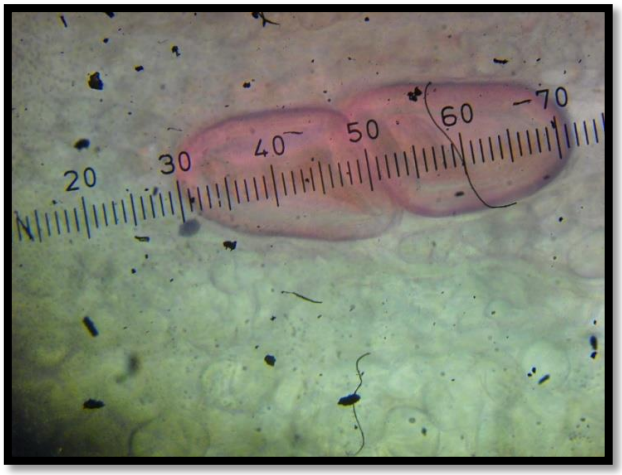
V- Shaped vascular bundle



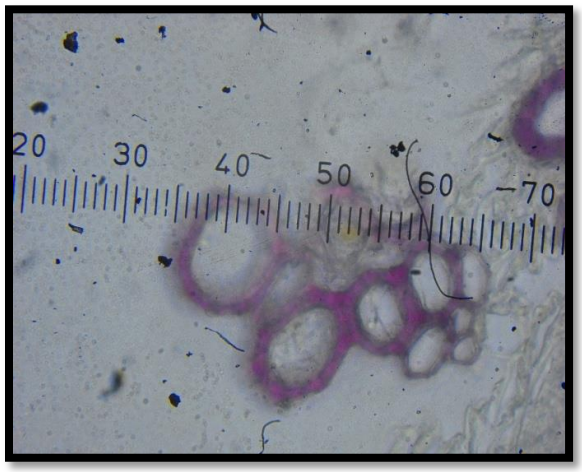
Vascular bundle with Pholem and xylem vessels



Iodine stained cortical zone consists starch grains



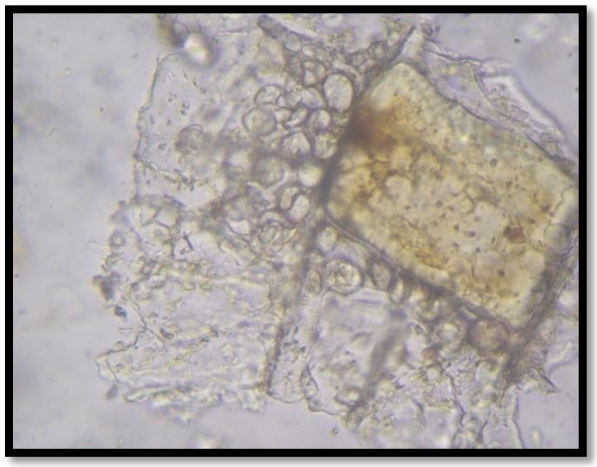
Stone cells with micromeasurment

	
Lignified xylem with micromeasurment	**Lignified stone cells and scleroids with micromeasurment**

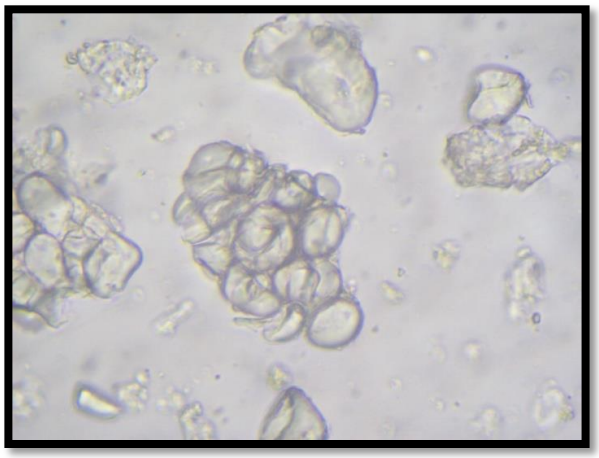
5.24.B.Pharmacognosy of *Shudhha Vatsanabh (Aconitum balfourii* Stapf)

Sample A

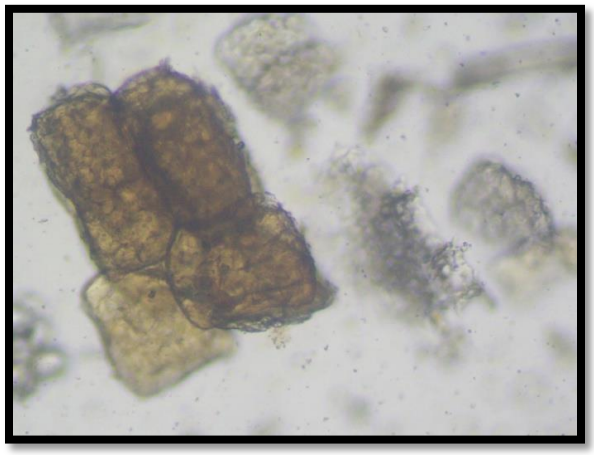
	
	Powder A, *Shuddha Vatsanabh* (*Aconitum balfourii* stapf)



Parenchyma cells with Starch grains



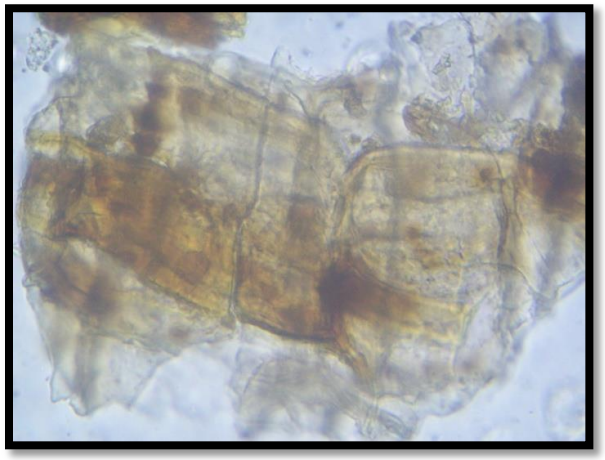
Group of starch grains



Cells with brown cantant



Stone cells along with scleroids



Exodermal cells with brown cantant



Simple starch grains



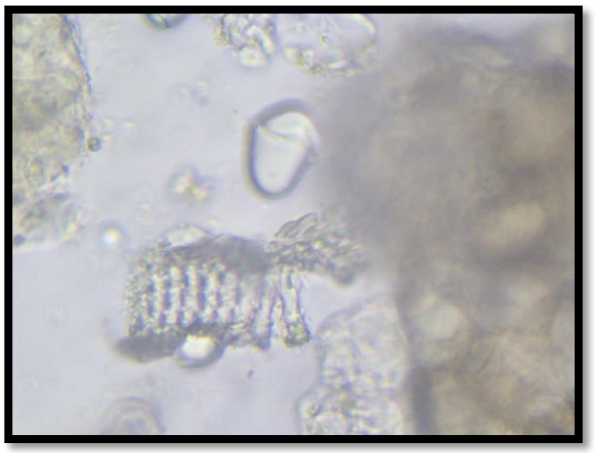
Stone cells with brown cantant



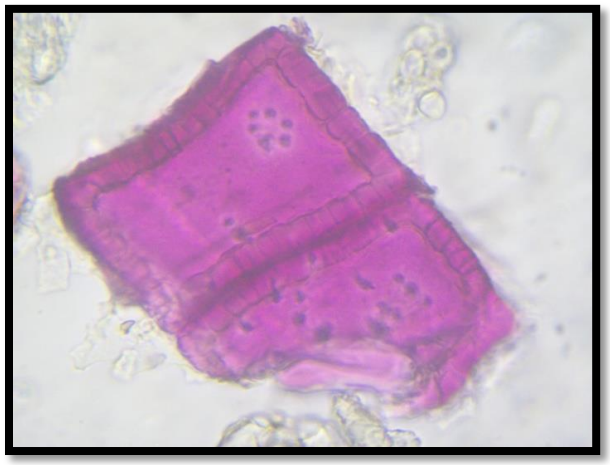
Scleroid

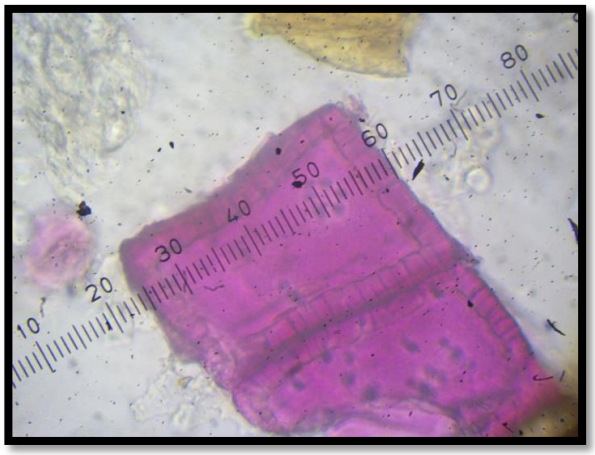


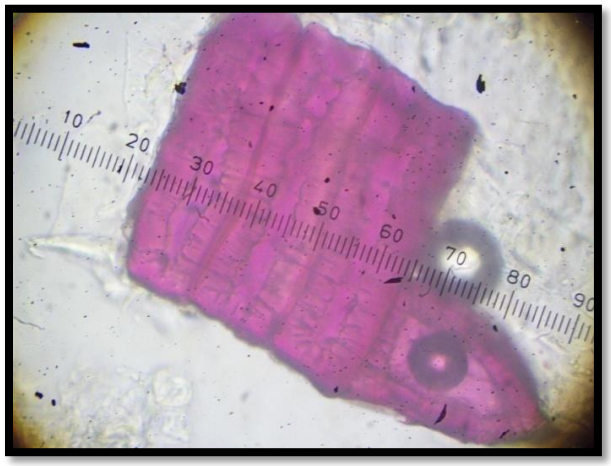
Group of stone cells



Pitted vessels





Lignified stone cells	**Lignified stone cells along with micromeasurment**
	
Lignified sclroids along with micromeasurment	**Pitted and annular vessels**

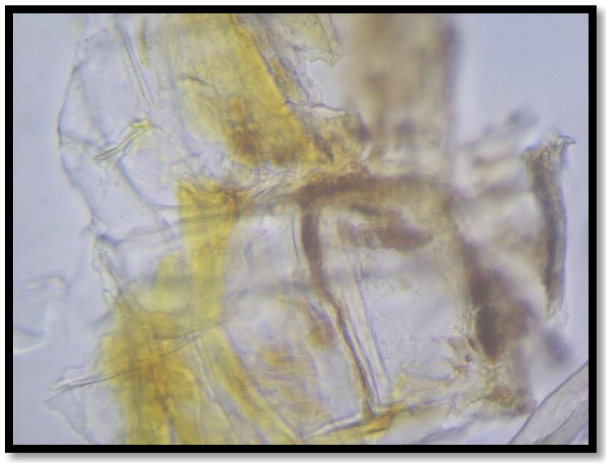
5.24.C.Pharmacognosy of Shudhha *Vatsanabh (Aconitum balfourii* Stapf)

Sample B

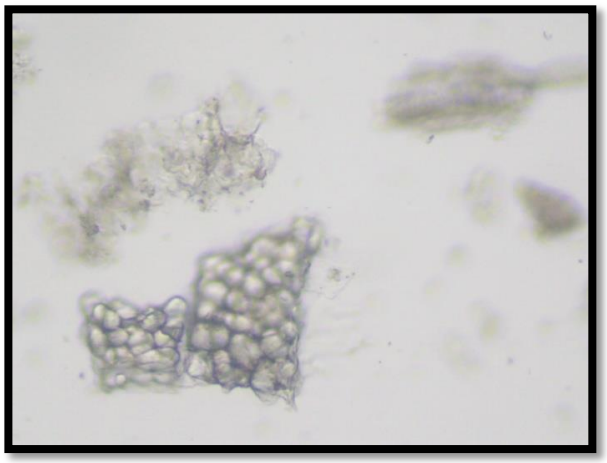




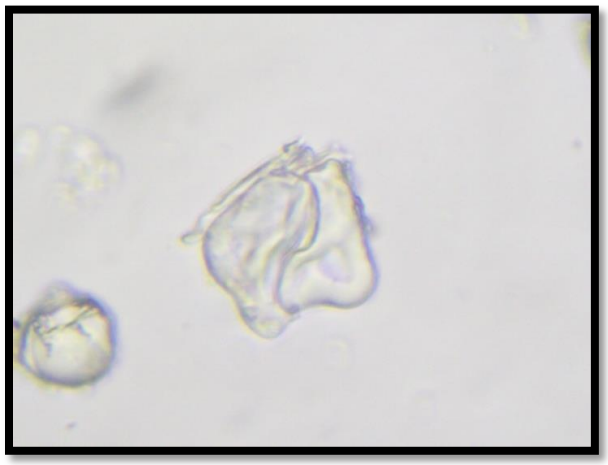
Figure 1, Sample B *Shuddha Vatsanabh*, (*Aconitum balfourii* stapf)



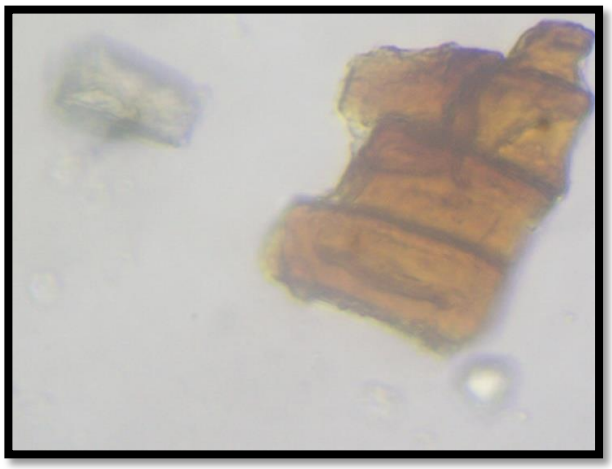
Exoderant



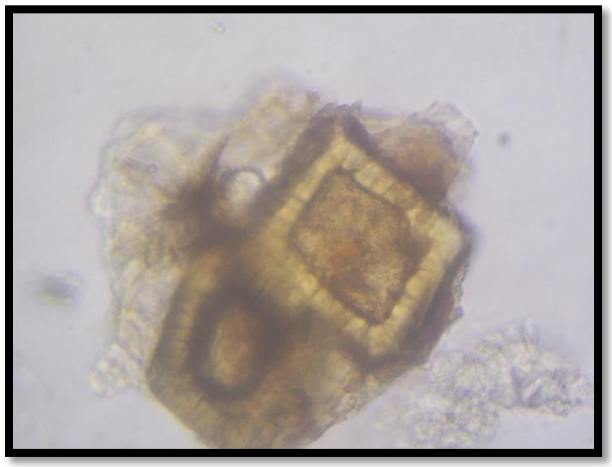
Group of starch grains



Starch grains with hilum



Sclroids with brown cantant



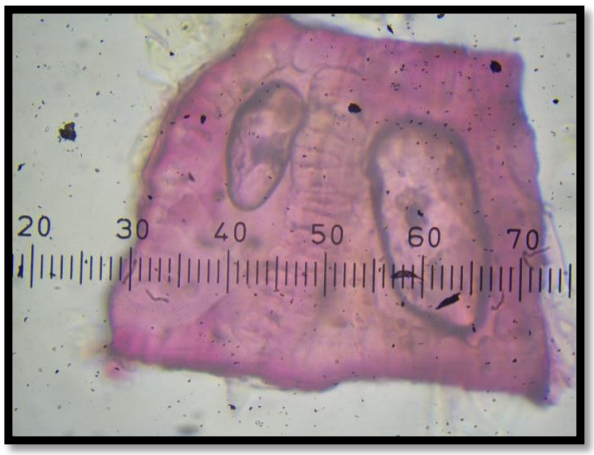
Stone cells with brown cantant



Simple fibre



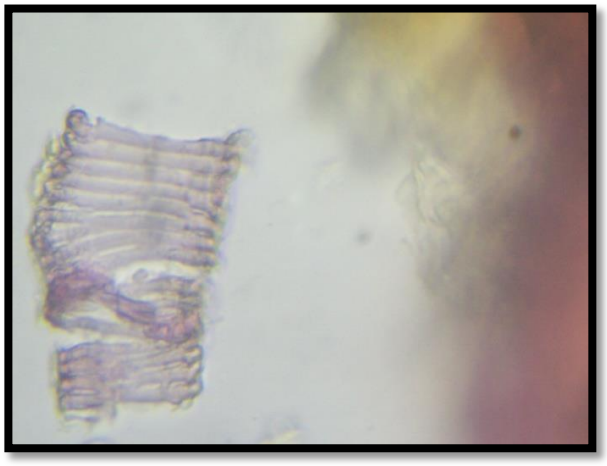
Lignified stone cells



Lignified stone cells with micromeasurment





Lignified scleroids	**Lignified scleroidss with micromeasurment**
	
Annular vessels	**Scalariform vessels**

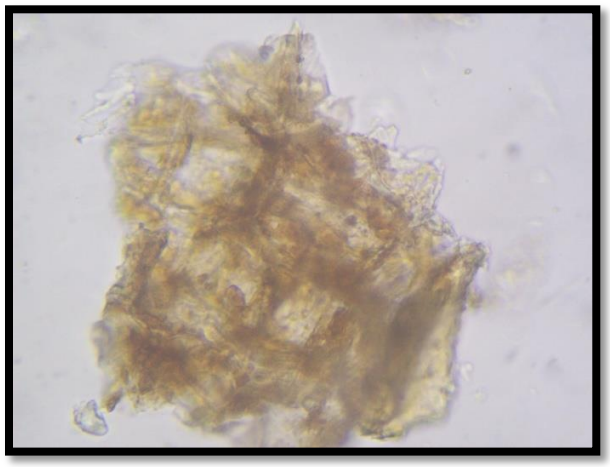
5.24.D.Pharmacognosy of *Shudhha Vatsanabh (Aconitum balfourii* Stapf)

Sample C

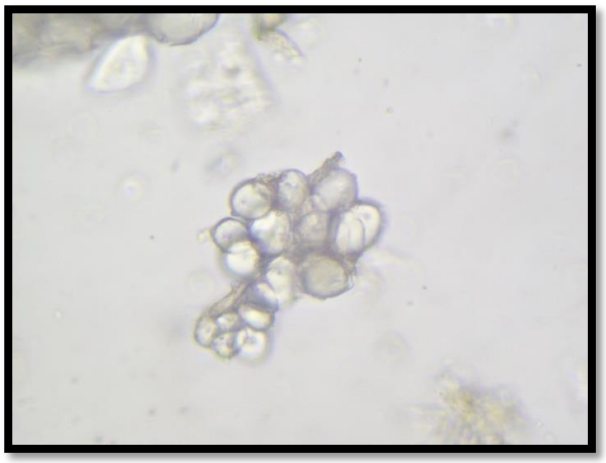




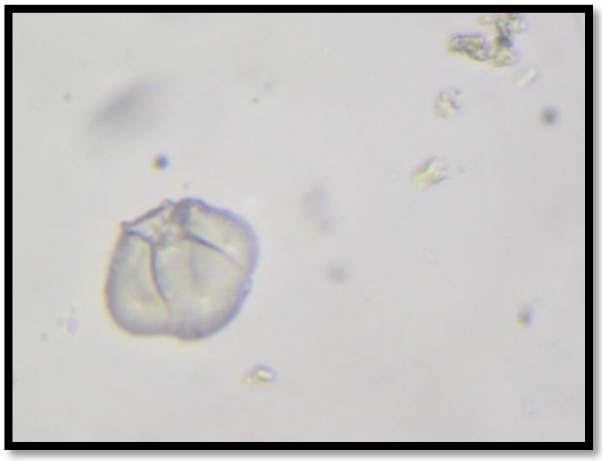
Sample C *Shuddha Vatsanabh* (*Aconitum balfourii* stapf)



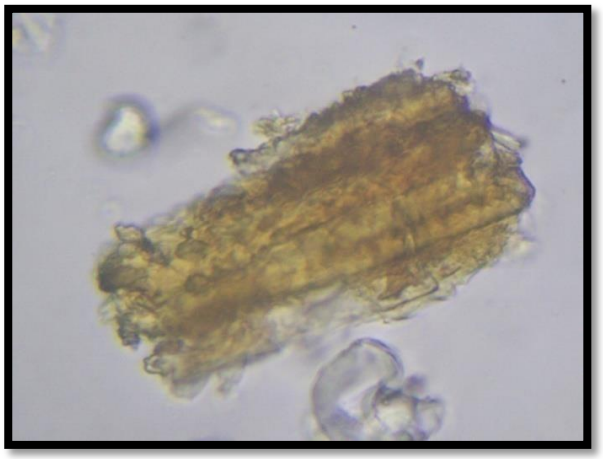
Exodermal cells with brown cantant



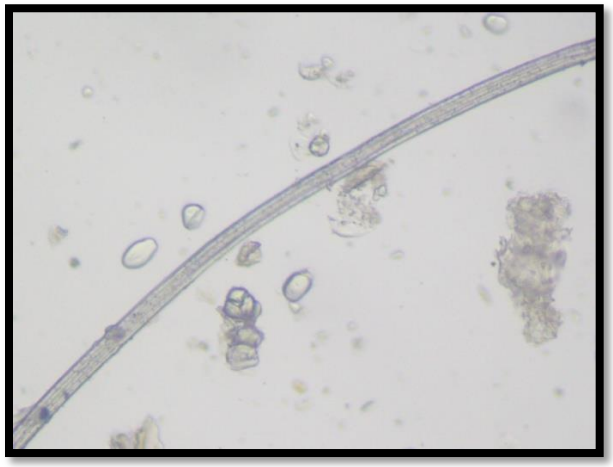
Group of starch grains



Simple starch grains with hilum



Scleroids with brown cantant



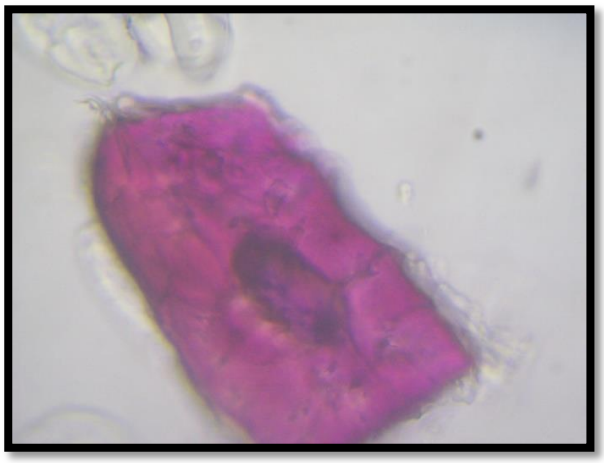
Simple fibre



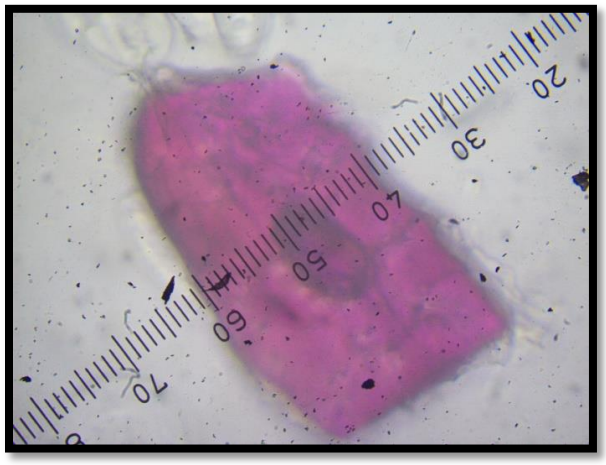
Stone cell



Group of Stone cells



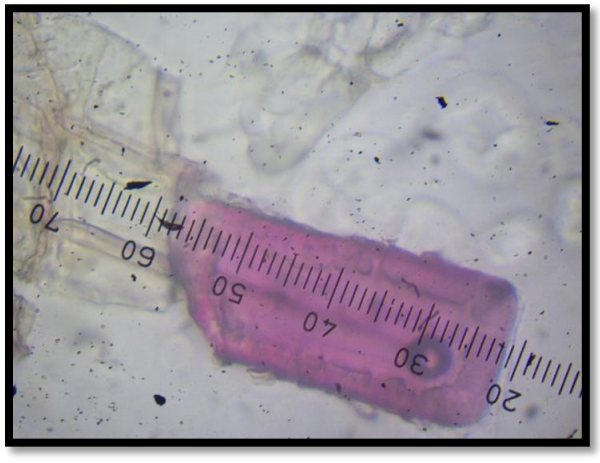
Lignified stone cell



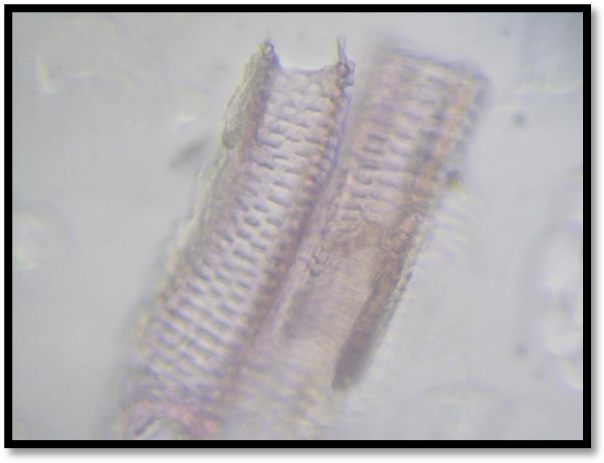
Lignified stone cell with micromeasurment



Lignified stone cell

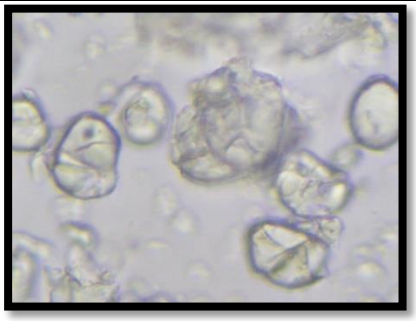
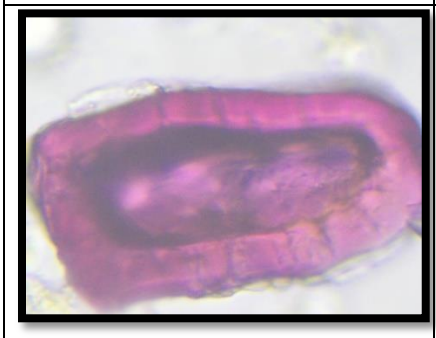
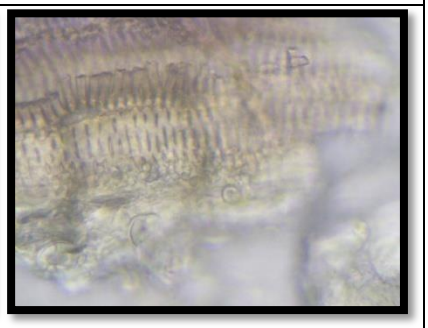


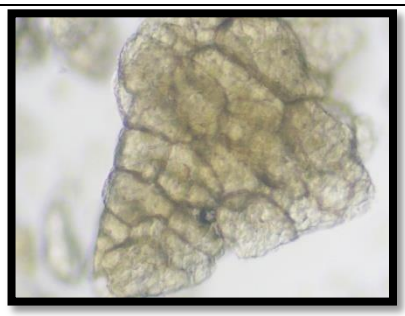
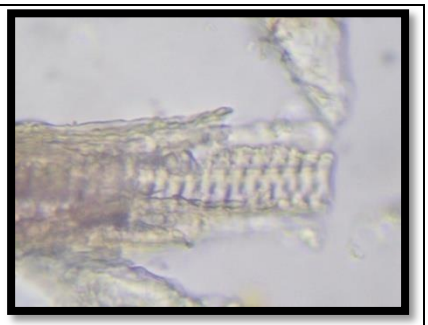
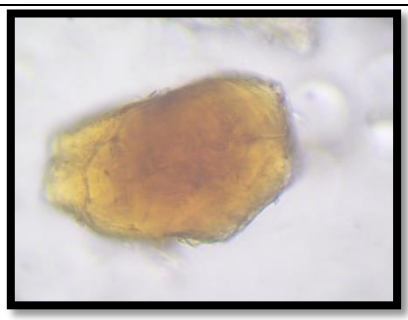
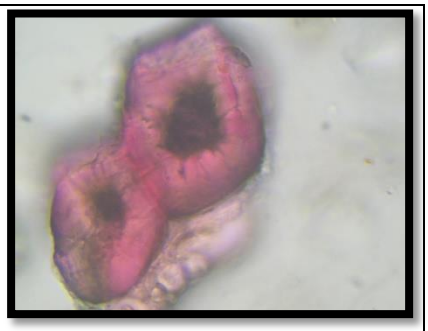
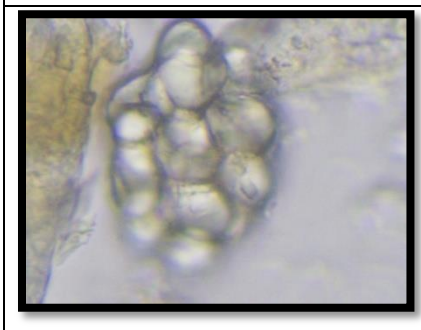
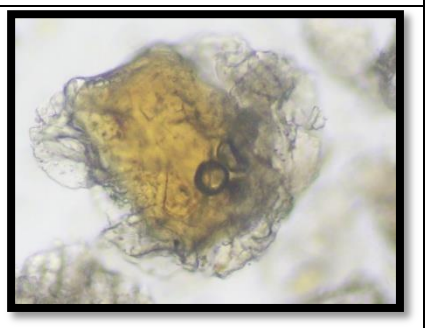
Lignified scleroids with micromeasurment



Pitted vessels

5.24.E.Photos of Pharmacognosy of *Shudhha* (A+B+C) composite *Vatsanabh (Aconitum balfourii Stapf):*

 **Triangular stone cells of A**	 **Stone cells without pits of A**	 **Stone cells from B**
 **Stone cell from B**	 **Starch grains of B**	 **Simple and compound Starch grains of C**
 **Stone cell with wide lumen of C**	 **Sclariform vessels of B**	 **Pitted vessels of C**

		
Parenchyma cells with brown contant of A	**Parenchyma cells of C**	**Lignified Pitted**
		
Lignified angulared stone cells of A	**Light Brown contant of B**	**Group of Stone cells without pits**
		
Group of starch grains of A	**Fibres of C**	**Brown contant of A**

***5.25 Hinguleshvara Rasa* Results:**

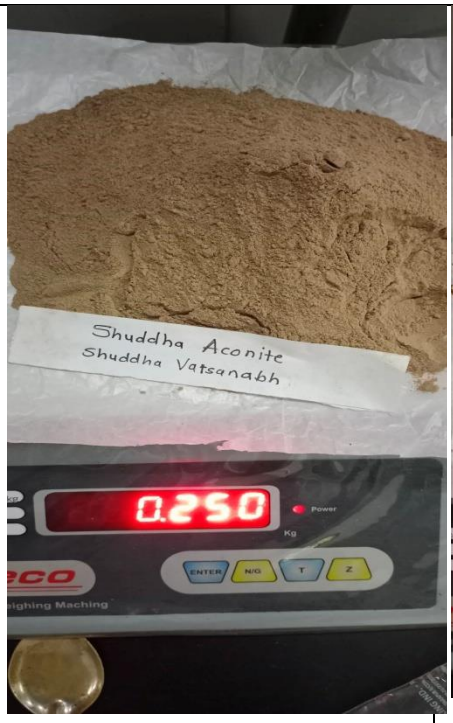
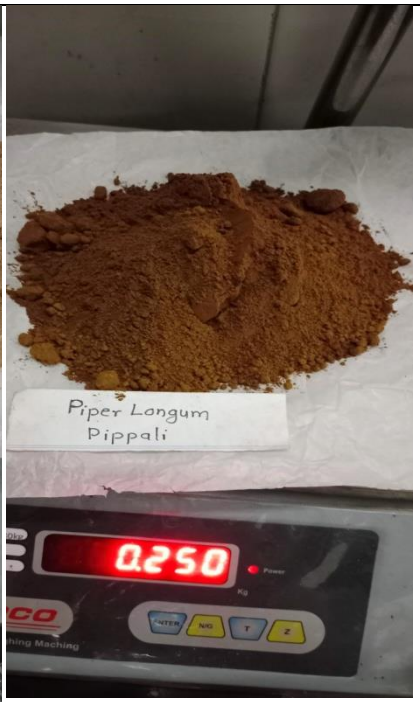
5.25.1Preperation of *Hinguleshvara Rasa*:

Materials and methodsof preparation of *Hinguleshvara Rasa* is as described in 4.9.

Total quantity obtained=736 gms

This *Hinguleshvara Rasa was* then subjected to characterization by particle size analyzer, SEM, FTIR and XRD, physicochemical, phytochemical analysis.

5.25.2 Photos of **Preperation of *Hinguleshvara Rasa* as per described in 4.9**

		
ShuddhaHingula **(Red Cinnabar:HgS)**	***Shuddha Vatsanabh*** ***(Aconitum balfourii* Stapf)**	***Pippali(Piper longum* Linn)**



***Hinguleshvara Rasa* by Ball mill**



***Hinguleshvara Rasa* by trituration**

5.25.3.Organoleptic character *of Hinguleshvara Rasa*

characteristic	***Hinguleshvara Rasa***
Colour	**Brownish dull Red**
Odor	**Typical strong odor not specific to any constituent.**
Taste	**Pungent**
Touch	**Smooth**

5.26 Physicochemical Analysis of *Hinguleshvara Rasa* is as follows

No.	**Test Name**	**Result Obtained %**
1	Loss on drying(as per 4.1a)	5.91%
2	Loss on Ignition(as per 4.1d)	94.16%
3	Total ash(as per 4.1b)	5.84
4	Acid insoluble Ash(as per 4.1c)	1.06
5	Water Soluble Ash	1.45

5.27 Microbial load of *Hinguleshvara Rasa*(as per method 4.5i)

Sample	Total Viable count CFU/gm	Total Fungal count CFU/gm
Hinguleshvara Rasa	160	40

5.28 Photos of microbial Viable and fungal count of *Hinguleshvara Rasa*:

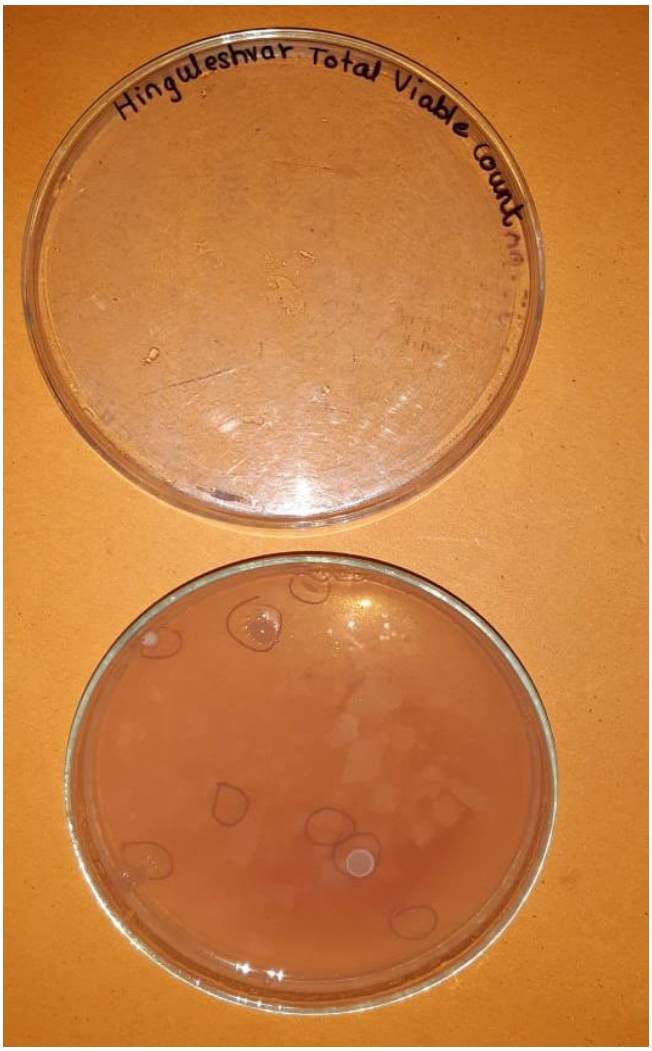


Fig 5.28 of Total viable count of *Hinguleshvara Rasa*

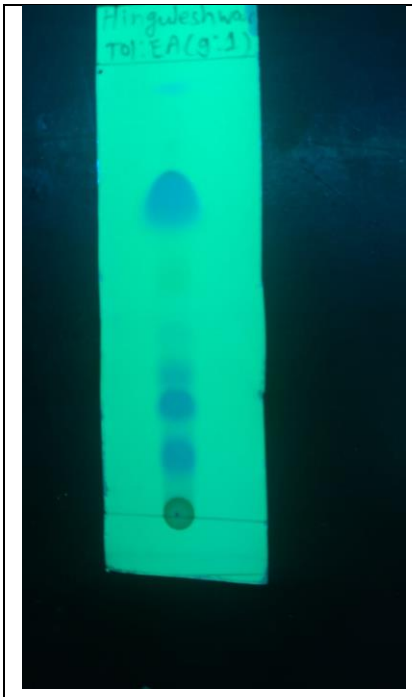
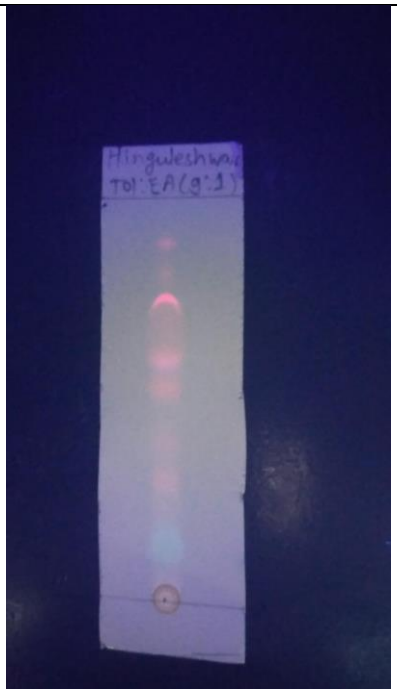
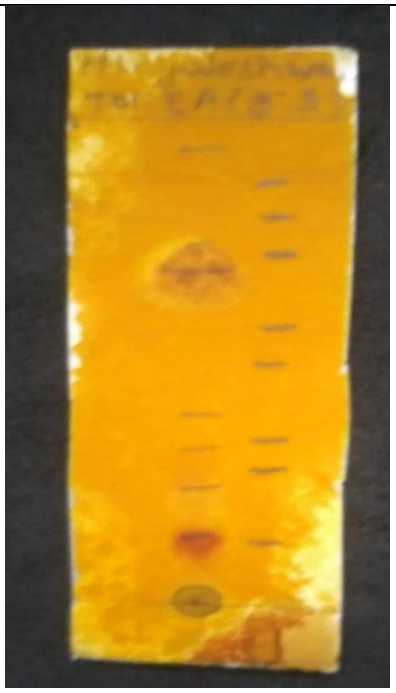


Fig 5.28 of Total fungal count of *Hinguleshvara Rasa*

5.29 TLC of *Hinguleshvara Rasa*

In UV at 254nm	**InUV at 365nm**	**After Derivatization with Dragendorff reagent**
0.14,0.26,0.34,0.40,0.71,0.77 ,0.96 (All Yellow)	0.14,0.29,0.51,0.59,0.74, 0.81,0.89 (All Blue)	0.14,0.74 (All orange)

5.30 Photos of T.L.C of *Hinguleshvara Rasa*

		
***Hinguleshvara Rasa*@254**	***Hinguleshvara Rasa*@365**	***Hinguleshvara Rasa* @ dragondroff reagent**

5.31 HPLC quantification report:

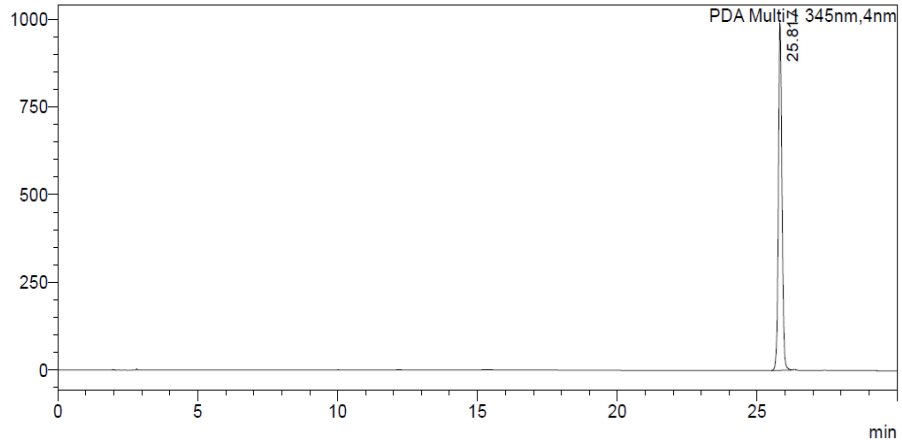
It includes estimation of piperine as well as aconitine content from *Hinguleshvara Rasa.* Total alkaloid from *Hinguleshvara Rasa* were also estimated :

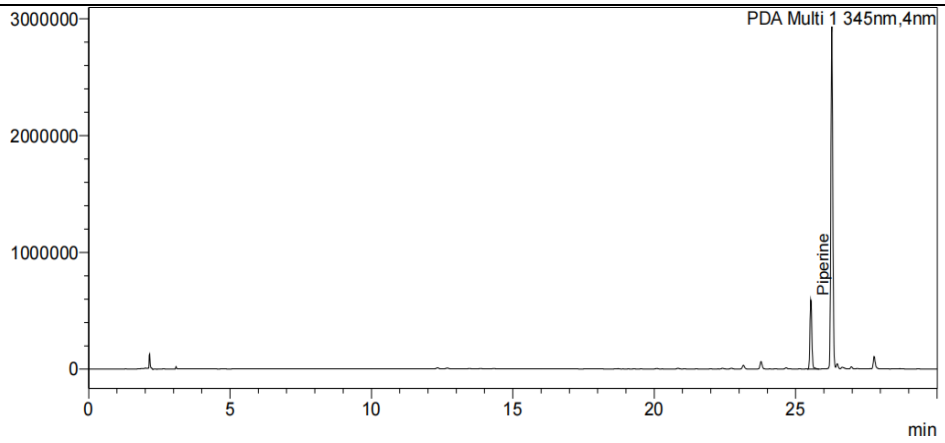
Sample	**Piperine Standard**	***Hinguleshvara Rasa***
Rt (min.)	25.817	25.539
Area	8817543	2736664
% Piperine	-	0.059 %

Sr no	Parameters	Results
1	% Total Alkaloids (By Gravimetry) (4.6.c)	0.48 %
2	% Piperine (By HPLC)	0.059 %
3	% Aconitine (By HPLC)	0.0022 %

5.32 HPLC Chromatographs of *Hinguleshvara Rasa*

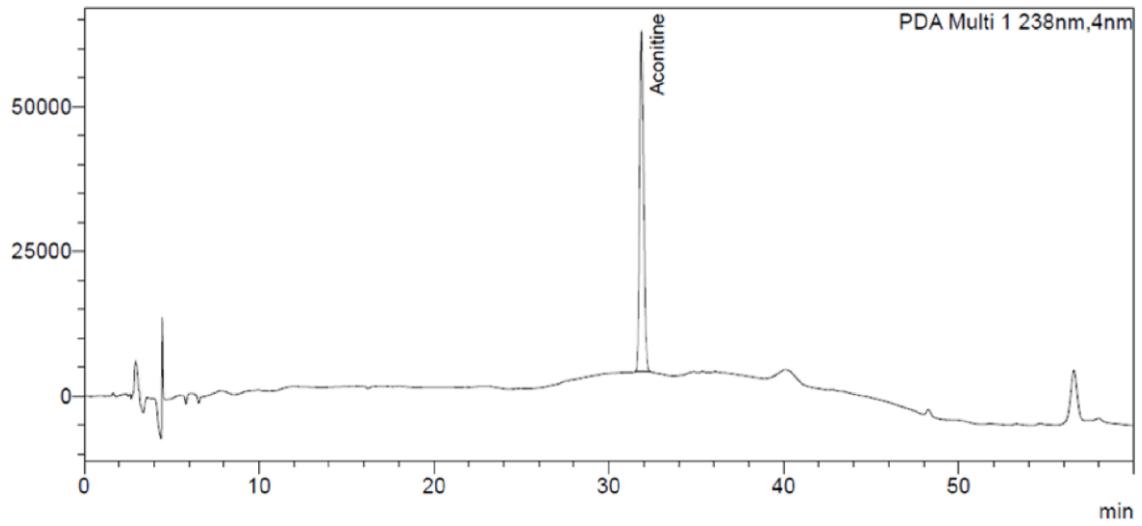
5.32.1 HPLC Chromatogram of Standard Piperine



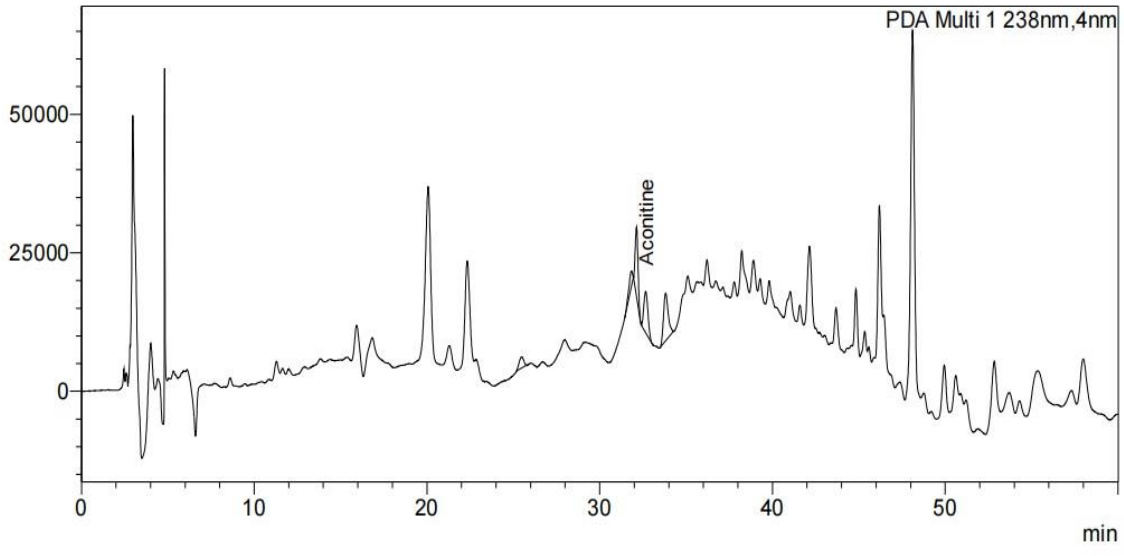


5.32.2 HPLC Chromatogram of piperine from *Hinguleshvara Rasa*

5.32.3 HPLC chromatogram of Standard Aconitine



5.32.4 HPLC chromatogram of Aconitine from *Hinguleshvara Rasa*



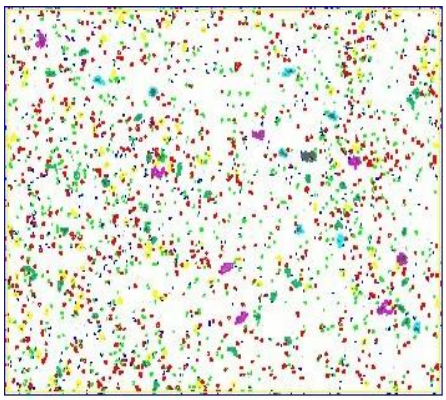
From the Characterization:

For the characterization of *Ashuddha Hingula* (Impure Red Cinnabar: HgS), *Shuddha Hingula* (Red Cinnabar processed with ginger juice).,*Hinguleshvara Rasa* prepared by ball mill and *Hinguleshvara Rasa* prepared by trituration (manual grinding) following finding observed such as:

5.33. Results of **Particle Size Analysis (as per methods 4.3.b)**of *Ashuddha Hingula* (Impure Red Cinnabar: HgS), *Shuddha Hingula* (Red Cinnabar processed with ginger juice). *Hinguleshvara Rasa* prepared by ball mill and *Hinguleshvara Rasa* prepared by manual grinding(trituration) are as follows:

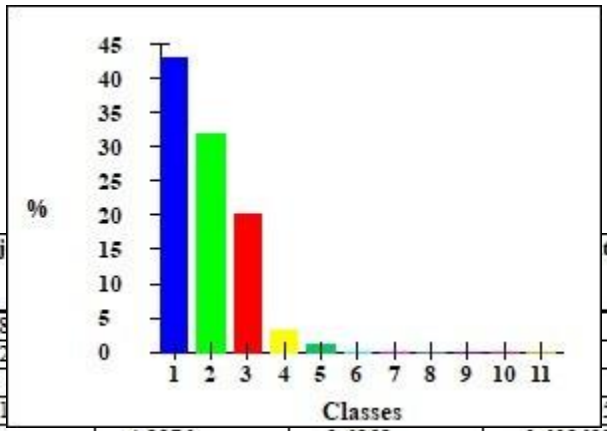
Ashuddha Hingula

5.33-1 *Ashuddha Hingula* (Particle Size)



Results Summary

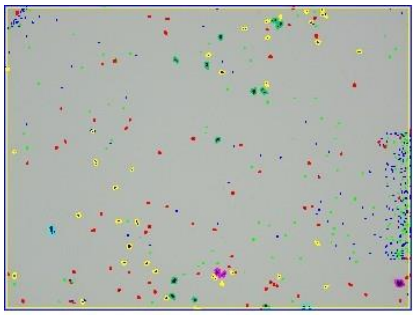
Fields	5
Field Area sq mm	6.3454
Total Count	14900
In Range Count	14900
In Range %	100



	sq microns		Axis(Maj microns			ty(Eqv
Min	0.6609822	0	0.81298			
Max	11794.57	11.1779	691.022			
Mean	22.9399	1.5178	4.9895			
Sum	341803.8	22615.46	74343.1			5
Std.Dev	118.7882	0.8398427	7.8175	4.2976	3.6952	0.6096271

Classes	Count	%Count	%Count(Total)	Count/area mm
#1: 0 to 2.5	6425	43.1208	43.1208	0.000000001
#2: 2.5 to 5	4701	31.5503	31.5503	0.000000001
#3: 5 to 10	3020	20.2685	20.2685	0.000000001
#4: 10 to 15	489	3.2819	3.2819	0.000000001
#5: 15 to 20	156	1.047	1.047	0.000000001
#6: 20 to 25	59	0.3959731	0.3959731	0.000000001
#7: 25 to 30	22	0.147651	0.147651	0.000000001
#8: 30 to 40	21	0.1409396	0.1409396	0.000000001
#9: 40 to 50	3	0.000000001	0.000000001	0.000000001
#10: 50 to 100	3	0.000000001	0.000000001	0.000000001
#11: 100Above	1	0.000000001	0.000000001	0.000000001

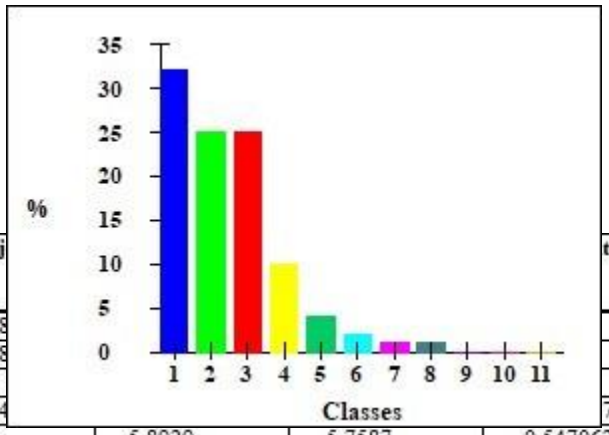
5.33-2 *Shuddha Hingula* (Particle Size)



Classes	Count	%Count	%Count(Total)	Count/area mm
#1: 0 to 2.5	986	32.275	32.275	0.000000001
#2: 2.5 to 5	760	24.8773	24.8773	0.000000001
#3: 5 to 10	774	25.3355	25.3355	0.000000001
#4: 10 to 15	310	10.1473	10.1473	0.000000001
#5: 15 to 20	107	3.5025	3.5025	0.000000001
#6: 20 to 25	75	2.455	2.455	0.000000001
#7: 25 to 30	22	0.7201309	0.7201309	0.000000001
#8: 30 to 40	18	0.5891981	0.5891981	0.000000001
#9: 40 to 50	3	0.000000001	0.000000001	0.000000001
#10: 50 to 100	0	0	0	0
#11: 100Above	0	0	0	0

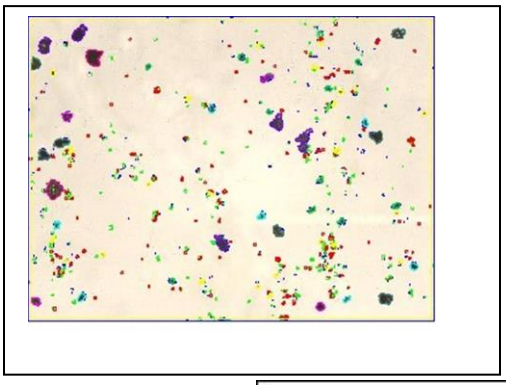
Results Summary

Fields	5
Field Area sq mm	6.3454
Total Count	3055
In Range Count	3055
In Range %	100



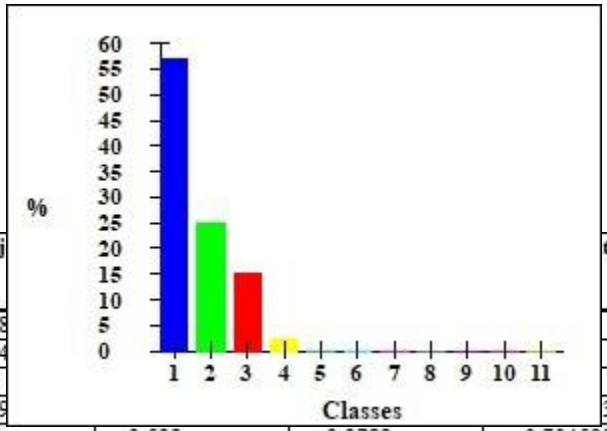
	sq microns		Axis(Maj microns			ty(Eqv
Min	0.6609822	0	0.81298			
Max	1348.404	66580.02	73.0668			
Mean	54.7795	23.1856	7.5396			
Sum	167351.4	70831.97	23033.4			7
Std.Dev	119.3167	1204.562	8.3558	5.8929	5.7587	0.5470629

5.33.3 *Hinguleshvara Rasa* by Ball mill (Particle Size)



Results Summary

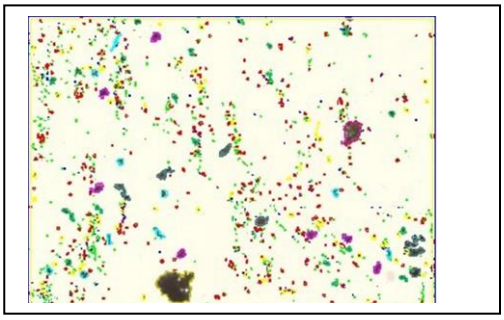
Fields	5
Field Area sq mm	6.3454
Total Count	13495
In Range Count	13495
In Range %	100



	sq microns		Axis(Maj microns			ty(Eqv
Min	0.6609822	0	0.81298			
Max	2758.279	56724.31	87.4504			
Mean	17.2092	13.6195	4.0624			
Sum	232238.8	183795.5	54821.9			3
Std.Dev	80.24	811.5098	4.9093	3.539	3.3782	0.7045337

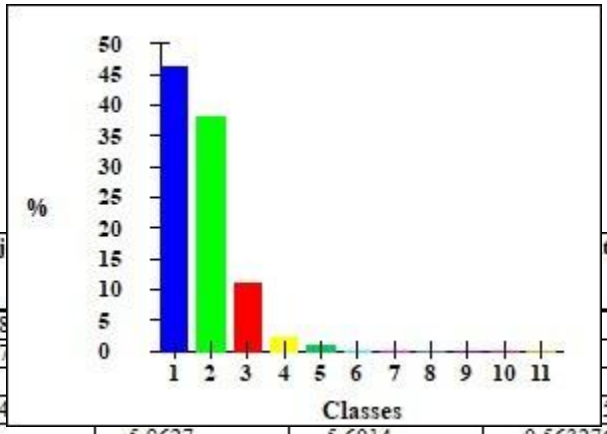
Classes	Count	%Count	%Count(Total)	Count/area mm
#1: 0 to 2.5	7717	57.1841	57.1841	0.000000001
#2: 2.5 to 5	3395	25.1575	25.1575	0.000000001
#3: 5 to 10	2011	14.9018	14.9018	0.000000001
#4: 10 to 15	225	1.6673	1.6673	0.000000001
#5: 15 to 20	64	0.4742497	0.4742497	0.00001009
#6: 20 to 25	34	0.2519452	0.2519452	0.000000001
#7: 25 to 30	13	0.000000001	0.000000001	0.000000001
#8: 30 to 40	18	0.1333827	0.1333827	0.000000001
#9: 40 to 50	15	0.1111523	0.1111523	0.000000001
#10: 50 to 100	3	0.000000001	0.000000001	0.000000001
#11: 100Above	0	0	0	0

5.33.4 *Hinguleshvara Rasa* by Trituration/Manual grinding (Particle Size)



Results Summary

Fields	5
Field Area sq mm	6.3454
Total Count	15438
In Range Count	15438
In Range %	100



	sq microns		Axis(Maj microns			ty(Eqv
Min	0.6609822	0	0.81298			
Max	8706.458	17.9268	193.237			
Mean	36.7143	1.3771	4.6817			
Sum	566795.6	21260.16	72275.4			5
Std.Dev	250.6582	0.732951	8.5605	5.9627	5.6014	0.5632769

Classes	Count	%Count	%Count(Total)	Count/area mm
#1: 0 to 2.5	7036	45.5759	45.5759	0.000000001
#2: 2.5 to 5	5895	38.185	38.185	0.000000001
#3: 5 to 10	1769	11.4587	11.4587	0.000000001
#4: 10 to 15	318	2.0599	2.0599	0.000000001
#5: 15 to 20	133	0.8615106	0.8615106	0.000000001
#6: 20 to 25	73	0.4728592	0.4728592	0.000000001
#7: 25 to 30	57	0.3692188	0.3692188	0.000000001
#8: 30 to 40	72	0.4663817	0.4663817	0.000000001
#9: 40 to 50	32	0.2072807	0.2072807	0.0000050
#10: 50 to 100	50	0.3238761	0.3238761	0.000000001
#11: 100Above	3	0.000000001	0.000000001	0.000000001

5.34 Reports of Field Emmission Scanning Electron Microscopy (FESEM)(as per methods4.3.a) of *Ashuddha Hingula* (Impure Red Cinnabar HgS), *Shuddha Hingula* (Red Cinnabar processed with ginger juice). *Hinguleshvara Rasa* prepared by ball mill and *Hinguleshvara Rasa* preparedbymanualgrinding(trituration) are as follows:

The Scale of 20 micrometer was used for measuring the particle size of all the samples in SEM image.

5.34.1 SEM of *Ashuddha Hingula* (Impure Red Cinnabar: HgS):-

The SEM image of *Ashuddha Hingula* (Impure Red Cinnabar: HgS) shows particles of various sizes and shapes. Particles of irregular shapes are observed in the field. Triangular, cylindrical, pentagonal, hexagonal, particles can be seen in the image. SEM image of particles appear compact agglumerated. Size of the particles of *Ashuddha Hingula* (Impure Red Cinnabar: HgS) from image observed at 8000 magnification from SEM was as follows:

Sr. No	Particle size in micrometer	Average size in micrometer
1	0.777	1.291
2	0.7951	
3	0.8012	

4	0.9094	
5	1.113	
6	1.134	
7	1.145	
8	1.160	
9	1.213	
10	1.369	
11	1.601	
12	1.604	
13	1.999	
14	2.466	

5.34.2 SEM of *Shuddha Hingula* (Red Cinnabar: HgS processed with Ginger juice):-

Size of the particles of ***Shuddha Hingula*** **(Red Cinnabar: HgS processed with Ginger juice)** from image observed at 8000 magnification from SEM was as follows:

Sr.No	Particle size in micrometer	Average size in micrometer
1	0.917	1.8302
2	0.934	
3	1.051	
4	1.256	
5	1.327	

6	1.409	
7	1..549	
8	1.810	
9	1.866	
10	2.120	
11	2.226	
12	2.347	
13	2.481	
14	3.003	
15	3.157	

5.34.3 SEM of *Hinguleshvara Rasa prepared* by Ball Mill:

Size of the particles of ***Hinguleshvara Rasa prepared*** **by Ball Mill**from image observed at 8000 magnification from SEM was as follows

Sr.No	Particle size in micrometer	Average size in micrometer
1	1.862	3.131
2	2.307	
3	2.433	
4	2.842	
5	3.028	
6	3.549	

7	4.466	
8	4.568	

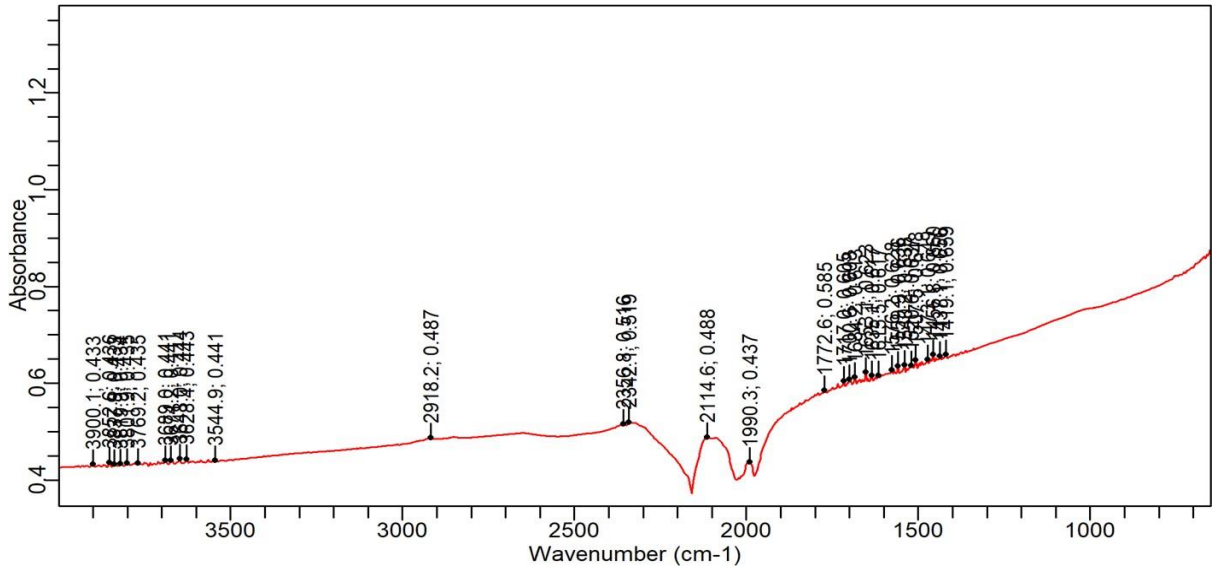
5.34.4 SEM of *Hinguleshvara Rasa* prepared by Trituration:

Size of the particles of ***Hinguleshvara Rasa*** **prepared by Trituration** from image observed at 8000 magnification from SEM was as follows:

Sr. No.	**Particle size in Micrometer**	**Average size in micrometer**
1	2.089	2.488
2	2.262	
3	2.520	
4	2.730	
5	3.276	
6	3.723	
7	**4.092**	

5.35 Reports of Fourier Transform Infrared Spectroscopy (FTIR) (as per methods 4.3.c)of *Ashuddha Hingula* (Impure Red Cinnabar: HgS), *Shuddha Hingula* (Red Cinnabar processed with ginger juice). *Hinguleshvara Rasa* prepared by ball mill and *Hinguleshvara Rasa* prepared by manual grinding(trituration) are as follows:

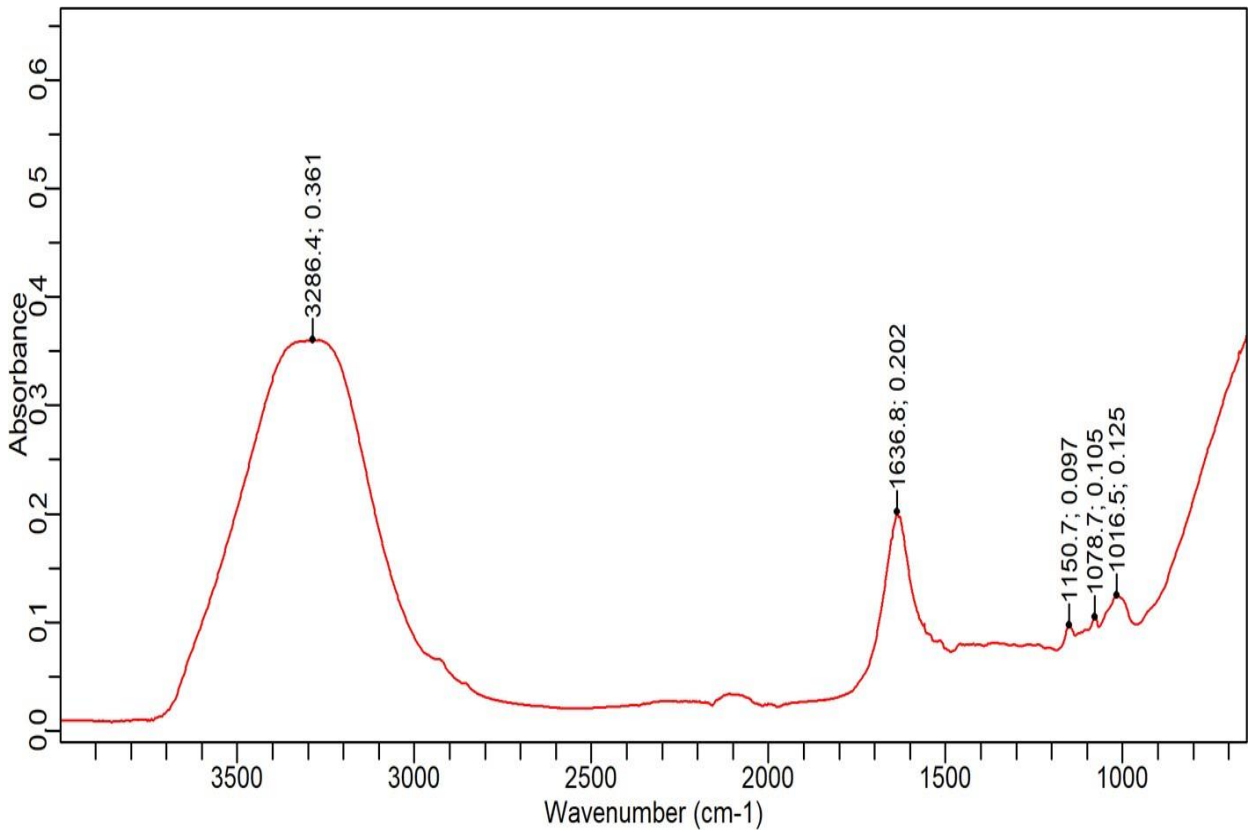
5.35.1 FTIR *Ashuddha Hingula (*Red Cinnabar :HgS)



Peak Number	Wavenumber (cm^{-1})	Intensity
1	1419.11628	0.65908
2	1437.11794	0.65647
3	1456.75611	0.65985
4	1473.12126	0.64945
5	1507.48806	0.64826
6	1520.58017	0.63671
7	1540.21834	0.63827
8	1559.85652	0.63598

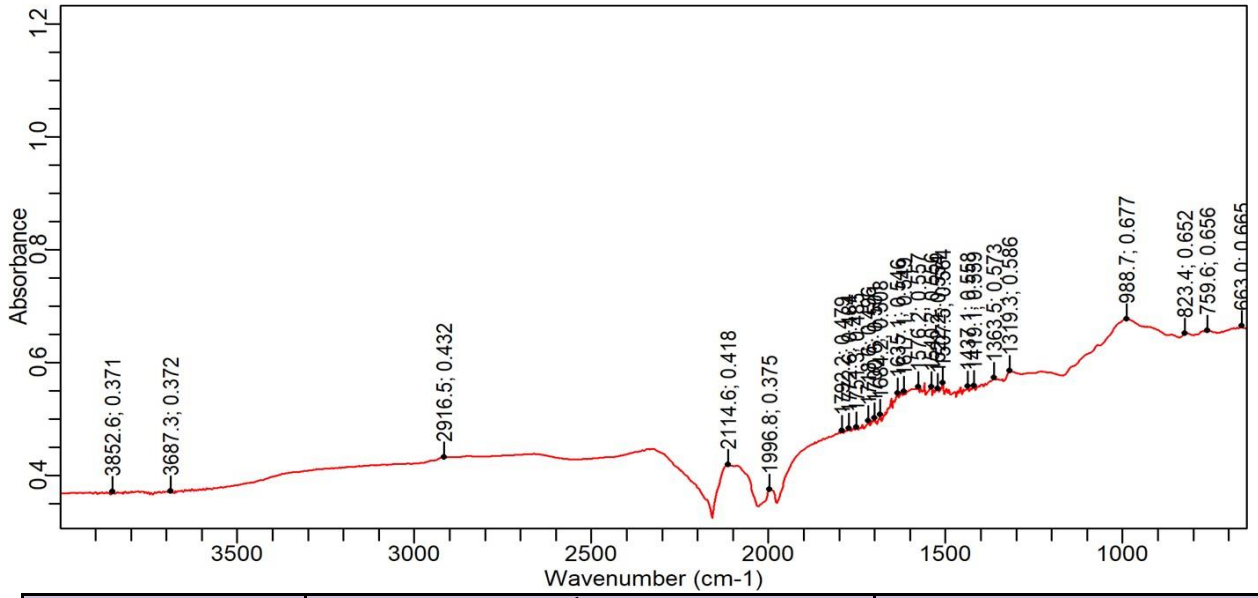
9	1576.22166	0.62801
10	1615.49800	0.61654
11	1635.13618	0.61660
12	1653.13783	0.62258
13	1684.23161	0.61296
14	1700.59675	0.60774
15	1716.96189	0.60497
16	1772.60338	0.58518
17	1990.25979	0.43708
18	2114.63488	0.48842
19	2342.11038	0.51892
20	2356.83901	0.51596
21	2918.16343	0.48701
22	3544.94843	0.44084
23	3628.41066	0.44287
24	3648.04883	0.44417
25	3674.23306	0.44053
26	3688.96169	0.44082
27	3769.15089	0.43504
28	3801.88118	0.43470
29	3819.88284	0.43382
30	3837.88450	0.43324
31	3852.61312	0.43626
32	3900.07204	0.43275

5.35.2 Ginger Juice (Juice of *Zinziber officinale* Rosc)



Peak Number	Wavenumber (cm^{-1})	Intensity
1	1016.53375	0.12491
2	1078.72130	0.10483
3	1150.72793	0.09717
4	1636.77269	0.20205
5	3286.37916	0.36055

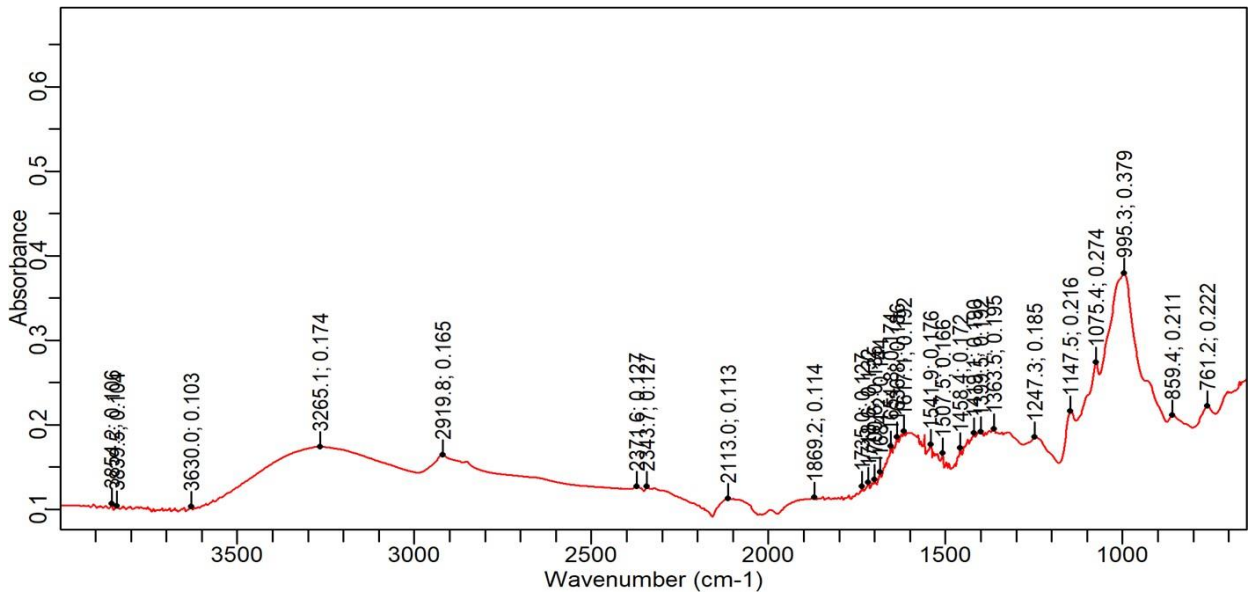
5.35.3 **FTIR *Shuddha Hingula* (Red Cinnabar :HgS)**



Peak Number	Wavenumber (cm^{-1})	Intensity
1	663.04665	0.66451
2	759.60100	0.65650
3	823.42506	0.65200
4	988.71301	0.67719
5	1319.28891	0.58551
6	1363.47479	0.57299
7	1419.11628	0.55905
8	1437.11794	0.55760
9	1507.48806	0.56422
10	1522.21669	0.55409
11	1540.21834	0.55629
12	1576.22166	0.55720
13	1617.13452	0.54878

14	1635.13618	0.54557
15	1684.23161	0.50752
16	1700.59675	0.50138
17	1718.59841	0.49633
18	1751.32870	0.48479
19	1772.60338	0.48357
20	1792.24155	0.47866
21	1996.80585	0.37478
22	2114.63488	0.41842
23	2916.52691	0.43249
24	3687.32518	0.37243
25	3852.61312	0.37134

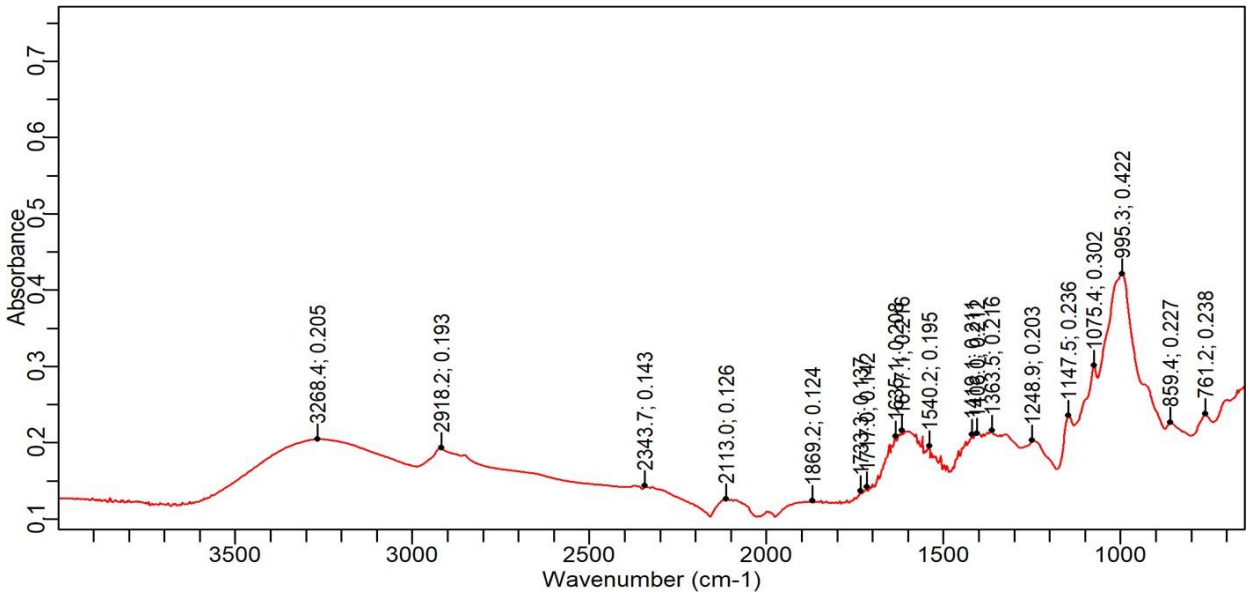
5.35.4 FTIR of *Hinguleshvara Rasa* By Ball mill



Peak Number	Wavenumber (cm^{-1})	Intensity
1	761.23751	0.22206
2	859.42837	0.21103
3	995.25906	0.37910
4	1075.44827	0.27398
5	1147.45490	0.21601
6	1247.28227	0.18530
7	1363.47479	0.19512
8	1399.47811	0.19178
9	1419.11628	0.19050
10	1458.39263	0.17229
11	1507.48806	0.16616
12	1541.85486	0.17633
13	1617.13452	0.19212
14	1636.77269	0.18566
15	1654.77435	0.17444
16	1684.23161	0.14398
17	1700.59675	0.13497
18	1718.59841	0.13161
19	1734.96355	0.12665
20	1869.15773	0.11428
21	2112.99837	0.11291
22	2343.74689	0.12660
23	2371.56764	0.12675

24	2919.79994	0.16451
25	3265.10447	0.17399
26	3630.04717	0.10312
27	3839.52101	0.10362
28	3854.24964	0.10624

5.35.5 FTIR of *Hinguleshvara Rasa* By Trituration

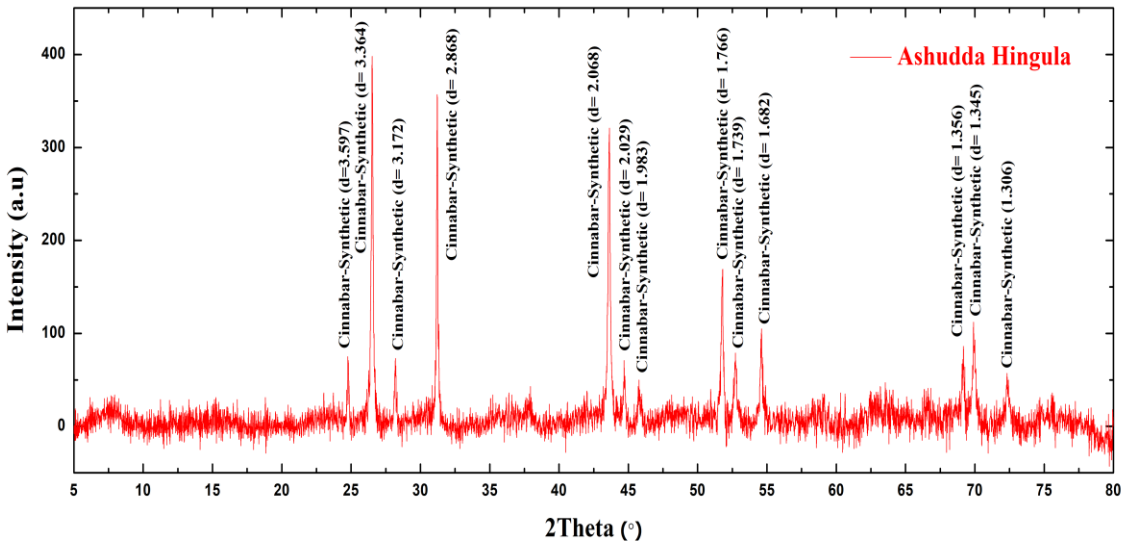


Peak Number	Wavenumber (cm^{-1})	Intensity
1	761.23751	0.23771
2	859.42837	0.22672
3	995.25906	0.42175
4	1075.44827	0.30170
5	1147.45490	0.23551
6	1248.91879	0.20334
7	1363.47479	0.21583

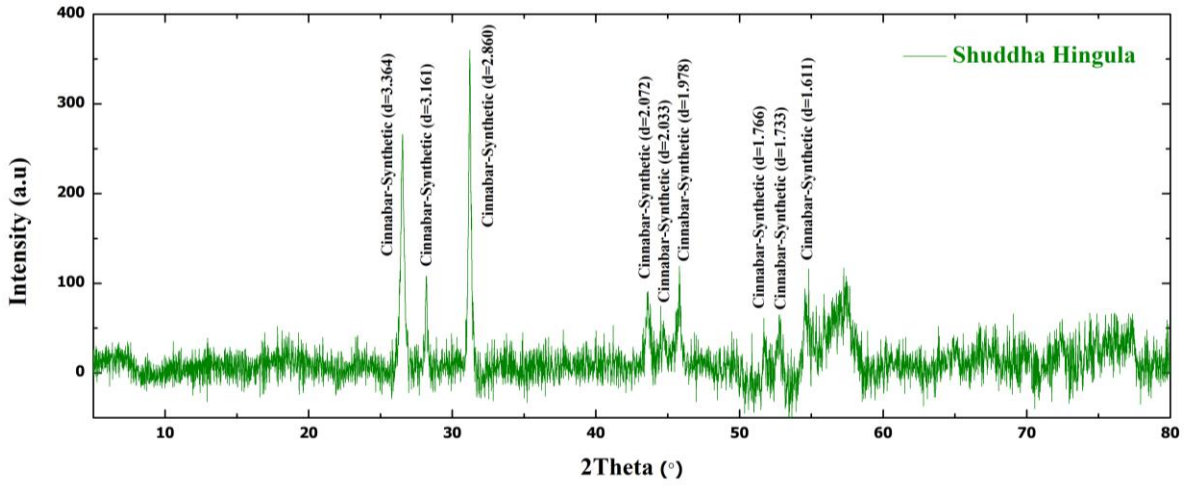
8	1406.02417	0.21222
9	1419.11628	0.21062
10	1540.21834	0.19547
11	1617.13452	0.21622
12	1635.13618	0.20843
13	1716.96189	0.14245
14	1733.32704	0.13714
15	1869.15773	0.12433
16	2112.99837	0.12601
17	2343.74689	0.14346
18	2918.16343	0.19317
19	3268.37750	0.20501

5.36 Results of X-Ray Diffraction (XRD) (as per metods 4.3.d)of *Ashuddha Hingula* (Impure Red Cinnabar: HgS), *Shuddha Hingula* (Red Cinnabar processed with ginger juice). *Hinguleshvara Rasa* prepared by Ball Mill and *Hinguleshvara Rasa* prepared by manual grinding(trituration) are as follows:

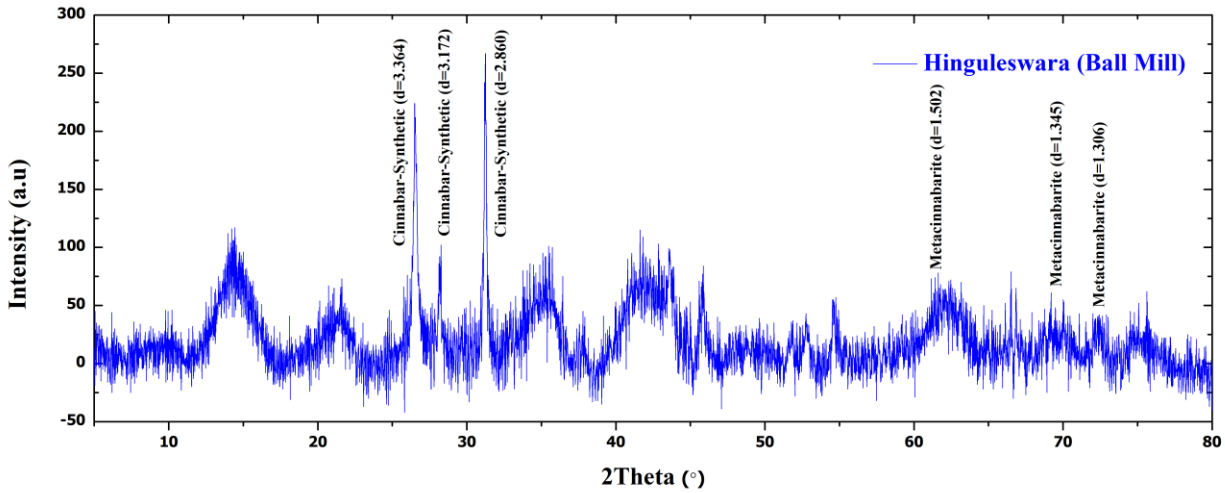
5.36.1 XRD of *Ashuddha Hingula* (Red Cinnabar :HgS)



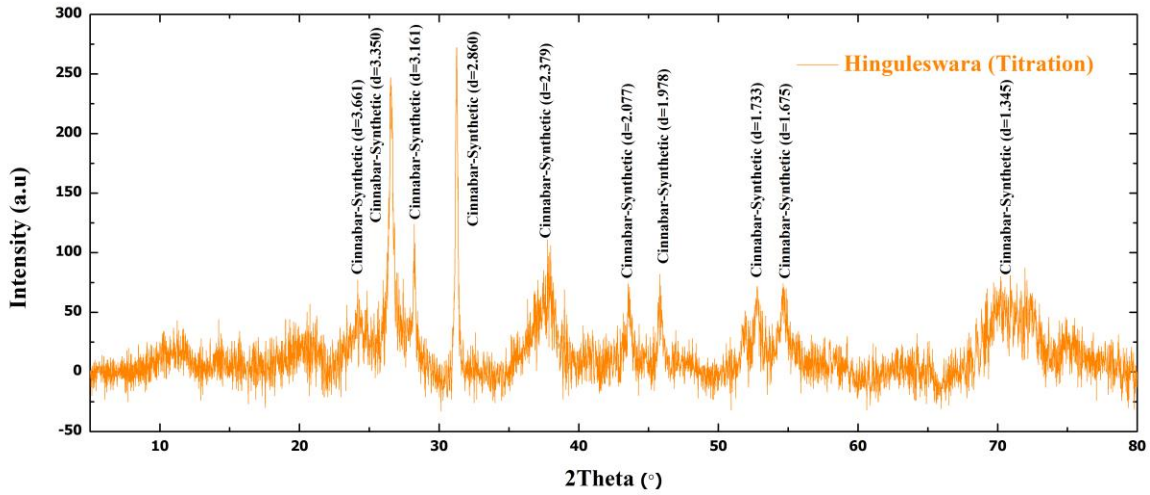
5.36.2 XRD of *Shuddha Hingula (Red* Cinnabar : HgS)



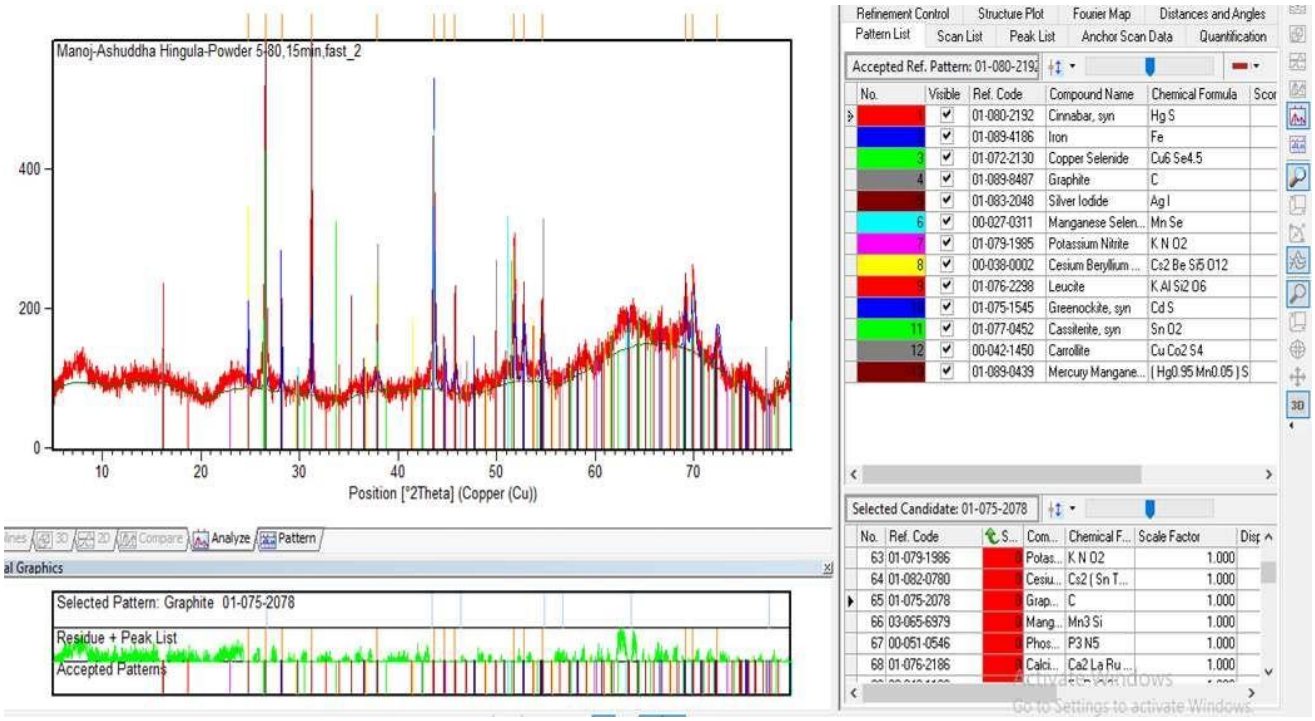
5.36.3 XRD of *Hinguleshvara Rasa* By Ball Mill



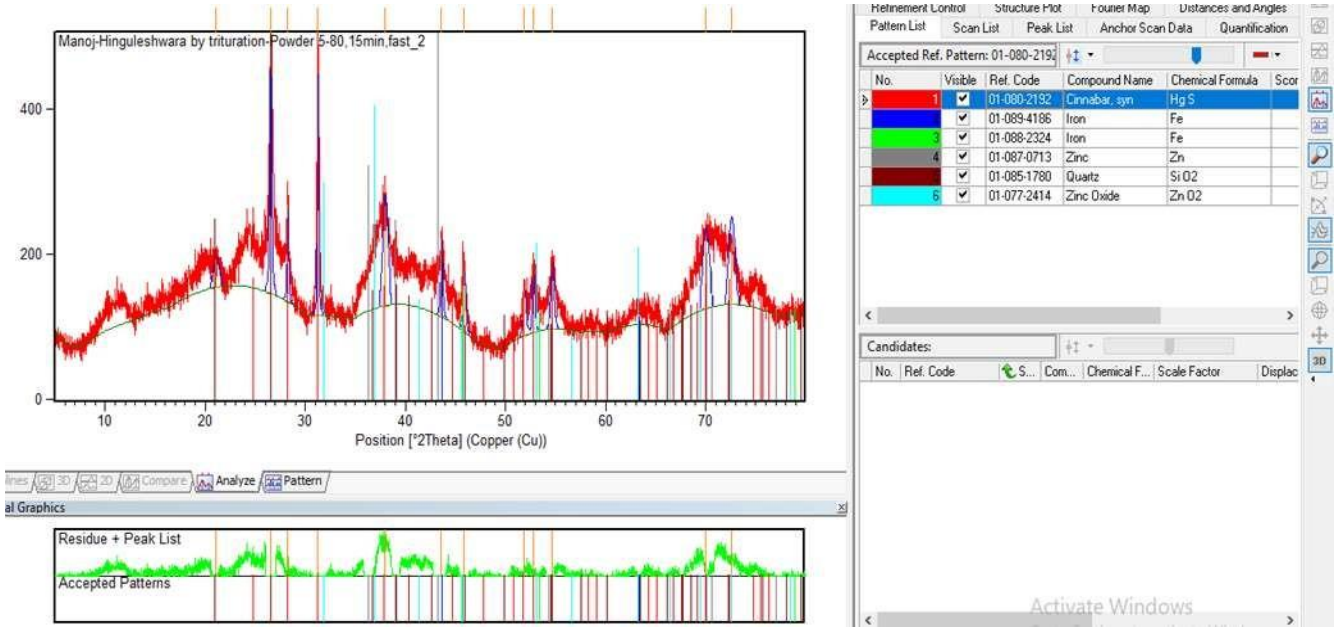
5.36.4 XRD of *Hinguleshvara Rasa* By Trituration



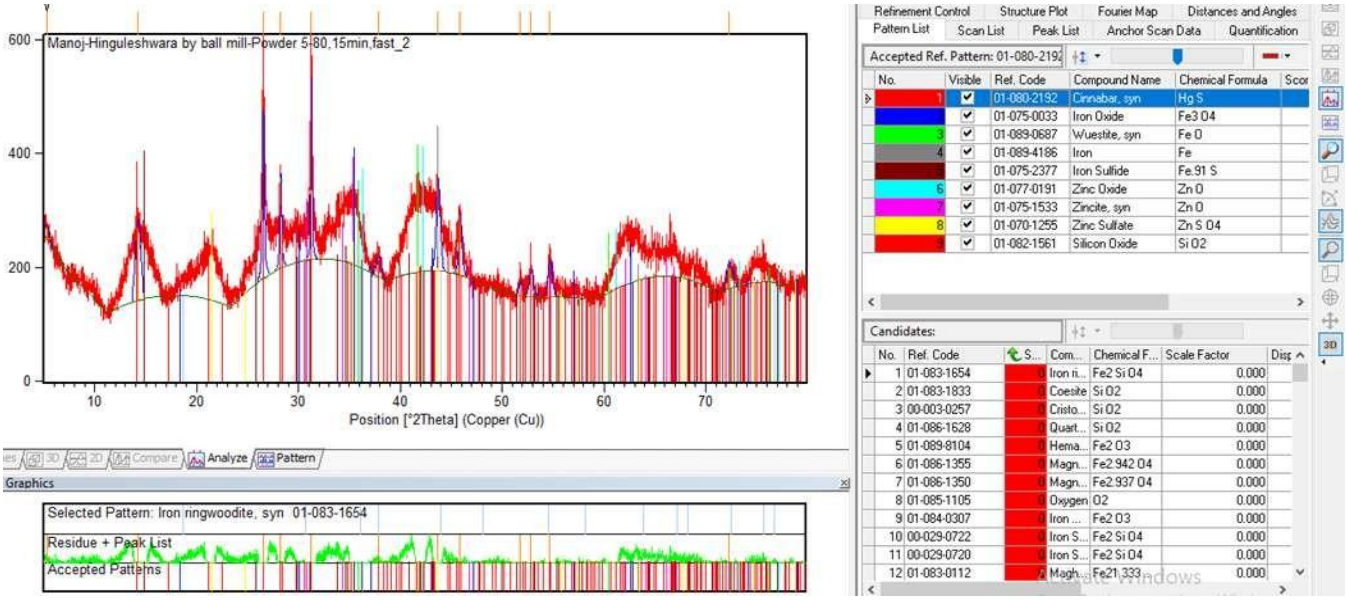
XRD phase of *Ashuddha Hingula*



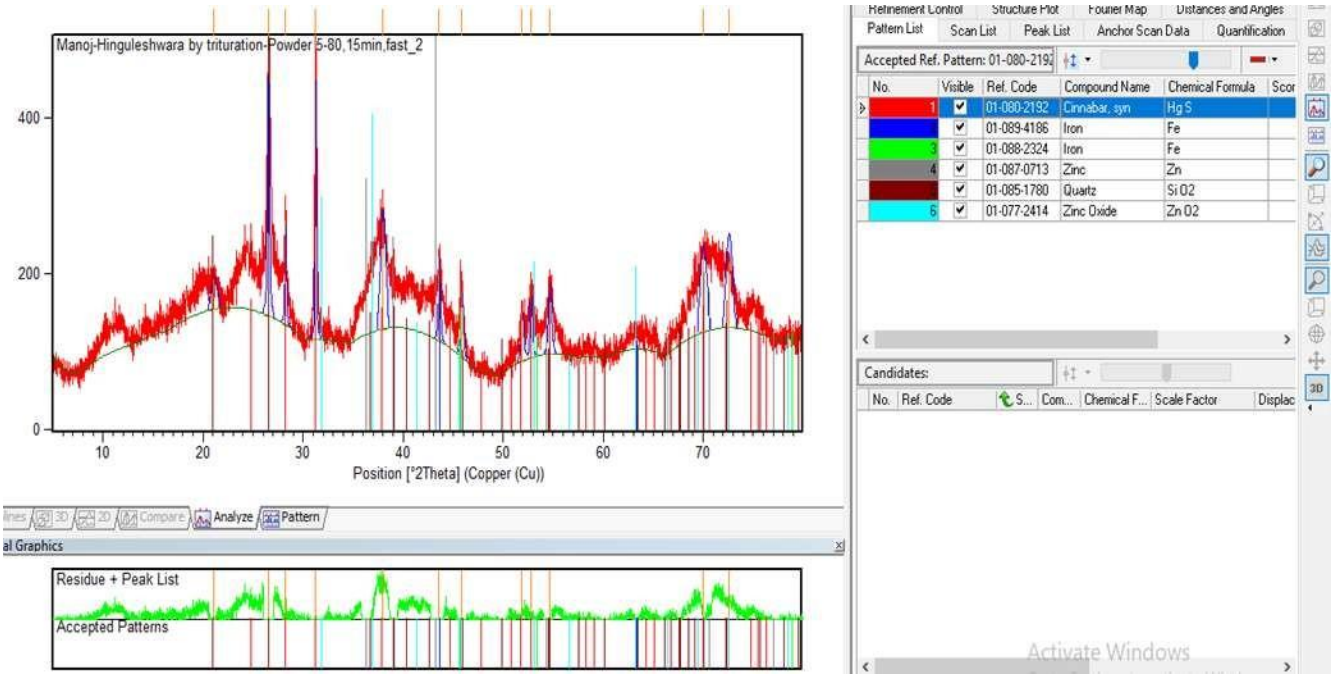
XRD phase of *Shuddha Hingula*

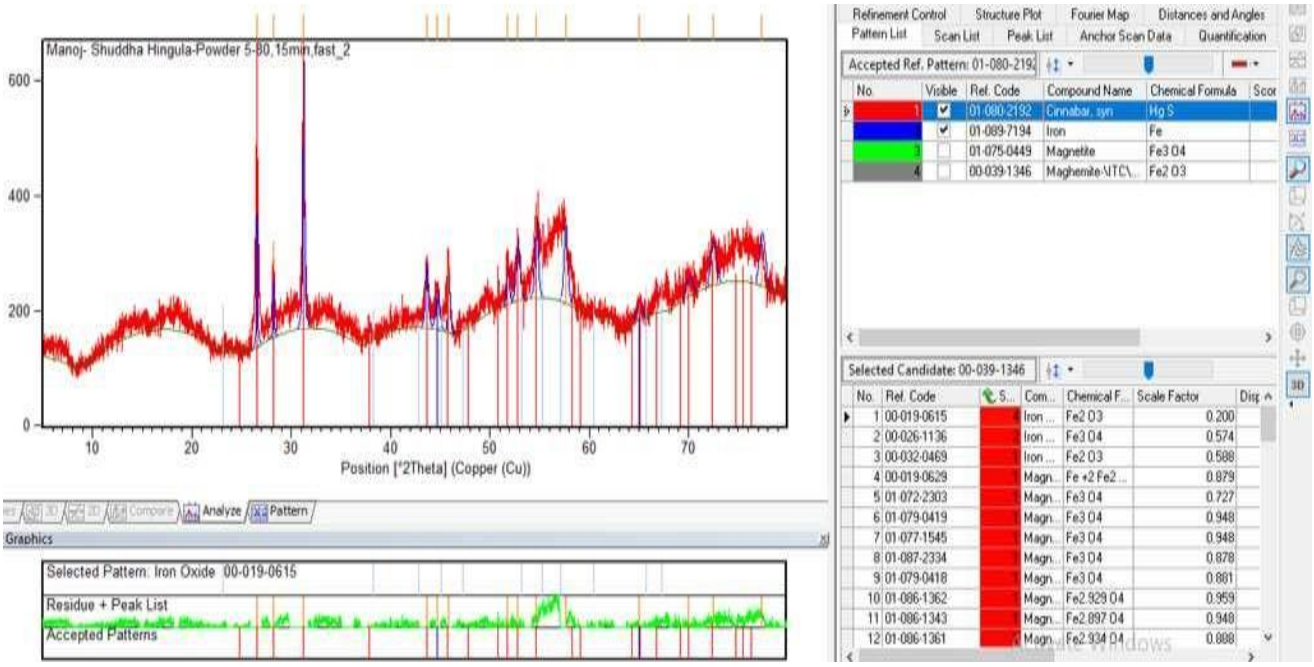


XRD phase of *Hinguleshvara Rasa* by Ball mill



XRD phase of *Hinguleshvara Rasa* By Trituration




Manoj- Shuddha Hingula-Powder 5-80,15min,fast_2
600
400
200
0
10
20
30
40
50
60
70
Position [°2Theta] (Copper (Cu))
Analyze
Pattern
Graphics
Selected Pattern: Iron Oxide 00-019-0615
Residue + Peak List
Accepted Patterns
Refinement Control
Structure Plot
Fourier Map
Distances and Angles
Pattern List
Scan List
Peak List
Anchor Scan Data
Quantification
Accepted Ref. Pattern: 01-080-2192
No.
Visible
Ref. Code
Compound Name
Chemical Formula
01-080-2192 Cinnabar, syn Hg S
01-089-7194 Iron Fe
01-075-0449 Magnetite Fe3 O4
00-039-1346 Maghemite-\ITC\.. Fe2 O3
Selected Candidate: 00-039-1346
No. Ref. Code Com... Chemical F... Scale Factor
1 00-019-0615 Iron ... Fe2 O3 0.200
2 00-026-1136 Iron ... Fe3 O4 0.574
3 00-032-0469 Iron ... Fe2 O3 0.588
4 00-019-0629 Magn... Fe +2 Fe2 ... 0.879
5 01-072-2303 Magn... Fe3 O4 0.727
6 01-079-0419 Magn... Fe3 O4 0.948
7 01-077-1545 Magn... Fe3 O4 0.948
8 01-087-2334 Magn... Fe3 O4 0.878
9 01-079-0418 Magn... Fe3 O4 0.881
10 01-086-1362 Magn... Fe2.929 O4 0.959
11 01-086-1343 Magn... Fe2.897 O4 0.948
12 01-086-1361 Magn... Fe2.934 O4 0.898

Experimental study

As described in chapter materials and methods(4.10,4.11&4.12) This study was comprised of three parts:

- 5.37 Study of Acute oral toxicity of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfouri* Stapf*), & Shuddha Hingula (*Red Cinnabar:HgS) by following OECD 423 guidelines(as per4.10)
- **Part A:** 5.38 Study of antipyretic activity of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*), & Shuddha Hingula (*Red Cinnabar:HgS) *& Pippali (Piper longum* Linn*)*
  (as per 4.11)
- **Part B:** 5.40 Study of Analgesic activity of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*),& Shuddha Hingula (*Red Cinnabar:HgS) *& Pippali (Piper longum* Linn*)*
  (as per 4.12)

5.37 Acute Oral Toxicity Study Reports:

The present study was undertaken to determine the acute oral toxicity of *Hinguleshvara Rasa* and its ingredients namely *Shuddha Vatsanabh (Aconitum balfourii* Stapf) and *Shuddha Hingula* (Red Cinnabar:HgS) in experimental animals as described in chapter materials and methods(4.10)-

TEST MATERIAL

1) **Test material :"*Hinguleshvara Rasa"***
   Physical State : Solid
   Colour : Orange
2) **Test material : "*Shuddha Hingula* (Red Cinnabar:HgS)"**
   Physical State : Solid
   Colour : Orange
3) **Test material : "*Shuddha Vatsanabh (Aconitum balfourii* Stapf)"**
   Physical State : Solid
   Colour : Yellow

Body weight data (gm)

As described in materials and methods4.10d reports are as follows:

5.37.1.Step 1 (300 mg/kg)

Groups	Animal No.	Marking	Sex	0 Day	7 Day	14 Day
Test-1 (*Hinguleshvara Rasa*) 300mg/kg	**1**	**1**	**F**	23.0	25.5	27.5
	2	**2**	**F**	25.0	28.0	31.5
	3	**3**	**F**	27.0	30.0	34.0
	MEAN			**25.0**	**27.8**	**31.0**
	SD			**2.0**	**2.3**	**3.3**
Test-2 *(Shuddha Vatsanabh*) (*Aconitum balfourii* Stapf) 300mg/kg	**4**	**1**	**F**	27.0	28.5	30.5
	5	**2**	**F**	20.0	22.5	26.0
	6	**3**	**F**	20.0	22.0	25.0
	MEAN			**22.3**	**24.3**	**27.2**
	SD			**4.0**	**3.6**	**2.9**
Test-3 (*Shuddha Hingula*) Red Cinnabar :HgS) 300mg/kg	**7**	**1**	**F**	20.0	23.0	24.5
	8	**2**	**F**	27.0	29.0	30.0
	9	**3**	**F**	24.0	26.5	29.0
	MEAN			**23.7**	**26.2**	**27.8**
	SD			**3.5**	**3.0**	**2.9**

5.37.2.Step 2 (300 mg/kg)

Groups	Animal No.	Marking	Sex	0 Day	7 Day	14 Day
Test-1 (*Hinguleshvara Rasa*) 300mg/kg	**1**	**1**	**F**	24.0	29.0	34.0
	2	**2**	**F**	22.0	26.0	30.0
	3	**3**	**F**	28.0	27.0	29.0
	MEAN			**24.7**	**27.3**	**31.0**
	SD			**3.1**	**1.5**	**2.6**
Test-2 *(Shuddha Vatsanabh)* (*Aconitum balfourii* Stapf) 300mg/kg	**4**	**1**	**F**	29.5	32.0	35.0
	5	**2**	**F**	29.0	32.0	34.0
	6	**3**	**F**	30.5	31.5	38.0
	MEAN			**29.7**	**31.8**	**35.7**
	SD			**0.8**	**0.3**	**2.1**
Test-3 *(Shuddha Hingula)* Red Cinnabar :HgS) 300mg/kg	**7**	**1**	**F**	29.0	30.0	31.5
	8	**2**	**F**	28.0	28.5	32.0
	9	**3**	**F**	26.0	32.0	31.0
	MEAN			**27.7**	**30.2**	**31.5**
	SD			**1.5**	**1.8**	**0.5**

5.37.3.Step 3 (2000 mg/kg)

Groups	Animal No.	Marking	Sex	0 Day	7 Day	14 Day
Test-1 (*Hinguleshvara Rasa)* 2000mg/kg	**1**	**1**	**F**	21.0	27.5	28.0
	2	**2**	**F**	21.0	30.0	32.5
	3	**3**	**F**	18.0	25.0	29.5
	MEAN			**20.0**	**27.5**	**30.0**
	SD			**1.7**	**2.5**	**2.3**
Test-2 *(Shuddha Vatsanabh*) (Aconitum balfourii Stapf) 2000mg/kg	**4**	**1**	**F**	18.0	Dead	
	5	**2**	**F**	25.0		
	6	**3**	**F**	23.0		
	MEAN			**22.0**	**0.0**	**0.0**
	SD			**3.6**	**0.0**	**0.0**
Test-3 *(Shuddha Hingula)* Red Cinnabar :HgS) 2000mg/kg	**7**	**1**	**F**	17.0	20.0	24.5
	8	**2**	**F**	27.0	30.5	31.5
	9	**3**	**F**	22.0	29.0	33.5
	MEAN			**22.0**	**26.5**	**29.8**
	SD			**5.0**	**5.7**	**4.7**

***F- Female**

5.37.4.Step 4 (2000 mg/kg)

Group	Animal No.	Marking	Sex	0 Day	7 Day	14 Day
Test-1 (*Hinguleshvara Rasa*) 2000mg/kg	**1**	**1**	**F**	24.0	28.0	33.5
	2	**2**	**F**	22.5	27.0	32.5
	3	**3**	**F**	21.0	26.0	28.5
	MEAN			**22.5**	**27.0**	**31.5**
	SD			**1.5**	**1.0**	**2.6**
Test-3 *(Shuddha Hingula*) Red Cinnabar :HgS) 2000mg/kg	**7**	**1**	**F**	22.0	25.0	30.0
	8	**2**	**F**	21.5	30.0	32.0
	9	**3**	**F**	23.0	28.5	33.0
	MEAN			**22.2**	**27.8**	**31.7**
	SD			**0.8**	**2.6**	**1.5**

5.37.5As described in materials and methods4.10d reports are as follows:

Groups	Step	Dose (mg/kg)	No. of Treated Rats	Terminal Sacrificed	Found Dead(X)
Hinguleshvara Rasa	**1**	300	03	03	0
***Shuddha Vatsanabh* (*Aconitum balfourii* Stapf)**			03	03	0
Shuddha Hingula			03	03	0

Red Cinnabar :HgS)					
Hinguleshvara Rasa	**2**	300	03	03	0
***Shuddha Vatsanabh* (*Aconitum balfourii* Stapf)**			03	03	0
***Shuddha Hingula* Red Cinnabar :HgS)**			03	03	0
Hinguleshvara Rasa	**3**	2000	03	03	0
***Shuddha Vatsanabh* (*Aconitum balfourii* Stapf)**			03	00	03
***Shuddha Hingula* Red Cinnabar :HgS)**			03	03	0
Hinguleshvara Rasa	**4**	2000	03	03	0
***Shuddha Hingula* Red Cinnabar :HgS)**			03	03	0
	TOTAL	-	**33**	**30**	**03**

Clinical signs for *Hinguleshvara Rasa* and its ingredients observed in acute toxicity study:

Step I and II – 30.00 mg/ml: The test *materials* ***"Hinguleshvara Rasa, Shuddha Hingula and Shuddha Vatsanabh"*** *c*aused **NO mortality**. All the animals appeared normal and showed no clinical signs of intoxication after dosing till the end of the study and also in repeated dose.

Step III – 200.00 mg/ml: The test materials **"*Hinguleshvara Rasa and Shuddha Hingula*"** caused **NO mortality**. All the animals in both the groups appeared normal and showed no clinical signs of intoxication after dosing till the end of the study and also in repeated dose.

The test material ***"Shuddha Vatsanabh"*** caused **mortality**. Three animals were found dead in 72 hrs.

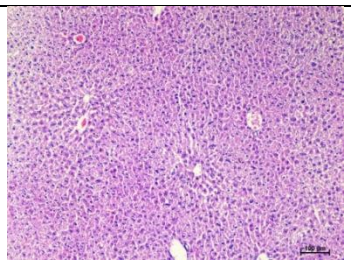
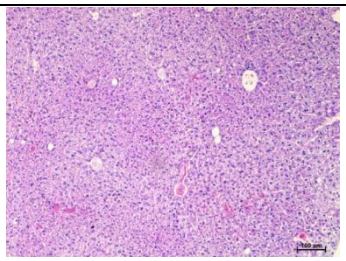
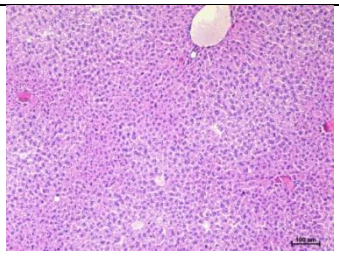
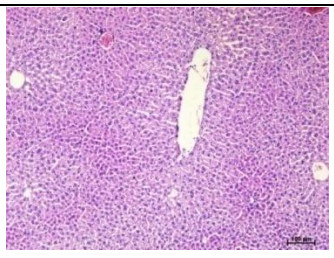
Necropsy: No gross pathological alterations were encountered in any of the female mice of all three groups dosed at 300 mg/kg dose, as evident during their necropsy.

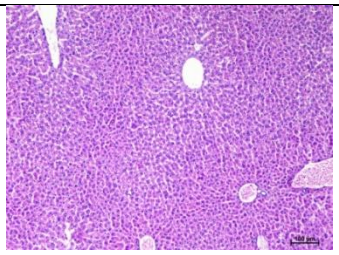
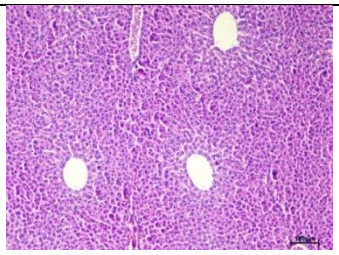
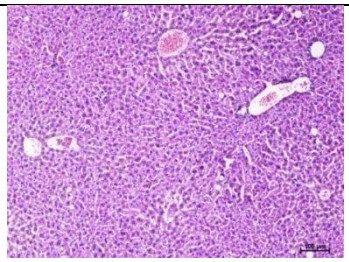
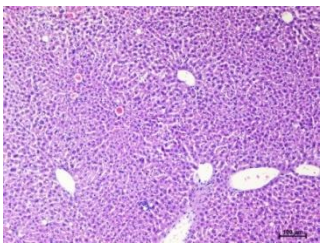
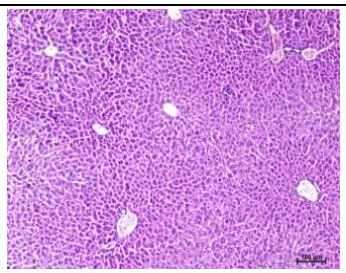
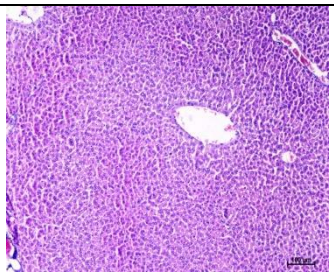
No gross pathological alterations were encountered in any of the female mice of group ***Hinguleshvara Rasa, Shuddha Hingula*** *dosed* at 2000 mg/kg dose, as evident during their necropsy.

In ***Shuddha Vatsanabh*** group, where the animals were dosed at 2000mg/ Kg dose, the animals may be died at night. Hence it was difficult to analyze the gross pathological alterations during their necropsy due to autolysis of the organs. Therefore the histopathological analysis was not possible. Histopathology of four organs performed namely liver,kidney,heart and gastric mucosa.

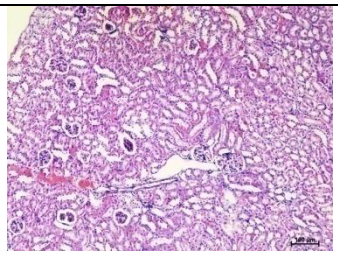
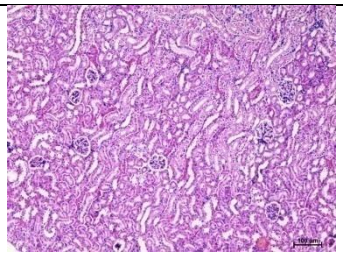
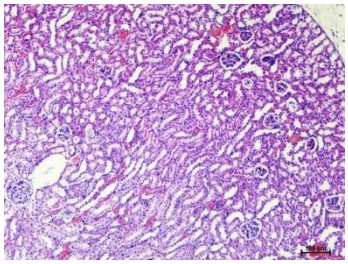
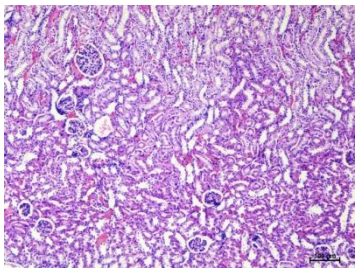
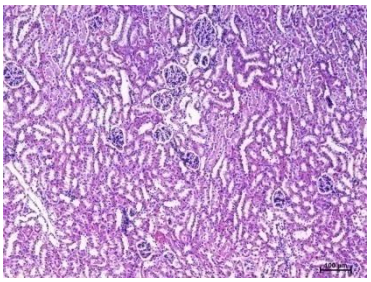
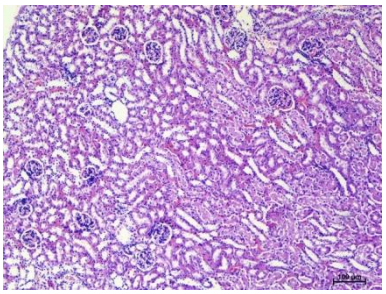
5.37.6.Photos of Histopathology Results after acute oral toxicity study

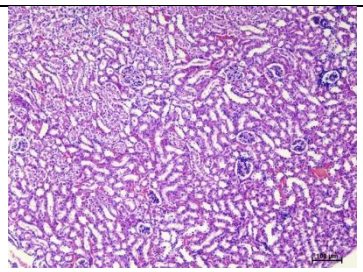
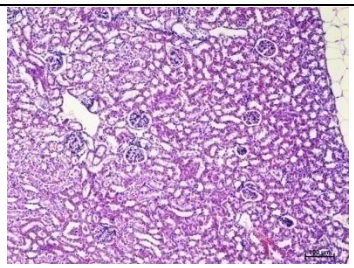
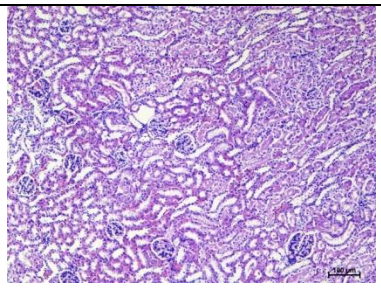
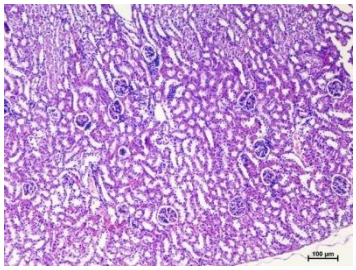
5.37.6.a photos of Histopathology Results Liver after acute oral toxicity study

	
***Hinguleshvara Rasa* (300 mg/Kg)**	***Hinguleshvara Rasa* (300 mg/Kg)**
	

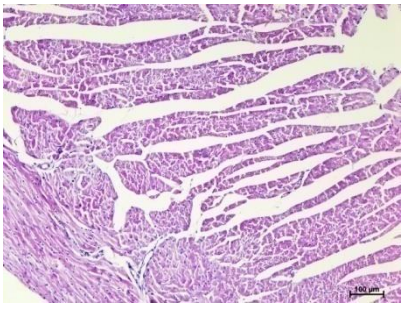
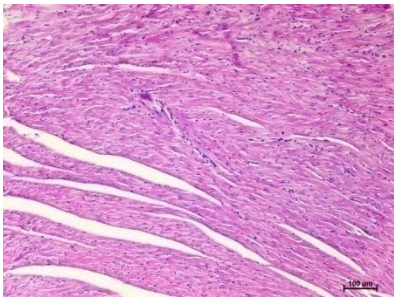
***Hinguleshvara Rasa* (2000 mg/Kg)**	***Hinguleshvara Rasa* (2000 mg/Kg)**
	
***Shuddha Hingula* (300 mg/Kg)**	***Shuddha Hingula* (300 mg/Kg)**
	
***Shuddha Hingula(*2000 mg/Kg)**	***Shuddha Hingul*a (2000 mg/Kg)**
	
Shuddha Vatsanabh*(Aconitum balfourii Stapf) *(300 mg/Kg)	***Shuddha Vatsanabh* (Aconitum balfourii Stapf) *(300 mg/Kg)***

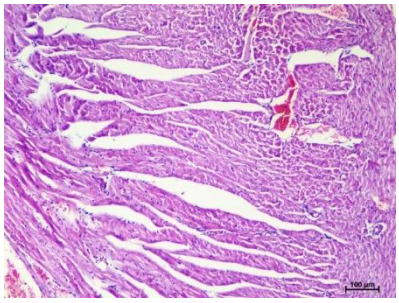
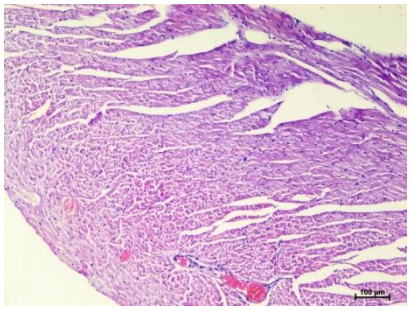
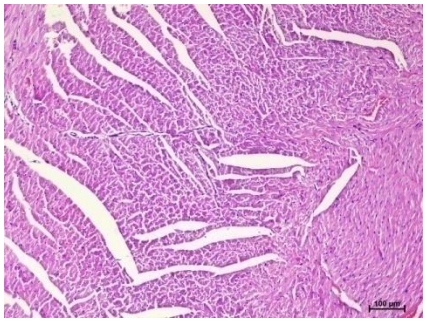
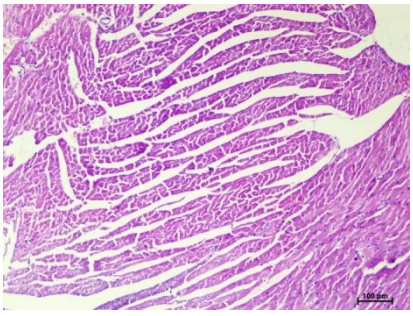
5.37.6.b.photos of Histopathology Results Kidney after acute oral toxicity study

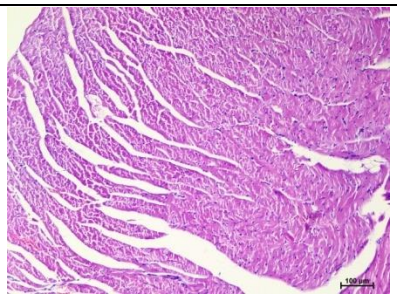
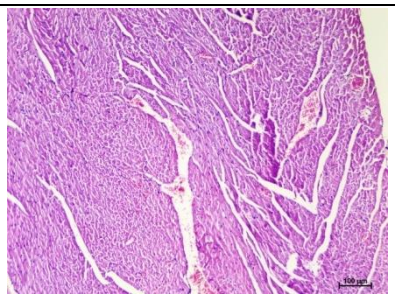
	
***Hinguleshvara Rasa* (300 mg/Kg)**	***Hinguleshvara Rasa* (300 mg/Kg)**
	
***Hinguleshvara Rasa* (2000 mg/Kg)**	***Hinguleshvara Rasa* (2000 mg/Kg)**
	
Shuddha Hingula(*Red Cinnabar:HgS) *(300 mg/Kg)	***Shuddha Hingula(*Red Cinnabar:HgS) *(300 mg/Kg)***

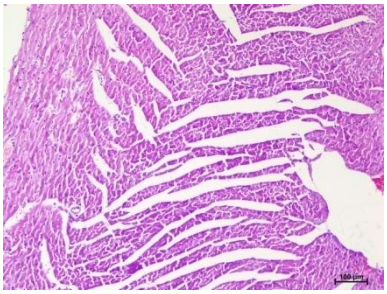
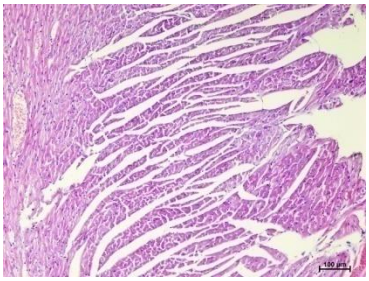
	
***Shuddha Hingula(*Red Cinnabar:HgS) (2000 mg/Kg)**	***Shuddha Hingula(*Red Cinnabar:HgS) (2000 mg/Kg)**
	
***Shuddha Vatsanabh*(Aconitum balfourii Stapf) (300 mg/Kg)**	***Shuddha Vatsanabh* (Aconitum balfourii Stapf) (300 mg/Kg)**

5.37.6.c.photos of Histopathology Results of **Heart after acute oral toxicity study**

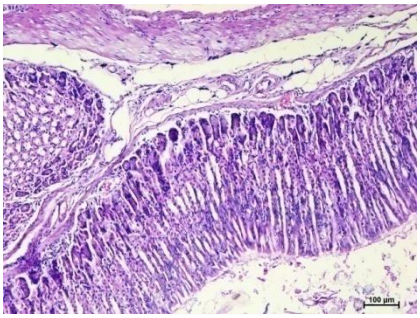
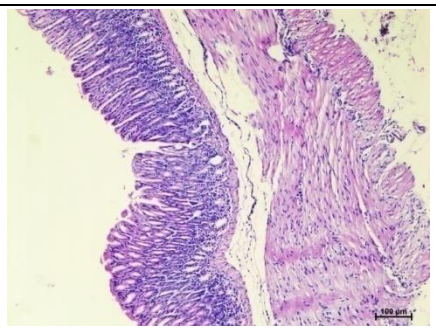
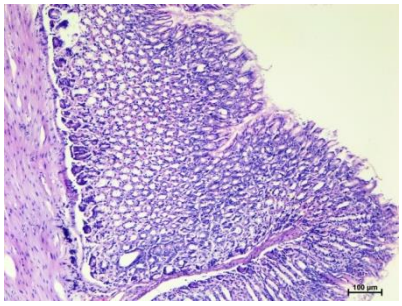
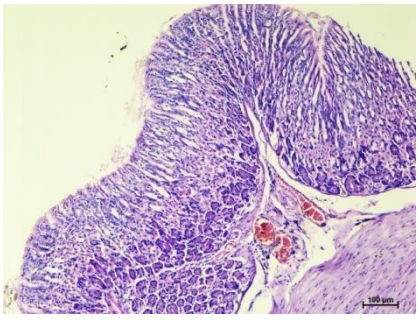
	
***Hinguleshvara Rasa* (300 mg/Kg)**	***Hinguleshvara Rasa* (300 mg/Kg)**

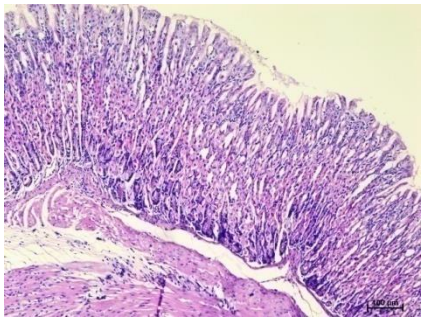
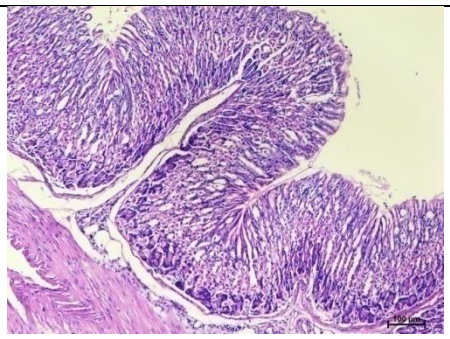
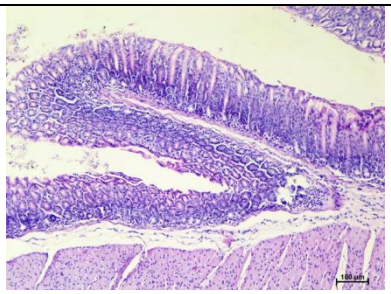
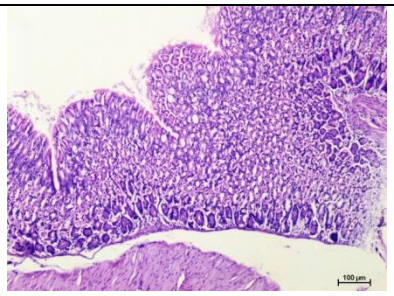
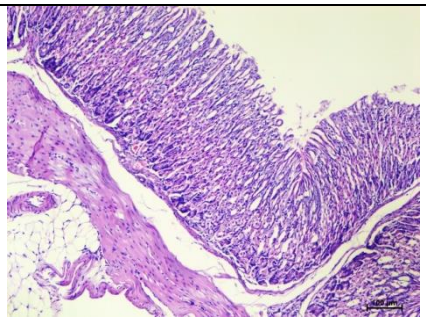
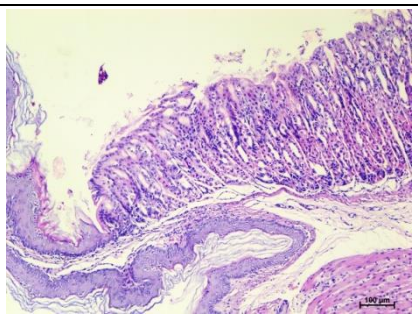
	
***Hinguleshvara Rasa* (2000 mg/Kg)**	***Hinguleshvara Rasa* (2000 mg/Kg)**
	
***Shuddha Hingula(*Red Cinnabar:HgS) (300 mg/Kg)**	***Shuddha Hingula(*Red Cinnabar:HgS) (300 mg/Kg)**

	
***Shuddha Hingula(*Red Cinnabar:HgS) (2000 mg/Kg)**	***Shuddha Hingula(*Red Cinnabar:HgS) (2000 mg/Kg)**

	
***Shuddha Vatsanabh*(Aconitum balfourii Stapf) (300 mg/Kg)**	***Shuddha Vatsanabh*(Aconitum balfourii Stapf) (300 mg/Kg)**

5.37.6.d.photos of Histopathology Results of **Gastric Muosa after acute oral toxicity study**

	
***Hinguleshvara Rasa* (300 mg/Kg)**	***Hinguleshvara Rasa* (300 mg/Kg)**
	
***Hinguleshvara Rasa* (2000 mg/Kg)**	***Hinguleshvara Rasa* (2000 mg/Kg)**

	
***Shuddha Hingula(*Red Cinnabar:HgS) (300 mg/Kg)**	***Shuddha Hingula(*Red Cinnabar:HgS) (300 mg/Kg)**
	
***Shuddha Hingula(*Red Cinnabar:HgS) (2000 mg/Kg)**	***Shuddha Hingula(*Red Cinnabar:HgS) (2000 mg/Kg)**
	
***Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) (300 mg/Kg)**	***Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) (300 mg/Kg)**

5.37.7.Histopathological observations after acute oral toxicity study

5.37.7.a.Histopathological observations of Liver after acute oral toxicity study

Sr. N.	Group /Slide Code	Histopathological observations 1.Liver	Overall Pathological Grade
1	***Hinguleshvara Rasa (300 mg/Kg)-1***	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
2	***Hinguleshvara Rasa (300 mg/Kg) -2***	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
3	***Hinguleshvara Rasa (2000 mg/Kg)-1***	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
4	***Hinguleshvara Rasa (2000 mg/Kg)-2***	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
5	***Shuddha Hingula(*Red Cinnabar:HgS)**	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue.	**NAD**

	(300 mg/Kg)-1	Absence of inflammatory or pathological changes in hepatic parenchyma.	
6	***Shuddha Hingula(*Red Cinnabar:HgS)** ***(300 mg/Kg)-2***	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
7	***Shuddha Hingula(*Red Cinnabar:HgS)** ***(2000 mg/Kg)-1***	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
8	***Shuddha Hingula(*Red Cinnabar:HgS)** ***(2000 mg/Kg)-2***	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
9	***Shuddha Vatsanabh* (*Aconitum balfourii* Stapf)** ***(300 mg/Kg)-1***	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
10	***Shuddha Vatsanabh*(*Aconitum balfourii* Stapf)** ***(300 mg/Kg)-2***	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
Note : Overall Grade score as- **NAD** =No Abnormality Detected, **Minimal changes** (+1), **Mild changes** (+2), **Moderate changes** (+3), **Severe changes** (+4).			

5.37.7.b.Histopathological observations of kidneys **after acute oral toxicity study**

Histopathology Report of Toxicity Study			
Sr. N.	**Group / Slide Code**	**Histopathological observations** **2. Kidney**	**Overall Pathological Grade**
1	***Hinguleshvara Rasa (300 mg/Kg)-1***	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
2	***Hinguleshvara Rasa (300 mg/Kg) -2***	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
3	***Hinguleshvara Rasa (2000 mg/Kg)-1***	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
4	***Hinguleshvara Rasa (2000 mg/Kg)-2***	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
5	***Shuddha Hingula(*Red Cinnabar:HgS)** ***(300 mg/Kg)-1***	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
6	***Shuddha Hingula(*Red Cinnabar:HgS)**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory	**NAD**

	(300 mg/Kg)-2	or pathological changes in renal parenchyma.	
7	***Shuddha Hingula(*Red Cinnabar:HgS)** ***(2000 mg/Kg)-1***	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
8	***Shuddha Hingula(*Red Cinnabar:HgS)** ***(2000 mg/Kg)-2***	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
9	***Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) *(300 mg/Kg)-1***	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
10	***Shuddha Vatsanabh* (Aconitum balfourii Stapf) *(300 mg/Kg)-2***	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
Note : Overall Grade score as- **NAD** =No Abnormality Detected, **Minimal changes** (+1), **Mild changes** (+2), **Moderate changes** (+3), **Severe changes** (+4).			

5.37.7.c.Histopathological observations of heart after acute oral toxicity study

Sr. N.	Group / Slide Code	Histopathological observations 3. Heart	Overall Pathological Grade
1	***Hinguleshvara***	Normal histomorphological features of cardiac	**NAD**

	Rasa (300 mg/Kg)-1	muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	
2	***Hinguleshvara Rasa (300 mg/Kg) -2***	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
3	***Hinguleshvara Rasa (2000 mg/Kg)-1***	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
4	***Hinguleshvara Rasa (2000 mg/Kg)-2***	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
5	***Shuddha Hingula(*Red Cinnabar:HgS) *(300 mg/Kg)-1***	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
6	***Shuddha Hingula(*Red Cinnabar:HgS) *(300 mg/Kg)-2***	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
7	***Shuddha Hingula(*Red Cinnabar:HgS) *(2000 mg/Kg)-1***	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
8	***Shuddha Hingula(*Red Cinnabar:HgS) *(2000 mg/Kg)-2***	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**

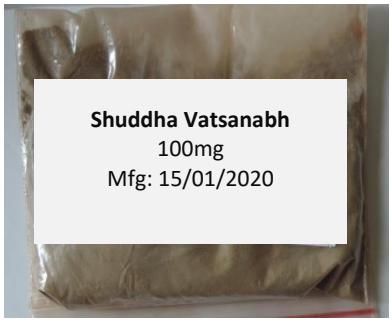
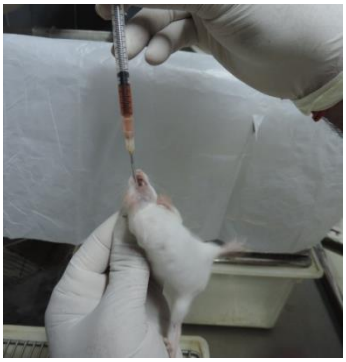
9	***Shuddha Vatsanabh ((Aconitum balfourii* Stapf) *300 mg/Kg)-1***	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
10	***Shuddha Vatsanabh (Aconitum balfourii* Stapf) *(300 mg/Kg)-2***	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
Note : Overall Grade score as- **NAD** =No Abnormality Detected, **Minimal changes** (+1), **Mild changes** (+2), **Moderate changes** (+3), **Severe changes** (+4).			

5.37.7.d.Histopathological observations of Gastric mucosa after acute oral toxicity study

Sr. N.	Group / Slide Code	Histopathological observations 4. Gastric mucosa	Overall Pathological Grade
1	***Hinguleshvara Rasa (300 mg/Kg)-1***	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
2	***Hinguleshvara Rasa (300 mg/Kg) -2***	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
3	***Hinguleshvara Rasa (2000 mg/Kg)-1***	Normal histological features of mucosal layers of glandular stomach with normal submucosal	**NAD**

		tissue.	
4	***Hinguleshvara Rasa (2000 mg/Kg)-2***	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
5	***Shuddha Hingula* (Red Cinnabar:HgS) *(300 mg/Kg)-1***	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
6	***Shuddha Hingula* (Red Cinnabar:HgS) *(300 mg/Kg)-2***	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
7	***Shuddha Hingula* (Red Cinnabar:HgS) *(2000 mg/Kg)-1***	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
8	***Shuddha Hingula* (Red Cinnabar:HgS) *(2000 mg/Kg)-2***	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
9	***Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) *(300 mg/Kg)-1***	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
10	***Shuddha Vatsanabh*(*Aconitum balfourii* Stapf) *(300 mg/Kg)-2***	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
Note : Overall Grade score as- **NAD** =No Abnormality Detected, **Minimal changes** (+1), **Mild changes** (+2), **Moderate changes** (+3), **Severe changes** (+4).			

Photos of 5.37) Acute Oral Toxicity Study of *Hinguleshvara Rasa, Shuddha Hingula (Red Cinnabar :HgS) & Shuddha Vatsanabh (Aconitum balfourii* Stapf)

		
Test Material- "*Hinguleshvara Rasa"*	**Test Material- *"Shuddha Vatsanabh(Aconitum balfourii* Stapf)**	**Test Material- "*Shuddha Hingula(*Red Cinnabar:HgS)**
		
Oral Dosing	**Gross Necropsy Step 1 *Hinguleshvara Rasa* 300 mg/Kg**	**Gross Necropsy Step 2 "*Shuddha Hingula(*Red Cinnabar:HgS 300 mg/Kg**
		
Gross Necropsy Step 1 *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) 300 mg/Kg	**Gross Necropsy Step 3 *Hinguleshvara Rasa* 2000 mg/Kg**	**Gross Necropsy Step 3 "*Shuddha Hingula (*Red Cinnabar:HgS 2000 mg/Kg**

5.38 RESULTS of ANTI-PYRETIC ACTIVITY

Part A: EXPERIMENT FOR ANTI-PYRETIC ACTIVITY was conducted as described in materials and methods 4.11 reports are as follows:-

5.38.1. Effect of test drug on body weight (g)

Group	Animal No.	Marking	Sex	0 Day	4th Day
DC-Brewer's Yeast	**1**	**1**	**M**	263.0	275.0
	2	**2**	**M**	287.5	311.5
	3	**3**	**M**	324.0	325.0
	4	**1**	**F**	183.5	188.0
	5	**2**	**F**	199.0	211.0
	6	**3**	**F**	185.0	182.0
	MEAN			240.3	248.8
	SD			59.6	63.3
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	**7**	**1**	**M**	204.0	216.0
	8	**2**	**M**	209.0	224.0
	9	**3**	**M**	209.0	218.0
	10	**1**	**F**	218.0	214.0
	11	**2**	**F**	201.0	190.0
	12	**3**	**F**	189.0	184.0
	MEAN			205.0	207.7
	SD			9.7	16.5
Brewer's Yeast +*Hinguleshvara Rasa (5*.63 mg/kg) T1	**13**	**1**	**M**	174.0	181.0
	14	**2**	**M**	234.0	241.0
	15	**3**	**M**	208.0	209.0
	16	**1**	**F**	216.0	225.0
	17	**2**	**F**	201.0	211.0
	18	**3**	**F**	178.0	187.0
	MEAN			201.8	209.0
	SD			22.9	22.6
Brewer's Yeast +Shuddha *Hingula* (Red Cinnabar:HgS) **(11.25 mg/Kg) T2**	**19**	**1**	**M**	189.0	210.0
	20	**2**	**M**	249.0	246.0
	21	**3**	**M**	180.0	189.0
	22	**1**	**F**	177.0	183.0
	23	**2**	**F**	178.0	197.0
	24	**3**	**F**	179.0	183.0
	MEAN			192.0	201.3
	SD			28.3	24.1
Brewer's Yeast +*Shuddha Vatsanabh* (*Aconitum balfourii* Stapf)	**25**	**1**	**M**	213.0	235.0
	26	**2**	**M**	174.0	191.0
	27	**3**	**M**	237.5	245.0
	28	**1**	**F**	174.0	181.0
	29	**2**	**F**	210.0	216.0

(1.40 mg/Kg) T3	**30**	**3**	**F**	198.0	203.0
	MEAN			201.1	211.8
	SD			24.6	25.0
Brewers Yeast+*Pippali* (*Piper longum* Linn) (67.5 mg/Kg) T4	**31**	**1**	**M**	191.0	189.0
	32	**2**	**M**	253.0	270.0
	33	**3**	**M**	242.0	229.0
	34	**1**	**F**	202.0	200.0
	35	**2**	**F**	237.0	234.0
	36	**3**	**F**	143.5	143.5
	MEAN			211.4	210.9
	SD			41.1	43.6

***M: Male; F: Female**

Mean of body weight

Groups	0 Day	Last Day
DC-Brewer's Yeast	240.3	248.8
STD- Brewer's Yeast+ Pracetamol	205.0	207.7
Brewer's Yeast +Hinguleshvara Rasa	201.8	209.0
Brewer's Yeast +*Shuddha Hingula (Red Cinnabar:HgS)*	192.0	201.3
Brewer's Yeast +*Shuddha Vatsanabh (Aconitum balfourii* Stapf)	201.1	211.8
***Brewers Yeast+ Pippali (Piper longum* Linn)**	211.42	210.92

5.38.2 Effect of test drug on Brewer's Yeast induced pyrexia (ºC)

Group	Animal No.	Marking	Sex	Before Yeast inj	After Yeast inj	After Drug Administration						
						30 min	1 hr	2 hr	4 hr	6 hr	24 hr	48 hr
DC-Brewer's Yeast	**1**	**1**	**M**	37.1	38.5	39.2	38.3	38.5	38.4	38.6	37.7	36.3
	2	**2**	**M**	36.8	38.0	38.3	38.8	39.4	36.9	37.6	38.0	36.6
	3	**3**	**M**	36.4	38.5	37.8	38.9	37.8	37.5	39.1	37.5	37.6
	4	**1**	**F**	36.0	38.8	38.7	39.0	38.4	39.3	39.2	37.9	38.5
	5	**2**	**F**	36.2	39.1	39.6	39.5	39.0	38.7	39.5	38.4	38.4

	6	**3**	**F**	36.2	39.2	39.4	38.7	38.9	38.1	39.6	38.5	37.8
	MEAN			36.5	38.7	38.8	38.9	38.7	38.2	38.9	38.0	37.5
	SD			0.4	0.4	0.7	0.4	0.6	0.9	0.7	0.4	0.9
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	**7**	**1**	**M**	35.2	38.0	37.5	36.9	37.1	36.4	37.4	37.7	36.0
	8	**2**	**M**	34.4	37.8	36.9	36.5	35.8	35.7	36.1	37.3	36.3
	9	**3**	**M**	36.4	37.7	37.5	37.0	36.7	36.4	37.1	37.2	35.9
	10	**1**	**F**	37.6	39.3	38.2	37.0	36.8	36.9	39.1	39.2	37.2
	11	**2**	**F**	36.7	39.2	38.3	37.6	37.6	38.4	37.7	39.5	38.3
	12	**3**	**F**	37.3	38.2	37.8	37.7	37.4	37.3	38.2	37.8	35.5
	MEAN			36.3	38.4	37.7	37.1	36.9	36.9	37.6	38.1	36.5
	SD			1.2	0.7	0.5	0.5	0.6	0.9	1.0	1.0	1.0
Brewer's Yeast +*Hinguleshvara Rasa* (5.63 mg/kg) T1	**13**	**1**	**M**	35.6	38.2	38.7	38.6	38.6	38.4	38.4	37.7	36.3
	14	**2**	**M**	37.1	38.5	39.0	39.1	38.9	38.7	38.9	37.8	37.2
	15	**3**	**M**	36.4	39.4	40.1	40.3	39.9	39.0	39.1	38.3	37.6
	16	**1**	**F**	37.0	38.1	38.4	38.7	38.8	38.1	37.9	38.3	36.4
	17	**2**	**F**	36.5	38.7	39.1	39.4	37.8	38.2	37.9	38.0	37.1
	18	**3**	**F**	37.0	39.4	39.3	39.9	38.9	38.7	39.0	38.4	38.1
	MEAN			36.6	38.7	39.1	39.3	38.8	38.5	38.5	38.1	37.1
	SD			0.6	0.6	0.6	0.7	0.7	0.3	0.5	0.3	0.7

Group	Animal No.	Marking	Sex	Before Yeast inj	After Yeast inj	After Drug Administration						
						30 min	1 hr	2 hr	4 hr	6 hr	24 hr	48 hr
Brewer's Yeast +*Shuddha* Hingiula*(Red Cinnabar:HgS)* (11.25 mg/Kg) T2	**19**	**1**	**M**	35.9	37.7	37.8	38.3	38.3	37.0	38.5	37.3	37.5
	20	**2**	**M**	37.5	38.1	37.9	39.1	39.5	38.7	39.8	37.4	37.0
	21	**3**	**M**	36.4	37.7	38.1	37.6	38.3	38.2	39.8	37.3	36.8
	22	**1**	**F**	36.6	38.4	38.6	39.0	37.6	38.1	38.5	38.1	37.8
	23	**2**	**F**	37.1	38.4	38.6	38.2	38.9	38.4	38.1	38.5	37.6
	24	**3**	**F**	36.6	38.5	38.5	39.0	38.9	38.3	39.9	38.3	37.9
	MEAN			36.7	38.1	38.3	38.5	38.6	38.1	39.1	37.8	37.4
	SD			0.6	0.4	0.4	0.6	0.7	0.6	0.8	0.5	0.4
Brewer's Yeast + *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf)(1.40 mg/Kg) T3	**25**	**1**	**M**	36.6	38.3	38.0	36.9	38.1	37.6	39.1	38.9	37.7
	26	**2**	**M**	36.1	37.9	38.5	38.3	39.0	38.1	39.3	38.1	37.4
	27	**3**	**M**	36.8	38.9	38.3	39.0	38.9	38.0	39.0	38.3	37.8
	28	**1**	**F**	36.5	38.1	38.7	38.8	39.1	38.8	38.4	38.4	37.7
	29	**2**	**F**	36.6	38.4	38.5	38.0	38.7	38.1	38.9	38.3	37.8
	30	**3**	**F**	36.1	38.3	38.8	39.1	38.7	37.5	39.2	38.1	38.3
	MEAN			36.5	38.3	38.5	38.4	38.8	38.0	39.0	38.4	37.8
	SD			0.3	0.3	0.3	0.8	0.4	0.5	0.3	0.3	0.3
Brewers Yeast+ *Pippali Piper longum* Linn)T4 (67.5 mg/Kg)	**31**	**1**	**M**	35.1	37.9	37.4	37.0	37.6	36.0	38.9	38.1	37.8
	32	**2**	**M**	35.8	38.1	37.8	38.0	38.2	37.6	38.6	38.6	38.1
	33	**3**	**M**	36.5	38.2	35.8	35.9	38.2	38.0	38.0	38.2	38.1
	34	**1**	**F**	36.5	38.7	38.6	38.4	38.5	38.2	38.5	37.9	38.4
	35	**2**	**F**	37.1	38.8	36.7	36.6	37.6	38.5	38.0	38.4	38.0
	36	**3**	**F**	37.2	38.7	38.7	39.0	38.5	37.9	38.9	39.0	38.4
	MEAN			36.4	38.4	37.5	37.5	38.1	37.7	38.5	38.4	38.1
	SD			0.8	0.4	1.1	1.2	0.4	0.9	0.4	0.4	0.2

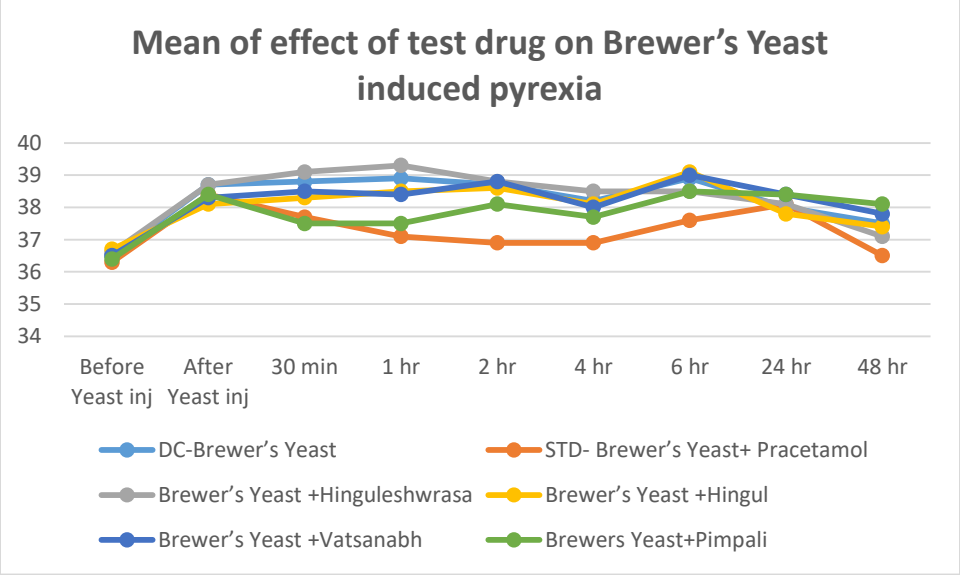
Mean of effect of test drug on Brewer's Yeast induced pyrexia

Groups	Before Yeast inj	After Yeast inj	After Drug Administration						
			30 min	1 hr	2 hr	4 hr	6 hr	24 hr	48 hr
DC-Brewer's Yeast	36.5	38.7	38.8	38.9	38.7	38.2	38.9	38.0	37.5
STD- Brewer's Yeast+ Pracetamol	36.3	38.4	37.7	37.1	36.9	36.9	37.6	38.1	36.5
Brewer's Yeast +Hinguleshvara RasaT1	36.6	38.7	39.1	39.3	38.8	38.5	38.5	38.1	37.1
Brewer's Yeast +Hingula (Red Cinnabar:HgS) T2	36.7	38.1	38.3	38.5	38.6	38.1	39.1	37.8	37.4
***Brewer's Yeast* + *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf)T3**	36.5	38.3	38.5	38.4	38.8	38.0	39.0	38.4	37.8
Brewers Yeast+*Pippali* *Piper longum* Linn)T4	36.4	38.4	37.5	37.5	38.1	37.7	38.5	38.4	38.1

Mean of effect of test drug on Brewer's Yeast induced pyrexia

Groups	Before Yeast inj	After Yeast inj	After Drug Administration								
			30 min	1 hr	2 hr	4 hr	6 hr	24 hr	48 hr	F-Value	P-Value
DC-Brewer's Yeast	36.5	38.7	38.8	38.9	38.7	38.2	38.9	38	37.5	1.593	0.154
STD- Brewer's Yeast+ Pracetamol	36.3	38.4	37.7	37.1	36.9	36.9	37.6	38.1	36.5	1.321	0.258
Brewer's Yeast + *Hinguleshvara Rasa*	36.6	38.7	39.1	39.3	38.8	38.5	38.5	38.1	37.1	1.325	0.256
Brewer's Yeast + Shuddha Hingula	36.7	38.1	38.3	38.5	38.6	38.1	39.1	37.8	37.4	1.526	0.175
Brewer's Yeast + Shuddha Vatsanabh	36.5	38.3	38.5	38.4	38.8	38	39	38.4	37.8	1.360	0.240
Brewers Yeast+Pimpali	36.4	38.4	37.5	37.5	38.1	37.7	38.5	38.4	38.1	1.281	0.278

Repeated Measures ANOVA Test is carried out to test significance in all groups at different time points. Above table shows that, P-Value for all six groups are greater than **0.05. Hence, we can conclude that, there is no significant change observed in mean at** different time points.



After 18 hrs of injecting Brewer's Yeast in the animals of all the groups except Normal Control, the rectal temperature was increased in all the groups. After oral administration of Standard (Paracetamol) and respective test drugs, no significant reduction in the rectal temperature was observed in all the treated groups.

Based on the above results obtained it can be concluded that the test drugs *Hinguleshvara Rasa* (T1), *Shodhita Hingula* (T2), *Shodhita Vatsanabh* (T3) and Pippali (T4) are having the potent analgesic activity but less anti-pyretic activity.

Overall results indicate that the standard drug Paracetamol is more effective in antipyretic activity than the test drugs *Hinguleshvara Rasa*, specifically in initial period like at the end of one hour, two hours. However in later stage like twenty-four hours and more the antipyretic activity of Paracetamol and *Hinguleshvara Rasa* is almost similar. Still it can be concluded that the antipyretic activity of *Hinguleshvara Rasa* doesn't match with that of standard drug Paracetamol

Actually *Hinguleshvara Rasa* is widely used for its antipyretic activity. In present study antipyretic activity of *Hinguleshvara Rasa* was compared with its all three ingredients namely *Shuddha Hingula* (Red Cinnabar:HgS), *Shuddha Vatsana*bh (*Aconitum balfourii* Stapf) and *Piper longum* Linn. It was found that all these four test drug exerted better analgesic activity than their known antipyretic action. In previous few study of *Hinguleshvara Rasa* research scholars mentioned that *Hinguleshvara Rasa* has good analgesic activity as well as antipyretic activity.

In one study research scholar mentioned that antipyretic activity of *Vatsanabh* when its *shodhana* performed in cow milk was more than when its *shodhana* performed in cow urine [**267**] In present study *Vatsanabh shodhana* was performed in cow urine and not in cow urine. So expected antipyretic activity was not found.

Hinguleshvara Rasa prepared with *bhavana* gave potent antipyretic effect while *Hinguleshvara Rasa* prepared without *bhavana* was exerted weak antipyretic activity**.**[**268]**

In present study as *Bhavana dravya* was not mentioned in classical reference of *Hinguleshvara Rasa* given in Bhaishajya Ratnavali, so *bhavana* were not performed. This is also the reason for not getting expected antipyretic activity of *Hinguleshvara Rasa*

If these earlier two findings compared with present study, it was clear in present study *shodhana of Vatsnabh Aconitum balfourii* Stapf was performed in cow urine and not in cow milk. As there was no dravya mentioned for wet grinding of *Hinguleshvara* in *Bhaishajya Ratnavali, bhavana* were not performed. This is the reason why an expected antipyretic activity of *Hinguleshvara Rasa* was not observed in this study.

5.38.3 Effect of test drug on Haematological parameters

Group	Animal No.	Marking	Sex	WBC	LYM	NEUT	RBC	HGB	HCT	PLT
				*10^9/L	*10^9/L	*10^9/L	*10^{12}/L	g/dL	%	*10^9/L
Normal Control NC	**1**	**1**	**M**	2.6	2.5	0.1	9.6	18.3	70.6	315.0
	2	**2**	**M**	2.9	2.8	0.1	8.1	14.5	57.6	546.0
	3	**3**	**M**	0.9	0.9	0.0	8.2	14.1	59.0	713.0
	4	**1**	**F**	2.9	2.9	0.0	7.2	13.3	55.2	496.0
	5	**2**	**F**	3.4	3.3	0.1	7.5	14.9	59.4	787.0
	6	**3**	**F**	1.4	1.3	0.1	7.1	13.3	53.8	372.0
	MEAN			2.4	2.3	0.1	7.9	14.7	59.3	538.2
	SD			1.0	1.0	0.1	1.0	1.9	6.0	185.3
Disease Control DC- Brewer's Yeast	**7**	**1**	**M**	2.8	2.6	0.2	8.1	14.6	62.5	527.0
	8	**2**	**M**	3.5	3.4	0.1	8.0	14.8	59.4	717.0
	9	**3**	**M**	3.1	3.0	0.0	8.0	14.7	57.8	548.0
	10	**1**	**F**	1.9	1.8	0.1	9.0	17.4	69.5	340.0
	11	**2**	**F**	2.4	2.3	0.0	8.3	16.1	63.2	584.0
	12	**3**	**F**	2.4	2.3	0.1	8.0	15.3	60.4	483.0
	MEAN			2.7	2.6	0.1	8.2	15.5	62.1	533.2
	SD			0.6	0.6	0.1	0.4	1.1	4.1	123.7
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	**13**	**1**	**M**	3.6	3.5	0.0	6.5	11.6	47.7	699.0
	14	**2**	**M**	7.6	7.4	0.1	7.1	12.5	52.8	946.0
	15	**3**	**M**	5.4	5.3	0.0	8.1	14.9	59.2	728.0
	16	**1**	**F**	1.7	1.6	0.1	6.1	10.6	43.8	438.0
	17	**2**	**F**	2.9	2.8	0.1	6.7	12.3	50.8	600.0
	18	**3**	**F**	3.7	3.6	0.1	6.8	13.1	53.6	783.0
	MEAN			4.2	4.0	0.1	6.9	12.5	51.3	699.0
	SD			2.1	2.0	0.1	0.7	1.5	5.3	171.4
Brewer's Yeast +*Hingules hvara Rasa* (5.63 mg/kg) T1	**19**	**1**	**M**	6.8	6.6	0.1	6.9	12.5	51.7	845.0
	20	**2**	**M**	6.4	6.2	0.1	7.3	13.0	53.9	733.0
	21	**3**	**M**	6.4	6.2	0.1	7.3	13.0	53.5	666.0
	22	**1**	**F**	3.9	3.7	0.1	6.3	11.8	49.1	687.0
	23	**2**	**F**	5.0	4.8	0.1	2.2	12.6	16.4	216.0
	24	**3**	**F**	4.5	4.3	0.1	0.0	12.8	0.0	451.5
	MEAN			5.5	5.3	0.1	5.0	12.6	37.4	599.8
	SD			1.2	1.2	0.0	3.1	0.4	23.3	227.7
Brewer's Yeast + *Shuddha Hingula (Red Cinnabar: HgS)* (11.25 mg/Kg) T2	**25**	**1**	**M**	3.6	3.5	0.1	6.8	13.1	50.7	585.0
	26	**2**	**M**	4.0	3.9	0.1	7.2	13.1	51.7	543.0
	27	**3**	**M**	4.6	4.5	0.0	7.2	12.8	49.8	653.0
	28	**1**	**F**	1.6	1.5	1.0	7.2	13.2	52.1	394.0
	29	**2**	**F**	2.9	2.8	0.1	8.4	16.4	62.5	360.0
	30	**3**	**F**	2.0	1.9	0.1	7.5	13.3	57.0	505.0
	MEAN			3.1	3.0	0.2	7.4	13.7	54.0	506.7
	SD			1.2	1.2	0.4	0.6	1.4	4.9	112.3

Group	Animal No.	Marking	Sex	WBC	LYM	NEUT	RBC	HGB	HCT	PLT
				*10^9/L	*10^9/L	*10^9/L	*10^{12}/L	g/dL	%	*10^9/L
Brewer's Yeast + *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) (1.40 mg/Kg) T3	**31**	**1**	**M**	1.9	1.8	0.1	10.6	22.0	80.4	224.0
	32	**2**	**M**	3.0	2.9	0.1	9.9	18.9	71.7	264.0
	33	**3**	**M**	3.0	2.9	0.1	7.7	13.8	54.9	501.0
	34	**1**	**F**	3.2	3.1	0.0	7.5	13.8	54.0	491.0
	35	**2**	**F**	2.8	2.7	0.1	8.8	18.0	67.5	331.0
	36	**3**	**F**	0.7	0.7	0.0	11.8	24.9	89.7	87.0
	MEAN			2.4	2.4	0.1	9.4	18.6	69.7	316.3
	SD			1.0	0.9	0.1	1.7	4.4	14.1	160.4
Brewers Yeast+ *Pippali Piper longum* Linn) T4 (67.5 mg/Kg)	**37**	**1**	**M**	2.8	2.7	0.1	10.0	20.0	73.7	256.0
	38	**2**	**M**	3.3	3.2	0.1	10.5	20.9	78.4	194.0
	39	**3**	**M**	1.4	1.4	0.0	11.2	23.0	83.0	156.0
	40	**1**	**F**	1.9	1.8	0.1	8.3	15.7	60.7	369.0
	41	**2**	**F**	2.2	2.1	0.1	10.6	21.5	80.2	231.0
	42	**3**	**F**	1.4	1.3	0.1	8.3	14.9	57.7	439.0
	MEAN			2.2	2.1	0.1	9.8	19.3	72.3	274.2
	SD			0.8	0.7	0.0	1.2	3.3	10.6	108.4

Mean of hematological parameters

Groups	WBC	LYM	NEUT	RBC	HGB	HCT	PLT
	$*10^9$/L	$*10^9$/L	$*10^9$/L	$*10^{12}$/ L	g/dL	%	$*10^9$/ L
NC	2.4	2.3	0.1	7.9	14.7	59.3	538.2
DC-Brewer's Yeast	2.7	2.6	0.1	8.2	15.5	62.1	533.2
STD- Brewer's Yeast+ Pracetamol	4.2	4.0	0.1	6.9	12.5	51.3	699.0
Brewer's Yeast + *Hinguleshvara RasaT1*	5.5	5.3	0.1	5.0	12.6	37.4	599.8
Brewer's Yeast + *Shuddha Hingula* (Red Cinnabar:HgS) T2	3.1	3.0	0.2	7.4	13.7	54.0	506.7
Brewer's Yeast +*Shuddha Vatsanabh(Aconitum balfourii* Stapf)T3	2.4	2.4	0.1	9.4	18.6	69.7	316.3
Brewers Yeast+*Pippali Piper longum* Linn)T4	2.2	2.1	0.1	9.8	19.3	72.3	274.2

5.38.4. Effect of test drug on Biochemical parameters

Group	Animal No.	Marking	Sex	TP	TB	ALB	Creat	ALP	Urea	AST	ALT
NC	**1**	**1**	**M**	6.9	0.22	4.5	1.8	134.6	44.2	163.0	22.6
	2	**2**	**M**	5.8	0.17	3.3	1.7	157.6	61.4	134.8	36.3
	3	**3**	**M**	5.1	0.24	4.5	1.7	86.7	42.2	145.5	19.8
	4	**1**	**F**	6.0	0.29	3.5	1.5	174.2	39.9	174.3	29.7
	5	**2**	**F**	6.0	0.22	4.6	2.1	141.8	38.3	154.9	21.6
	6	**3**	**F**	5.3	0.18	3.1	1.4	126.8	54.9	128.2	29.7
	MEAN			5.8	0.22	3.9	1.7	137.0	46.8	150.1	26.6
	SD			0.6	0.05	0.7	0.2	29.9	9.2	17.4	6.3
Dsease	**7**	**1**	**M**	9.0	0.19	3.8	1.7	150.1	45.2	128.5	40.0

Control	**8**	**2**	**M**	8.6	0.25	3.1	0.1	129.1	28.0	143.9	38.3
	9	**3**	**M**	8.6	0.25	4.1	1.7	140.2	39.3	196.0	28.6
	10	**1**	**F**	7.3	0.19	3.3	1.5	165.2	29.0	150.5	28.4
	11	**2**	**F**	7.8	0.22	2.7	1.5	135.9	50.1	121.5	39.7
	12	**3**	**F**	15.9	0.20	2.1	1.6	166.6	44.5	117.4	29.3
	MEAN			9.5	0.22	3.2	1.4	147.9	39.3	143.0	34.0
	SD			3.2	0.03	0.7	0.6	15.6	9.1	29.0	5.8
STD	**13**	**1**	**M**	7.2	0.16	2.8	1.0	154.3	42.2	162.5	28.2
	14	**2**	**M**	6.3	0.22	2.1	0.9	224.3	36.6	138.9	28.9
	15	**3**	**M**	5.9	0.12	4.8	1.6	201.3	53.4	238.3	19.7
	16	**1**	**F**	4.8	0.19	2.8	0.9	180.6	33.9	182.7	22.2
	17	**2**	**F**	4.3	0.27	3.6	1.5	163.2	64.1	157.8	32.7
	18	**3**	**F**	7.0	0.30	3.9	1.2	154.1	47.2	162.8	17.0
	MEAN			5.9	0.21	3.3	1.2	179.7	46.2	173.8	24.8
	SD			1.2	0.07	1.0	0.3	28.4	11.2	34.5	6.1
Brewer's Yeast + *Hingule shvara Rasa* T1	**19**	**1**	**M**	6.5	0.23	3.8	1.4	175.5	65.7	209.9	26.6
	20	**2**	**M**	5.6	0.19	3.4	1.9	207.5	40.0	265.8	20.9
	21	**3**	**M**	6.6	0.18	2.0	1.4	178.3	56.2	152.7	21.8
	22	**1**	**F**	4.5	0.25	3.4	1.0	208.1	43.4	149.0	26.7
	23	**2**	**F**	4.9	0.21	4.0	1.4	159.4	32.3	144.8	18.0
	24	**3**	**F**	5.1	0.29	3.6	1.3	171.9	39.0	162.8	27.4
	MEAN			5.5	0.22	3.4	1.4	183.4	46.1	180.8	23.6
	SD			0.9	0.04	0.7	0.3	19.9	12.4	47.9	3.9

Group	**Animal No.**	**Marking**	**Sex**	**TP**	**TB**	**ALB**	**Creat**	**ALP**	**Urea**	**AST**	**ALT**
Brewer's Yeast + Shuiddh	**25**	**1**	**M**	6.9	0.17	3.6	2.2	212.0	59.9	218.9	29.8
	26	**2**	**M**	6.7	0.28	4.6	1.7	220.7	40.2	230.5	26.4

***Hingula* (Red Cinnabar:HgS) T2**	**27**	**3**	**M**	6.5	0.34	3.3	1.4	195.4	51.8	213.0	29.8
	28	**1**	**F**	7.9	0.19	2.7	2.0	199.5	44.2	218.2	31.1
	29	**2**	**F**	7.1	0.28	3.0	2.0	167.9	56.0	221.6	28.5
	30	**3**	**F**	6.4	0.23	2.9	2.1	219.9	42.1	210.3	31.9
	MEAN			6.9	0.25	3.4	1.9	202.6	49.0	218.7	29.6
	SD			0.5	0.06	0.7	0.3	19.9	8.0	7.1	2.0
Brewer's Yeast + Shuiddha *Vatsanabh* (*Aconitum balfourii* Stapf) T3	**31**	**1**	**M**	9.2	0.34	2.3	1.7	152.1	46.3	295.1	27.0
	32	**2**	**M**	6.6	0.16	2.6	1.8	154.5	59.0	207.5	32.8
	33	**3**	**M**	7.5	0.18	3.4	1.9	182.5	39.0	166.4	37.1
	34	**1**	**F**	6.8	0.24	5.3	1.4	125.1	48.7	161.1	26.7
	35	**2**	**F**	6.7	0.21	4.8	1.6	225.8	43.7	203.1	24.0
	36	**3**	**F**	9.0	0.22	3.4	1.4	227.0	51.9	213.5	31.7
	MEAN			7.6	0.23	3.6	1.7	177.8	48.1	207.8	29.9
	SD			1.2	0.06	1.2	0.2	41.8	6.9	48.1	4.8
Brewers Yeast+*Pippal*	**37**	**1**	**M**	6.1	0.17	3.5	1.8	187.5	54.2	220.0	25.2

***i Piper longum* Linn)T4**	**38**	**2**	**M**	5.2	0.25	2.6	1.6	164.0	46.2	185.9	30.2
	39	**3**	**M**	6.1	0.21	4.3	1.8	178.5	58.3	212.7	38.4
	40	**1**	**F**	6.1	0.25	2.9	1.9	153.8	45.4	231.1	29.7
	41	**2**	**F**	6.2	0.21	3.4	1.8	209.6	51.3	232.0	38.8
	42	**3**	**F**	7.7	0.30	3.9	0.5	226.5	42.0	210.5	30.3
	MEAN			6.2	0.23	3.4	1.6	186.6	49.6	215.4	32.1
	SD			0.8	0.04	0.6	0.5	27.5	6.1	17.0	5.4

Mean of Biochemical parameters

Groups	**TP**	**TB**	**ALB**	**Creat**	**ALP**	**Urea**	**AST**	**ALT**
NC	5.8	0.2	3.9	1.7	137.0	46.8	150.1	26.6
DC-Brewer's Yeast	9.5	0.2	3.2	1.4	147.9	39.3	143.0	34.0
STD- Brewer's Yeast+ Pracetamol	5.9	0.2	3.3	1.2	179.7	46.2	173.8	24.8
Brewer's Yeast +*Hinguleshvara Rasa* T1	5.5	0.2	3.4	1.4	183.4	46.1	180.8	23.6
Brewer's Yeast +*Shuddha Hingula***(Red Cinnabar:HgS) T2**	6.9	0.2	3.4	1.9	202.6	49.0	218.7	29.6
Brewer's Yeast +*ShuddhaVatsanabh* **(*Aconitum balfourii* Stapf) T3**	7.6	0.2	3.6	1.7	177.8	48.1	207.8	29.9
Brewers Yeast+*Pippali* *Piper longum* Linn)T4	6.2	0.23	3.4	1.6	186.6	49.6	215.4	32.1

5.38.5. Effect of test drug on Electrolytes

Group	Animal No.	Marking	Sex	Na	K	Ca	Cl
Normal Control	**1**	**1**	**M**	211.9	9.2	1.3	152.4
	2	**2**	**M**	187.6	9.9	1.0	141.4
	3	**3**	**M**	180.6	11.2	1.3	133.0
	4	**1**	**F**	216.8	7.1	1.4	156.3
	5	**2**	**F**	215.9	9.9	1.3	156.5
	6	**3**	**F**	183.9	8.3	0.9	141.0
	MEAN			199.5	9.3	1.2	146.8
	SD			17.1	1.4	0.2	9.7
Disease Control Brewer's Yeast	**7**	**1**	**M**	148.5	6.4	0.7	110.1
	8	**2**	**M**	154.9	7.5	0.8	113.0
	9	**3**	**M**	160.9	6.9	0.8	120.7
	10	**1**	**F**	143.9	6.1	0.6	109.1
	11	**2**	**F**	168.8	6.7	0.9	121.4
	12	**3**	**F**	158.2	7.0	0.8	118.8
	MEAN			155.9	6.8	0.8	115.5
	SD			8.9	0.5	0.1	5.5
Brewer's Yeast + Paracetamol STD	**13**	**1**	**M**	229.6	10.6	1.4	164.5
	14	**2**	**M**	212.0	12.1	1.2	151.3
	15	**3**	**M**	197.7	11.3	1.1	141.3
	16	**1**	**F**	223.2	11.9	1.7	158.7
	17	**2**	**F**	158.4	12.3	1.0	113.7
	18	**3**	**F**	247.7	12.4	1.2	160.4
	MEAN			211.4	11.7	1.3	148.3
	SD			30.9	0.7	0.3	18.8
Brewer's Yeast + *Hinguleshvara*	**19**	**1**	**M**	149.0	9.1	0.7	111.7
	20	**2**	**M**	234.7	15.0	1.0	158.7
	21	**3**	**M**	191.2	12.9	0.9	136.4

Rasa	**22**	**1**	**F**	247.4	15.0	1.2	166.8
T1	**23**	**2**	**F**	171.3	10.5	1.2	127.3
	24	**3**	**F**	204.7	12.1	1.6	140.6
	MEAN			199.7	12.4	1.1	140.3
	SD			37.3	2.4	0.3	20.2

Group	**Animal No.**	**Marking**	**Sex**	**Na**	**K**	**Ca**	**Cl**
Brewer's Yeast + *Shuddha Hingula(Red Cinnabar:HgS) T2*	**25**	**1**	**M**	158.3	6.6	0.6	118.3
	26	**2**	**M**	142.4	5.5	0.5	106.5
	27	**3**	**M**	169.2	7.5	0.5	122.1
	28	**1**	**F**	157.9	5.8	0.7	115.1
	29	**2**	**F**	161.6	6.6	0.8	117.3
	30	**3**	**F**	151.6	11.6	0.0	114.4
	MEAN			156.8	7.3	0.5	115.6
	SD			9.1	2.2	0.3	5.2
Brewer's Yeast + Shiuddha *Vatsanabh* (*Aconitum balfourii* Stapf) T3	**31**	**1**	**M**	142.1	5.6	0.6	107.3
	32	**2**	**M**	142.1	5.8	0.6	109.2
	33	**3**	**M**	159.5	6.4	0.6	116.4
	34	**1**	**F**	156.9	6.5	0.8	117.7
	35	**2**	**F**	165.2	6.8	0.8	115.5
	36	**3**	**F**	154.4	5.4	0.9	110.7
	MEAN			153.4	6.1	0.7	112.8
	SD			9.4	0.6	0.1	4.3
Brewers Yeast+*Pippali* *Piper longum* Linn)T4	**37**	**1**	**M**	174.6	7.6	0.7	125.2
	38	**2**	**M**	121.2	5.1	0.7	93.0
	39	**3**	**M**	121.8	4.7	0.9	93.1
	40	**1**	**F**	150.9	5.9	0.7	112.6
	41	**2**	**F**	139.5	5.4	0.6	104.6

	42	**3**	**F**	155.2	5.8	0.7	116.6
	MEAN			143.9	5.7	0.7	107.5
	SD			20.7	1.0	0.1	13.0

Mean of electrolytes

Groups	**Na**	**K**	**Ca**	**Cl**
NC	199.5	9.3	1.2	146.8
DC-Brewer's Yeast	155.9	6.8	0.8	115.5
STD- Brewer's Yeast+ Pracetamol	211.4	11.7	1.3	148.3
Brewer's Yeast +*Hinguleshvrasa* T1	199.7	12.4	1.1	140.3
Brewer's Yeast + *Shuddha Hingula (Red Cinnabar:HgS) T2*	156.8	7.3	0.5	115.6
Brewer's Yeast +*Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) T3	153.4	6.1	0.7	112.8
Brewers Yeast+*Pippali* *Piper longum* Linn)T4	143.9	5.7	0.7	107.5

5.38.6. Effect of test drug on organ weight

Group	**Animal No.**	**Marking**	**Sex**	**Adrenals**	**Heart**	**Kidneys**	**Liver**	**Spleen**	**Lungs**	**Gonads**	**Brain**	**Pancreas**	**Stomach**
Normal Control	**1**	**1**	**M**	0.117	1.188	2.249	10.596	1.064	1.893	2.992	1.358	0.468	1.728
	2	**2**	**M**	0.046	1.306	3.182	15.317	1.378	2.529	3.311	1.816	0.510	2.130
	3	**3**	**M**	0.096	0.824	1.703	8.202	0.889	1.989	2.647	1.266	0.356	1.477

	4	**1**	**F**	0.093	1.003	1.271	7.343	0.864	1.411	0.555	1.356	0.305	1.438
	5	**2**	**F**	0.073	0.791	1.450	7.793	0.844	1.963	0.923	1.531	0.380	1.698
	6	**3**	**F**	0.102	0.898	1.335	8.561	0.901	1.847	0.672	1.452	0.237	1.611
	MEAN			0.088	1.002	1.865	9.635	0.990	1.939	1.850	1.463	0.376	1.680
	SD			0.025	0.207	0.737	3.002	0.206	0.358	1.265	0.195	0.101	0.249
Disease Control	**7**	**1**	**M**	0.103	0.981	2.020	9.875	1.944	1.807	3.169	1.557	0.418	1.780
	8	**2**	**M**	0.052	1.106	2.252	13.738	1.592	2.070	2.951	1.737	0.428	2.279
	9	**3**	**M**	0.098	1.291	2.467	13.142	1.879	2.711	3.107	1.492	0.353	2.610
	10	**1**	**F**	0.060	1.316	1.531	8.859	1.814	1.754	0.565	1.689	0.422	1.562
	11	**2**	**F**	0.085	0.992	1.548	10.867	1.762	1.823	0.732	1.505	0.249	1.418
	12	**3**	**F**	0.091	1.252	1.929	12.310	1.869	1.667	0.472	1.508	0.533	2.060
	MEAN			0.082	1.156	1.958	11.465	1.810	1.972	1.833	1.581	0.401	1.952
	SD			0.021	0.150	0.374	1.917	0.123	0.386	1.366	0.105	0.094	0.451
STD Brewer's Yeast + Paraceta	**13**	**1**	**M**	0.099	0.869	1.695	9.495	1.308	1.716	2.786	1.430	0.446	1.425
	14	**2**	**M**	0.090	0.783	1.402	8.896	1.409	1.544	2.286	1.246	0.401	1.274

mol	**15**	**3**	**M**	0.100	0.999	1.797	8.715	1.050	1.186	3.259	1.415	0.279	0.865
	16	**1**	**F**	0.124	0.820	1.573	10.018	1.633	1.675	0.654	1.413	0.493	1.641
	17	**2**	**F**	0.171	1.153	2.564	12.887	1.652	1.814	0.535	1.413	0.493	1.285
	18	**3**	**F**	0.095	0.715	1.235	7.126	1.376	1.393	0.596	1.407	0.503	1.334
	MEAN			0.113	0.890	1.711	9.523	1.405	1.555	1.686	1.387	0.436	1.304
	SD			0.031	0.160	0.464	1.916	0.223	0.232	1.235	0.070	0.086	0.254
Hingulesh vara Rasa	**19**	**1**	**M**	0.092	0.791	1.552	10.858	1.237	1.710	2.533	1.432	0.536	1.519
	20	**2**	**M**	0.062	0.699	1.323	7.993	0.880	1.070	1.209	0.929	0.164	1.184
	21	**3**	**M**	0.090	0.728	1.825	9.454	1.473	1.587	2.593	1.348	0.213	1.362
	22	**1**	**F**	0.153	0.745	1.581	8.650	1.591	1.747	0.437	1.528	0.598	1.444
	23	**2**	**F**	0.124	0.912	1.534	9.925	2.387	1.630	0.774	1.309	0.292	1.415
	24	**3**	**F**	0.086	0.970	1.511	7.950	1.066	1.255	0.425	1.260	0.253	1.184
	MEAN			0.101	0.808	1.554	9.138	1.439	1.500	1.329	1.301	0.343	1.351
	SD			0.032	0.109	0.161	1.152	0.532	0.274	0.998	0.205	0.180	0.139

Group	Animal No.	Marking	Sex	Adrenals	Heart	Kidneys	Liver	Spleen	Lungs	Gonads	Brain	Pancreas	Stomach
Suddha Hingula (Red Cinnabar:HgS)	**25**	**1**	**M**	0.081	0.969	1.851	9.896	1.039	1.906	2.729	1.494	0.432	1.494
	26	**2**	**M**	0.092	0.944	1.598	10.491	1.090	1.547	2.384	1.512	0.472	1.589
	27	**3**	**M**	0.078	0.711	1.410	8.759	1.148	2.806	2.267	1.300	0.212	1.249
	28	**1**	**F**	0.055	0.632	1.405	8.074	1.435	1.417	0.514	1.105	0.234	1.209
	29	**2**	**F**	0.093	0.641	1.281	0.589	1.078	1.485	0.539	1.010	0.635	1.383
	30	**3**	**F**	0.102	0.672	1.138	7.824	1.237	1.644	0.603	1.023	0.227	1.046
	MEAN			0.084	0.762	1.447	7.606	1.171	1.801	1.506	1.241	0.369	1.328
	SD			0.016	0.154	0.250	3.589	0.146	0.521	1.056	0.228	0.172	0.199
Shuddha Vatsanabh (Aconitum balfourii **Stapf)**	**31**	**1**	**M**	0.064	0.856	1.590	9.499	1.706	1.514	2.613	1.112	0.835	1.474
	32	**2**	**M**	0.098	0.690	1.302	7.855	1.319	1.325	2.115	1.299	0.336	1.214
	33	**3**	**M**	0.083	0.788	1.531	7.341	1.111	1.844	3.087	0.876	0.161	1.580
	34	**1**	**F**	0.132	0.779	1.522	8.354	1.062	1.411	0.681	1.397	0.389	1.202
	35	**2**	**F**	0.196	0.667	2.177	9.503	1.520	1.497	0.549	1.307	0.628	1.362
	36	**3**	**F**	0.138	0.7	1.50	11.4	1.6	1.63	0.69	1.38	0.324	1.13

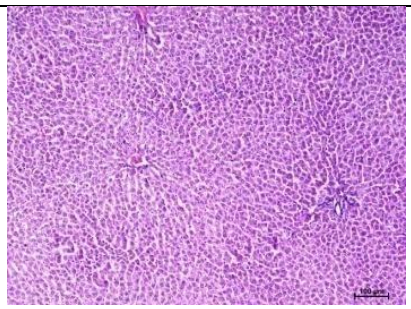
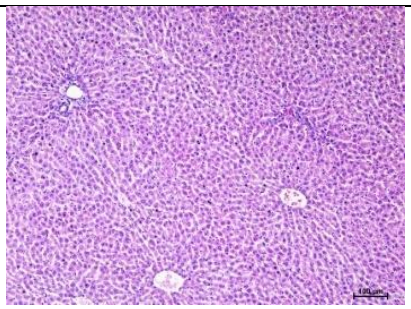
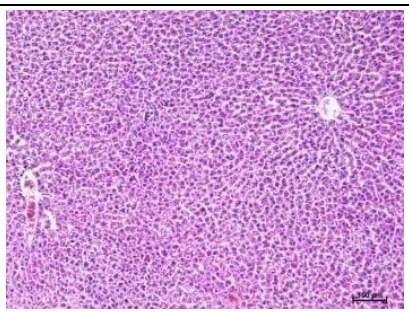
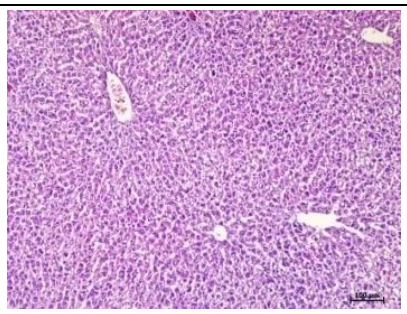
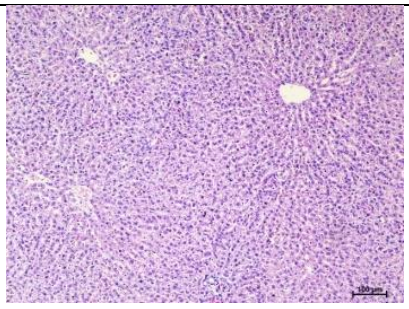
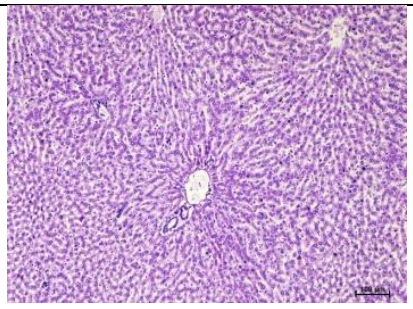
					06	3	44	92	1	4	7		7
	MEAN			0.119	0.748	1.604	8.999	1.402	1.537	1.623	1.230	0.446	1.328
	SD			0.047	0.072	0.297	1.480	0.282	0.182	1.120	0.201	0.243	0.174
Brewers Yeast+*Pippali Piper longum* Linn)T4	**37**	**1**	**M**	0.119	0.764	1.520	7.863	1.235	1.220	2.264	1.095	0.587	1.020
	38	**2**	**M**	0.101	0.944	1.715	9.535	1.123	1.732	2.637	1.361	0.399	1.350
	39	**3**	**M**	0.206	0.676	1.603	9.470	1.531	2.041	2.349	1.421	0.854	1.221
	40	**1**	**F**	0.236	0.606	1.257	7.581	1.181	1.735	1.797	1.298	0.642	1.439
	41	**2**	**F**	0.119	0.476	1.103	6.118	1.195	1.242	0.621	1.212	0.339	0.871
	42	**3**	**F**	0.143	0.758	1.768	12.019	1.648	1.653	0.812	1.142	0.438	1.511
	MEAN			0.154	0.704	1.494	8.764	1.319	1.604	1.747	1.255	0.543	1.235
	SD			0.054	0.159	0.263	2.045	0.216	0.318	0.845	0.127	0.191	0.249

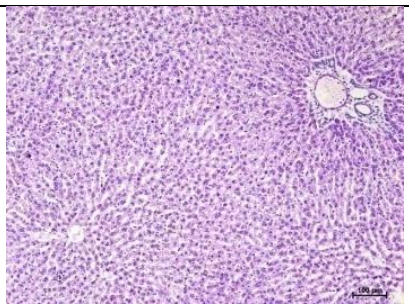
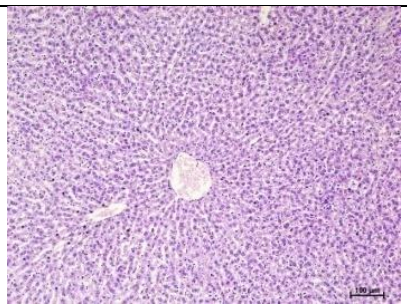
Mean of organ weight

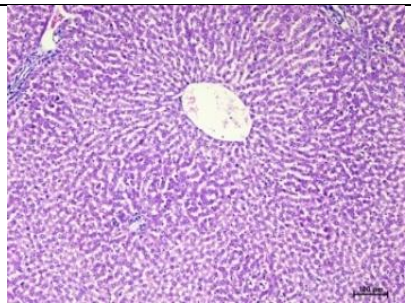
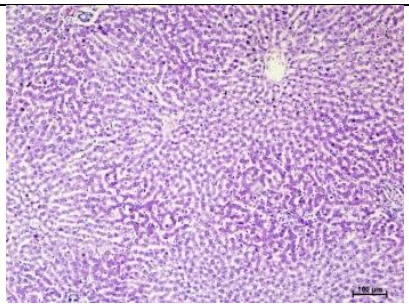
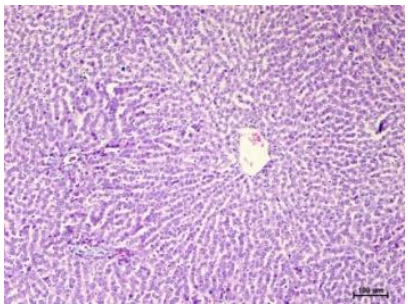
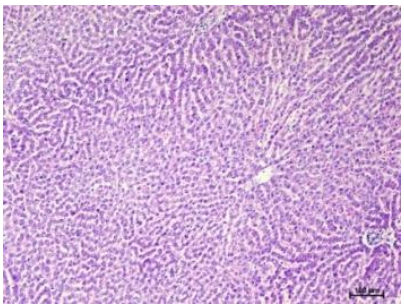
Groups	Adrenals	Heart	Kidneys	Liver	Spleen	Lungs	Gonads	Brain	Pancreas	Stomach
NC	0.088	1.002	1.865	9.635	0.990	1.939	1.850	1.463	0.376	1.680
DC-Brewer's Yeast	0.082	1.156	1.958	11.465	1.810	1.972	1.833	1.581	0.401	1.952
STD- Brewer's Yeast+ Pracetamol	0.113	0.890	1.711	9.523	1.405	1.555	1.686	1.387	0.436	1.304
Brewer's Yeast + ***HinguleshwrasaT 1***	0.101	0.808	1.554	9.138	1.439	1.500	1.329	1.301	0.343	1.351
Brewer's Yeast +*Hingula (Red Cinnabar:HgS)*T2	0.084	0.762	1.447	7.606	1.171	1.801	1.506	1.241	0.369	1.328
Brewer's Yeast +*Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) T3	0.119	0.748	1.604	8.999	1.402	1.537	1.623	1.230	0.446	1.328
Brewers Yeast+*Pippali Piper longum* Linn)T4	0.154	0.704	1.494	8.764	1.319	1.604	1.747	1.255	0.543	1.235

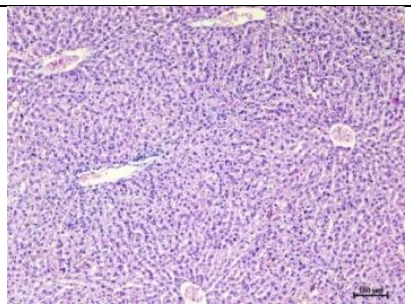
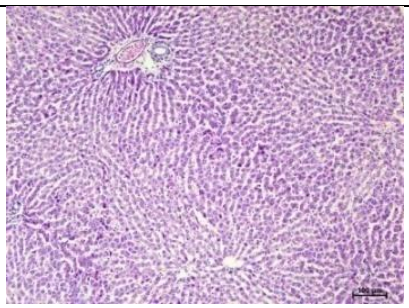
Photos and charts of Histopathology Results after antipyretic study:

5.38.7. Effect of test drug on histopathology of liver after antipyretic study

	
Normal Control 1	**Normal Control2**
	
Disease Control 1	**Disease Control 2**
	
Brewer's Yeast+ Paracetamol **STD-1** **1**	Brewer's Yeast+ Paracetamol **STD-2** **2**

	
Brewer's Yeast+ *Hinguleshvara Rasa* **T1- 1**	Brewer's Yeast+ *Hinguleshvara Rasa* **T1- 2**

	
Brewer's Yeast+*Shodhita Hingula* **(Red Cinnabar:HgS) T2- 1**	Brewer's Yeast+*Shodhita Hingula* **(Red Cinnabar:HgS) T2—2**
	
Brewer's Yeast+ ***Shuddha Vatsanabh*** **(*Aconitum balfourii* Stapf)T3- 1**	Brewer's Yeast+ ***Shuddha Vatsanabh*** **(*Aconitum balfourii* Stapf)T3- - 2**

	
Brewers Yeast+*Pippali* ***Piper longum* Linn)T4- 1**	**Brewers Yeast+*Pippali*** ***Piper longum* Linn)T4- 2**

Sr. N.	Group /Slide Code	Histopathological observations 1.Liver	Overall Pathological Grade
1	**Normal ControlNC-1**	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
2	**Normal ControlNC-2**	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
3	**Disease Control1**	Focal congested vascular tissue. Focal and occasional cellular swelling of hepatocytes. Minimal degenerative changes of hepatocytes with granular cytoplasmic changes. Absence of inflammatory changes in hepatic parenchyma.	**Minimal (+1)**
4	**Disease**	Focal and occasional cellular swelling of hepatocytes. Focal congested vascular tissue. Absence of	**Minimal**

	Control-2	inflammatory changes in hepatic parenchyma.	**(+1)**
5	Brewer's Yeast+ Paracetamol **STD -1**	Focal congested vascular tissue. Focal and occasional cellular swelling of hepatocytes. Minimal degenerative changes of hepatocytes with granular cytoplasmic changes. Absence of inflammatory changes in hepatic parenchyma.	**Minimal (+1)**
6	Brewer's Yeast+ Paracetamol **STD -2**	Focal congested vascular tissue. Focal and occasional cellular swelling of hepatocytes. Focal congested vascular tissue. Absence of inflammatory changes in hepatic parenchyma.	**Minimal (+1)**
7	Brewer's Yeast+ *Hingles hvara Rasa* **T1--1**	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
8	Brewer's Yeast+ *Hingles hvara Rasa* **T1--2**	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Focal and occasional cellular swelling of hepatocytes. Focal congested vascular tissue. Absence of inflammatory changes in hepatic parenchyma.	**NAD to Minimal (+1)**
9	Brewer's Yeast+*Shodhita Hingula*	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**

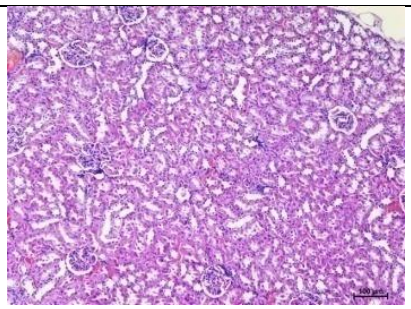
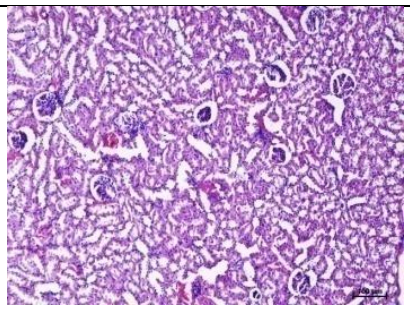
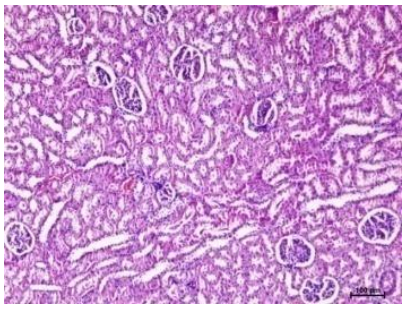
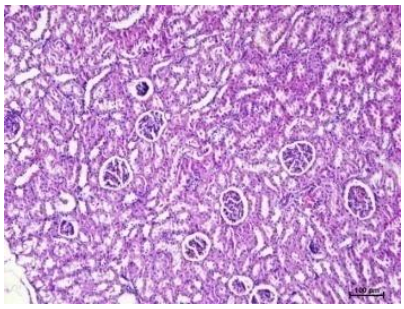
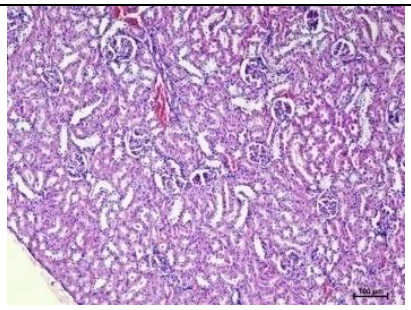
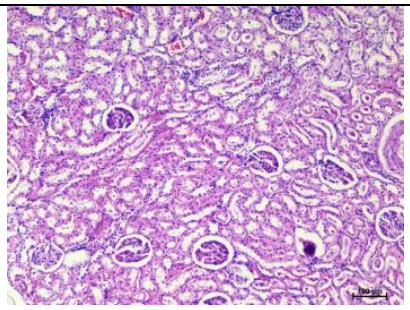
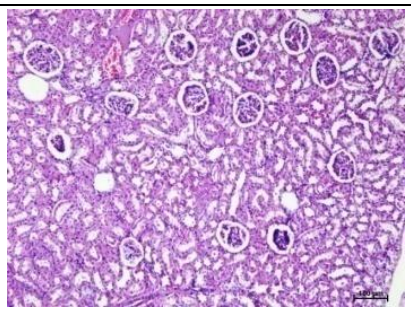
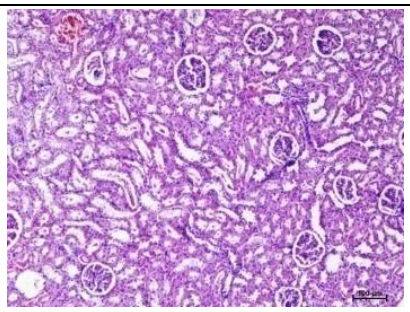
	T2--1		
10	Brewer's Yeast+*Shodhita Hingula* **T2--2**	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
Note : Overall Grade score as- **NAD** =No Abnormality Detected, **Minimal changes** (+1), **Mild changes** (+2), **Moderate changes** (+3), **Severe changes** (+4).			

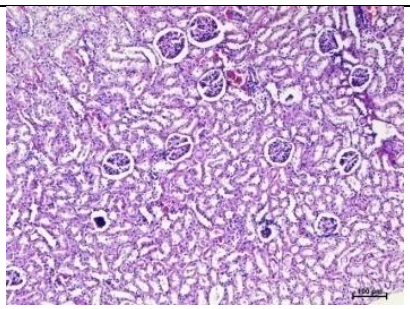
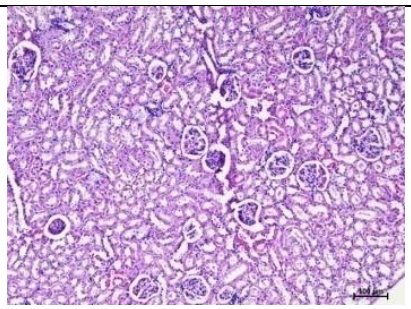
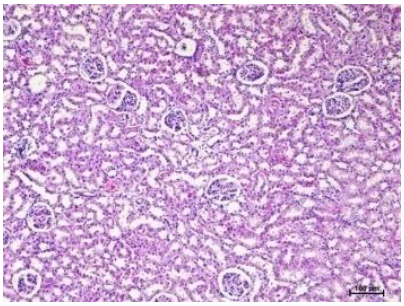
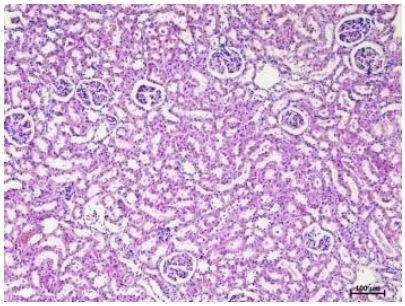
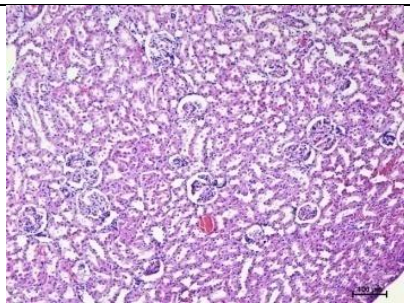
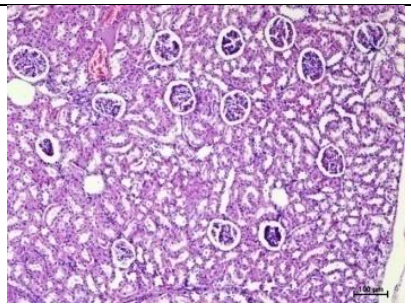
Sr. N.	Group /Slide Code	Histopathological observations 1.Liver	Overall Pathological Grade
11	Brewer's Yeast+ShodhitaVatsanabh **T3- -1**	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
12	Brewer's Yeast+ShodhitaVatsanabh **T3- 2**	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Absence of inflammatory or pathological changes in hepatic parenchyma.	**NAD**
13	Brewer's Yeast+ *Pippali***T4-1**	Focal congested vascular tissue. Focal and occasional cellular swelling of hepatocytes. Minimal degenerative changes of hepatocytes with granular cytoplasmic changes. Absence of inflammatory changes in hepatic parenchyma.	**Minimal (+1)**
14	Brewer's Yeast+ *Pippali***T4-2**	Normal histomorphological cellular features of hepatocytes with intact nucleus and cell borders. Focal congested vascular tissue. Focal and occasional cellular swelling of hepatocytes. Focal congested vascular tissue. Absence of inflammatory changes in hepatic parenchyma.	**NAD to Minimal (+1)**

Note : Overall Grade score as-

NAD =No Abnormality Detected, **Minimal changes** (+1), **Mild changes** (+2), **Moderate changes** (+3), **Severe changes** (+4).

5.38.8photos and charts of observations of Effect of test drug on histopathology of kidney after antipyretic study

	
Normal Control NC 1	**Normal Control NC 2**
	
Disease Control 1	**Disease Control 2**
	
Brewer's Yeast+ Paracetamol **STD 1**	Brewer's Yeast+ Paracetamol **STD2**
	
Brewer's Yeast+ *Hinguleshvara Rasa* **T1- 1**	Brewer's Yeast+ *Hinguleshvara Rasa* **T1- 2**

	
Brewer's Yeast+*Shodhita Hingula* **(Red Cinnabar:HgS)** **T2-- 1**	Brewer's Yeast+*Shodhita Hingula* **(Red Cinnabar:HgS)** **T2—2**
	
Brewer's Yeast+***Shuddha Vatsanabh*** **(*Aconitum balfourii* Stapf)T3- - 1**	Brewer's Yeast+ ***Shuddha Vatsanabh*** **(*Aconitum balfourii* Stapf)T3- - 2**
	
Brewers Yeast+*Pippali* ***Piper longum* Linn)T4- 1**	**Brewers Yeast+*Pippali*** ***Piper longum* Linn)T4- 2**

Effect of test drug on histopathology of kidney after antipyretic study

Sr. N.	Group / Slide Code	Histopathological observations 2. Kidney	Overall Pathological Grade
1	**Normal ControlNC -1**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
2	**Normal ControlNC -2**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
3	**Disease Control-1**	Focal congested vascular tissue and interstitial hemorrhages in renal parenchyma. Minimal and Focal and cellular swelling of tubular epithelium. Minimal degenerative changes of tubular epithelium with granular cytoplasmic changes. Absence of inflammatory changes in hepatic parenchyma.	**Minimal (+1)**
4	**Disease Control-2**	Focal congested vascular tissue and interstitial hemorrhages in renal parenchyma. Minimal and Focal and cellular swelling of tubular epithelium. Minimal degenerative changes of tubular epithelium with granular cytoplasmic changes. Absence of inflammatory changes in hepatic parenchyma.	**Minimal (+1)**
5	Brewer's Yeast+ Paracetamol **STD -1**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**

6	Brewer's Yeast+ Paracetamol **STD -2**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
7	Brewer's Yeast+ *Hinguleshvara Rasa* **T1-1**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
8	Brewer's Yeast+ *Hinguleshvara Rasa* **T1-2**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
9	Brewer's Yeast + *Shodhita Hingula* **T2--1**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
10	Brewer's Yeast+ *Shodhita Hingula* **T2--2**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**

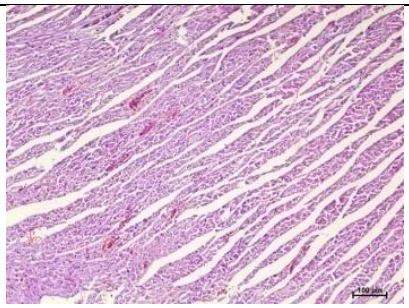
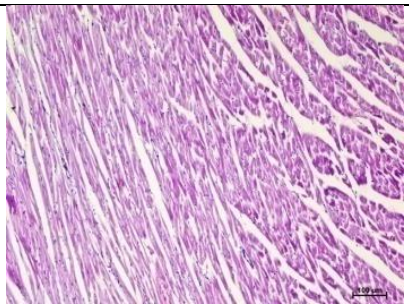
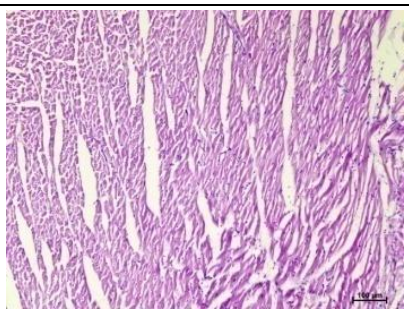
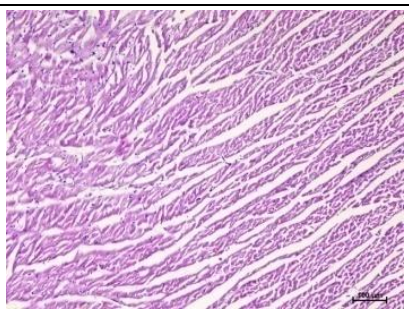
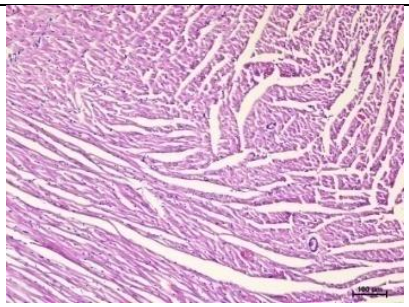
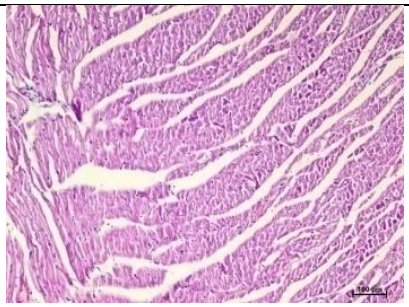
Note : Overall Grade score as-

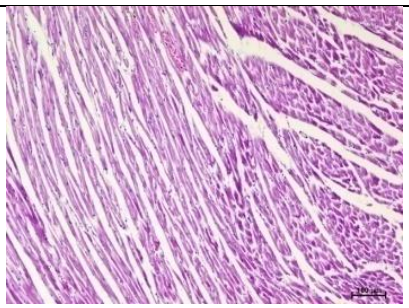
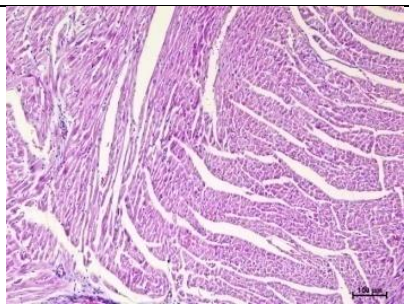
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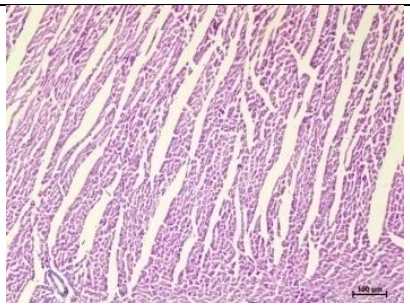
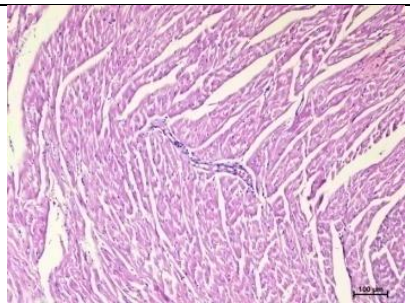
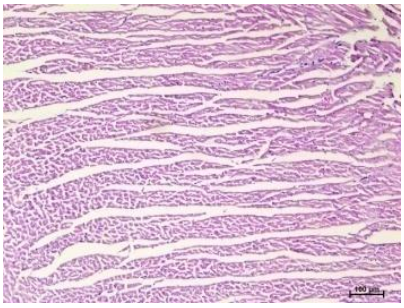
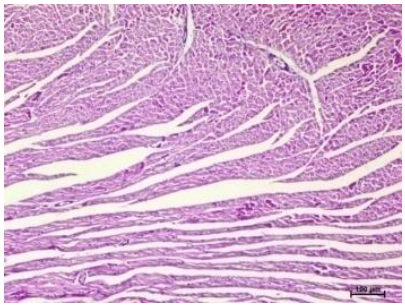
Sr. N.	Group /Slide Code	Histopathological observations 2. Kidney	Overall Pathological Grade
11	Brewer's Yeast+ShodhitaVatsanabh**T3- -1**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
12	Brewer's Yeast+ShodhitaVatsanabh**T3- -2**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
13	Brewer's Yeast+ *Pippali***T4-1**	Focal congested vascular tissue and interstitial hemorrhages in renal parenchyma. Minimal and Focal and cellular swelling of tubular epithelium. Minimal degenerative changes of tubular epithelium with granular cytoplasmic changes. Absence of inflammatory changes in hepatic parenchyma.	**Minimal (+1)**
14	Brewer's Yeast+ *Pippali***T4-2**	Normal histomorphological cellular features of renal tubules and glomeruli in the renal cortex and medulla region. Absence of inflammatory or pathological changes in renal parenchyma.	**NAD**
Note : Overall Grade score as- **NAD** =No Abnormality Detected, **Minimal changes** (+1), **Mild changes** (+2), **Moderate changes** (+3), **Severe changes** (+4).			

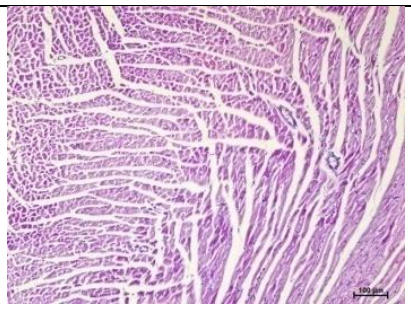
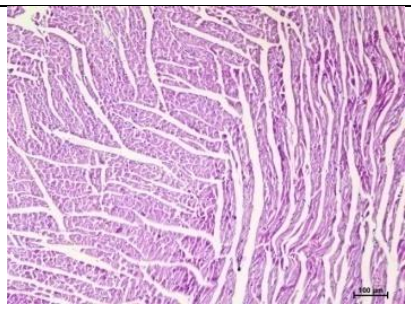
5.38.9. photos and charts of observations of Effect of test drug on histopathology of heart

Effect of test drug on histopathology of heart after antipyretic study

	
Normal Control NC 1	**Normal Control NC 2**
	
Disease Control1	**Disease Control 2**
	
Brewer's Yeast+ Paracetamol **STD1**	Brewer's Yeast+ Paracetamol **STD 2**

	
Brewer's Yeast+ *Hinguleshvara Rasa* **T1- 1**	Brewer's Yeast+ *Hinguleshvara Rasa* **T1- 2**

	
Brewer's Yeast+*Shodhita Hingula* **(Red Cinnabar:HgS) T2-- 1**	Brewer's Yeast+*Shodhita Hingula* **(Red Cinnabar:HgS) T2—2**
	
Brewer's Yeast+ ***Shuddha Vatsanabh*** **(*Aconitum balfourii* Stapf)T3- - 1**	Brewer's Yeast+ ***Shuddha Vatsanabh*** **(*Aconitum balfourii* Stapf)T3- - 2**

	
Brewers Yeast+*Pippali* ***Piper longum*** **Linn)T4- 1**	**Brewers Yeast+*Pippali*** ***Piper longum*** **Linn)T4- 2**

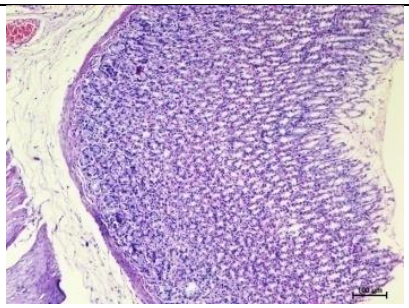
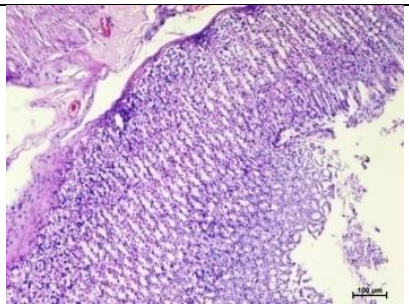
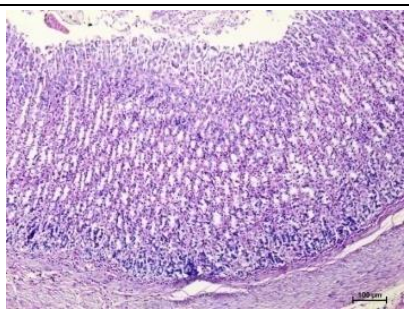
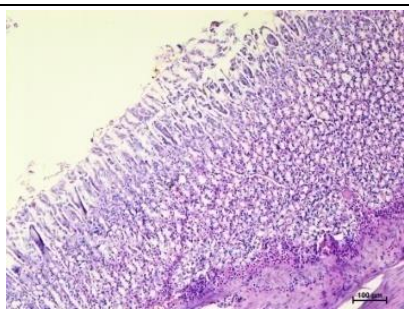
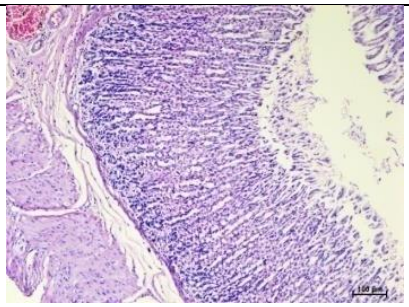
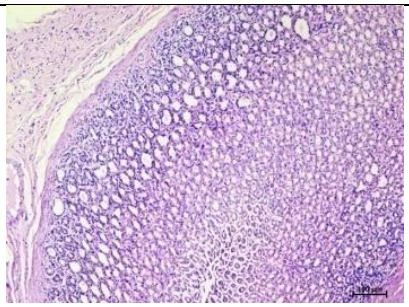
Sr. N.	Group / Slide Code	Histopathological observations 3. Heart	Overall Pathological Grade
1	**Norml Contrl NC-1**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
2	**Norml Contrl NC-2**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
3	**Disease Contrl-1**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Focal congested coronary vascular tissue and focal interstitial hemorrhages. Absence of inflammatory or pathological changes in heart.	**NAD**
4	**Disease Contrl-2**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**

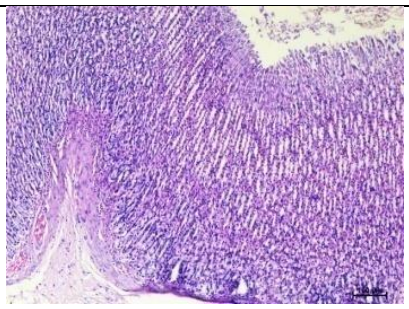
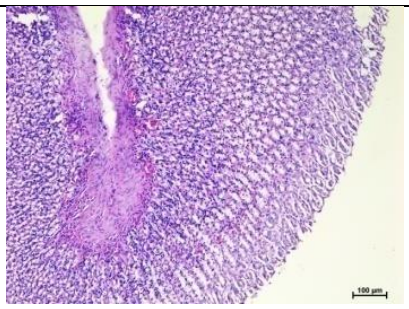
5	Brewer's Yeast+ Paracetamol **STD -1**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Focal congested coronary vascular tissue and focal interstitial hemorrhages. Absence of inflammatory or pathological changes in heart.	**NAD**
6	Brewer's Yeast+ Paracetamol **STD -2**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
7	Brewer's Yeast+ *Hinguleshvara Rasa* **T1-1**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Focal congested coronary vascular tissue and focal interstitial hemorrhages. Absence of inflammatory or pathological changes in heart.	**NAD**
8	Brewer's Yeast+ *Hinguleshvara Rasa* **T1-2**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
9	Brewer's Yeast+*ShodhitaHingula* **T2--1**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
10	Brewer's Yeast+*ShodhitaHingula*	Normal histomorphological features of cardiac muscle fibers in the myocardium. Focal congested coronary vascular tissue and focal interstitial hemorrhages.	**NAD**

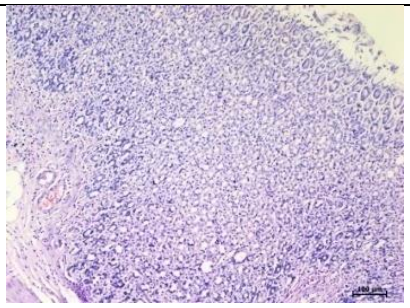
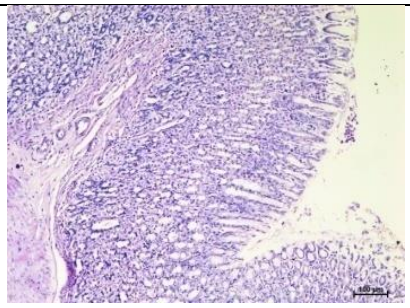
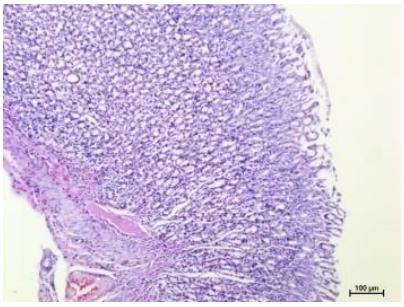
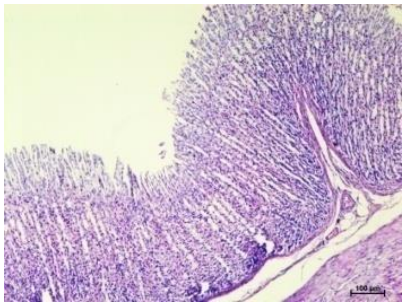
	T2--2	Absence of inflammatory or pathological changes in heart.	
Note : Overall Grade score as- **NAD** =No Abnormality Detected, **Minimal changes** (+1), **Mild changes** (+2), **Moderate changes** (+3), **Severe changes** (+4).			

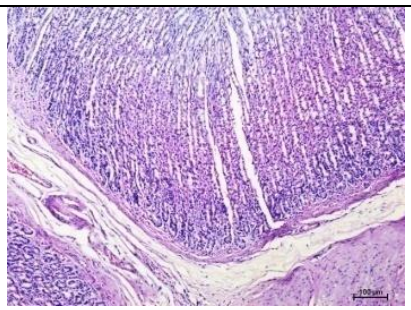
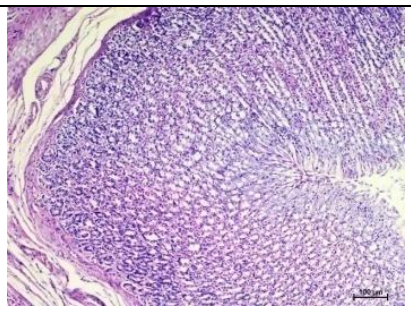
Sr. N.	**Group /Slide Code**	**Histopathological observations** **3. Heart**	**Overall Pathological Grade**
11	Brewer's Yeast+ShodhitaVatsanabh**T 3- -1**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
12	Brewer's Yeast+ShodhitaVatsanabh**T 3- -2**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
13	Brewer's Yeast+ *Pippali***T4-1**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
14	Brewer's Yeast+ *Pippali***T4-2**	Normal histomorphological features of cardiac muscle fibers in the myocardium. Absence of inflammatory or pathological changes in heart.	**NAD**
Note : Overall Grade score as- **NAD** =No Abnormality Detected, **Minimal changes** (+1), **Mild changes** (+2), **Moderate changes** (+3), **Severe changes** (+4).			

5.38.10.photos and charts of observations of Effect of test drug on histopathology of gastric mucosa after antipyretic study

	
Normal ControlNC 1	**Normal ControlNC 2**
	
Disease Control 1	**Disease Control 2**
	
Brewer's Yeast+ Paracetamol **STD 1**	Brewer's Yeast+ Paracetamol **STD 2**

	
Brewer's Yeast+ *Hinguleshvara Rasa* **T1- 1**	Brewer's Yeast+ *Hinguleshvara Rasa* **T1- 2**

	
Brewer's Yeast+*Shodhita Hingula* **(Red Cinnabar:HgS) T2-- 1**	Brewer's Yeast+*Shodhita Hingula* **(Red Cinnabar:HgS) T2—2**
	
Brewer's Yeast+ ***Shuddha Vatsanabh*** **(*Aconitum balfourii* Stapf)T3- - 1**	Brewer's Yeast+ ***Shuddha Vatsanabh*** **(*Aconitum balfourii* Stapf)T3- - 2**

	
Brewers Yeast+*Pippali* ***Piper longum* Linn)T4- 1**	**Brewers Yeast+*Pippali*** ***Piper longum* Linn)T4- 2**

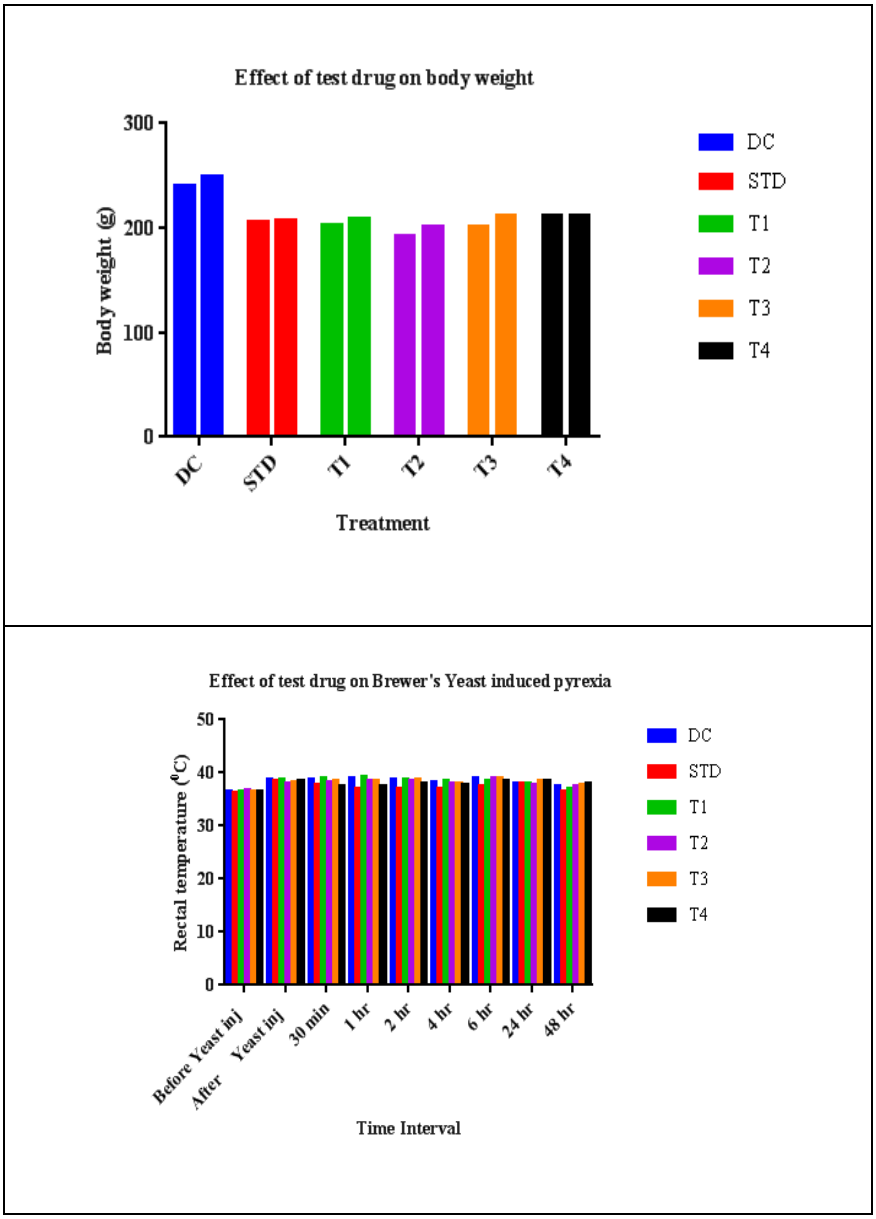
Sr. N.	Group / Slide Code	Histopathological observations 4. Gastric mucosa	Overall Pathological Grade
1	**Normal Control NC-1**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
2	**Normal Control NC-2**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
3	**Disease Control-1**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
4	**Disease Control-2**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
5	Brewer's Yeast+ Paracetamol **STD -1**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
6	Brewer's Yeast+ Paracetamol **STD -2**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
7	Brewer's Yeast+ *Hinguleshvara Rasa* **T1-1**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**

8	Brewer's Yeast+ *Hinguleshvara Rasa* **T1-2**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
9	Brewer's Yeast+*Shodhita Hingula* **T2--1**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
10	Brewer's Yeast+*ShodhitaHingula* **T2--2**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
Note : Overall Grade score as- **NAD** =No Abnormality Detected, **Minimal changes** (+1), **Mild changes** (+2), **Moderate changes** (+3), **Severe changes** (+4).			
Sr. N.	**Group /Slide Code**	**Histopathological observations** **4. Gastric mucosa**	**Overall Pathological Grade**
11	Brewer's Yeast+Shodhita Vatsanabh **T3- -1**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
12	Brewer's Yeast+Shodhita Vatsanabh **T3- -2**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
13	Brewer's Yeast+ *Pippali* **T4-1**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
14	Brewer's Yeast+ *Pippali* **T4-2**	Normal histological features of mucosal layers of glandular stomach with normal submucosal tissue.	**NAD**
Note : Overall Grade score as- **NAD** =No Abnormality Detected, **Minimal changes** (+1), **Mild changes** (+2), **Moderate changes** (+3), **Severe changes** (+4).			

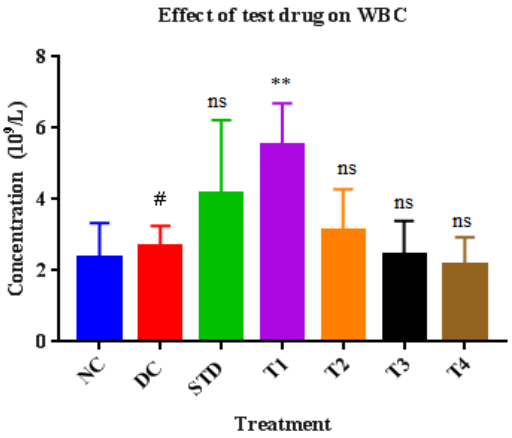
5.39 Statistical Analysis:

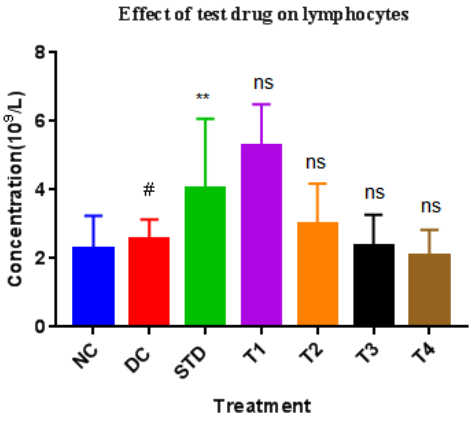
Part A: EXPERIMENT FOR ANTIPYRETIC ACTIVITY:-

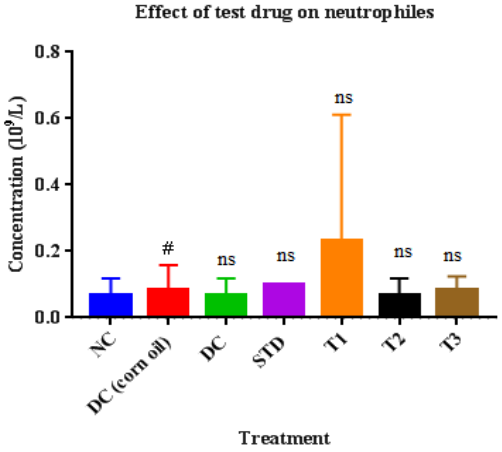
5.39.1. Effect test drug on body weight and body temperature in Brewer's Yeast induced pyrexia (n-6). Data is represented as mean ± SD.

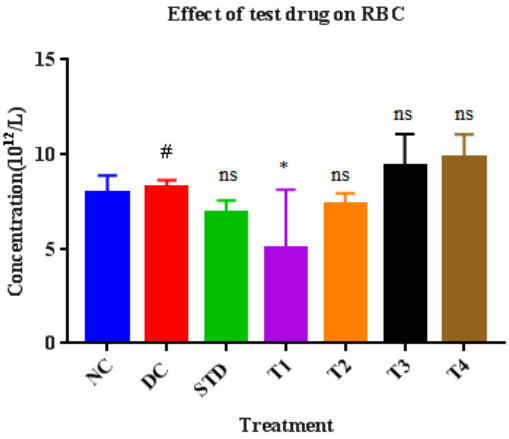


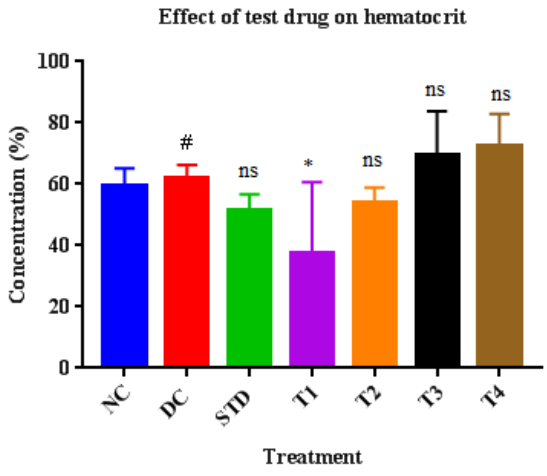
5.39.2. Effect test drug on hematological parameters in Brewer's Yeast induced pyrexia (n-6). Data is represented as mean ± SD. ####P<0.0001, ### P<0.001, ## P<0.01, #P<0.05 as compared to normal Control, ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, ns= nonsignificant

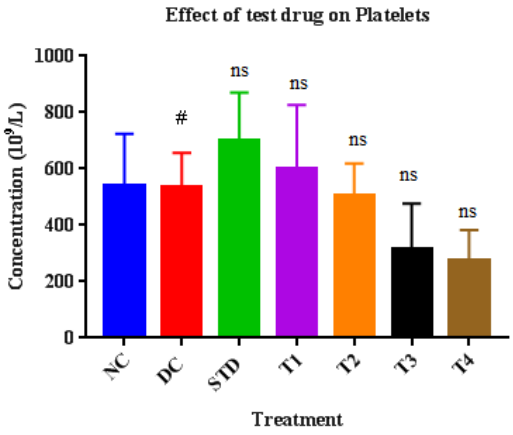




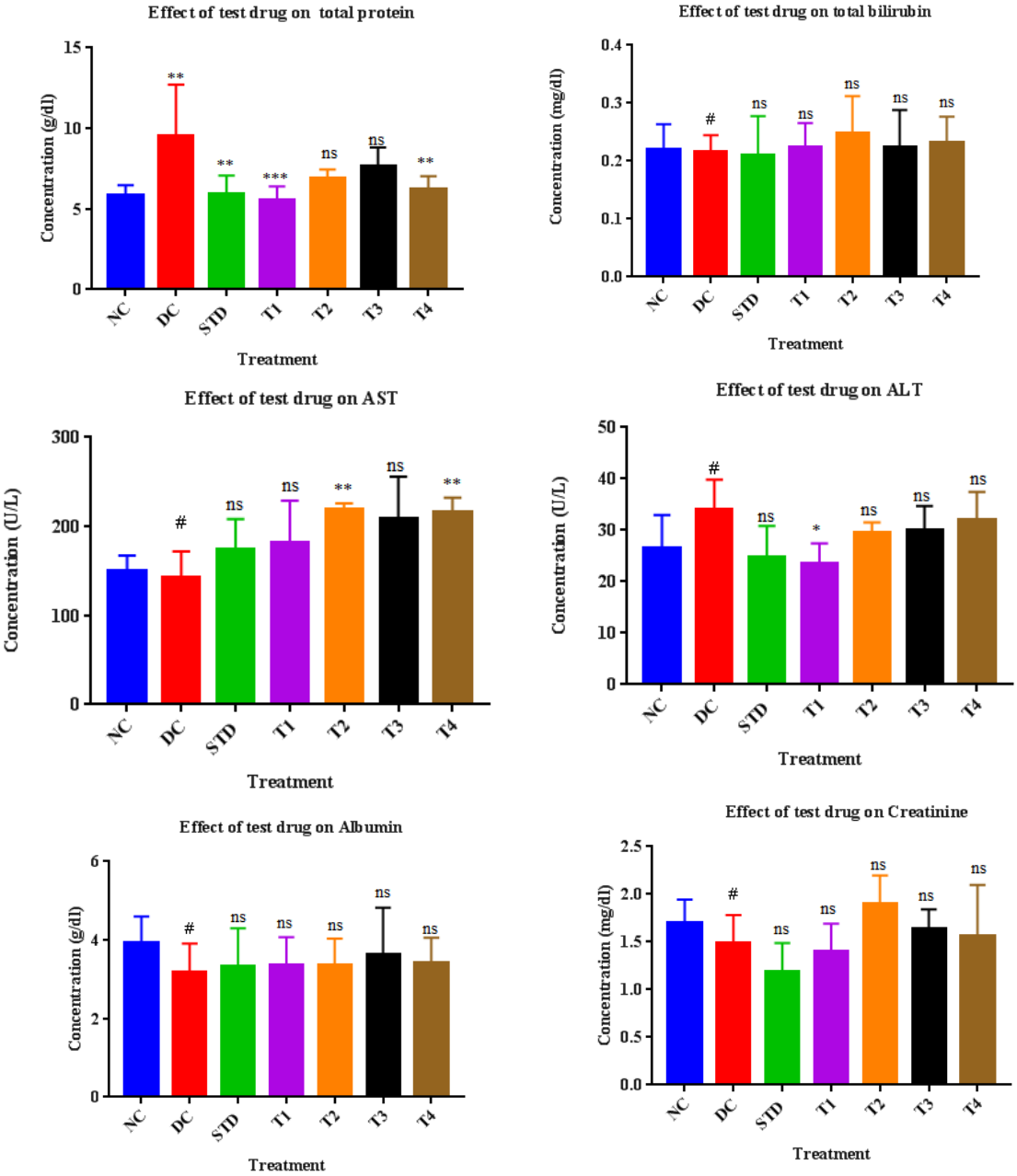


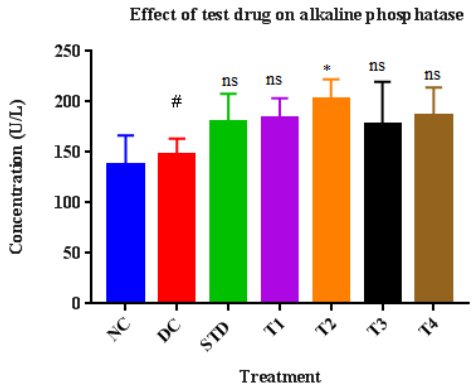


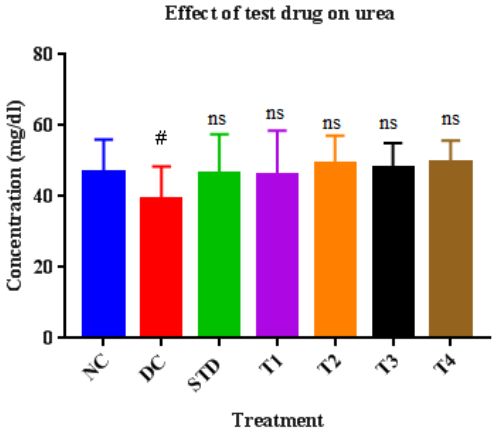




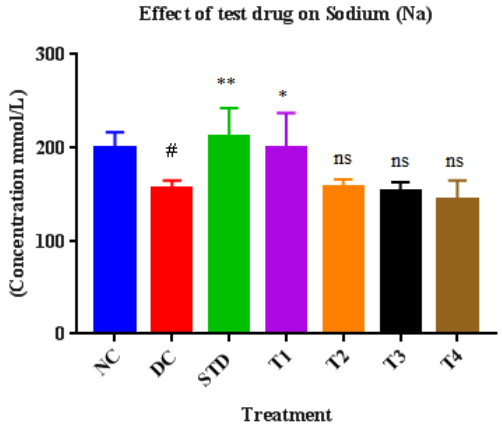
5.39.3. Effect test drug on biochemical parameters (n-6). Data is represented as mean ± SD. ####P<0.0001, ### P<0.001, ## P<0.01, #P<0.05 as compared to normal Control, ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, ns= nonsignificant.

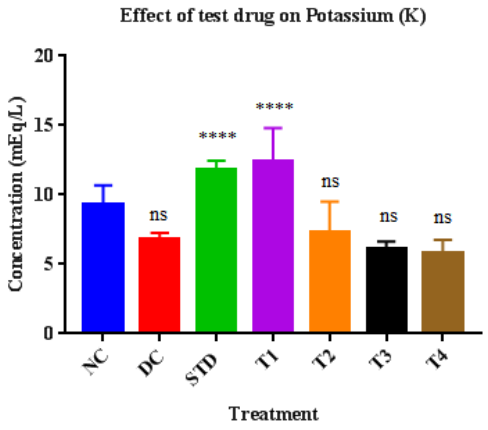


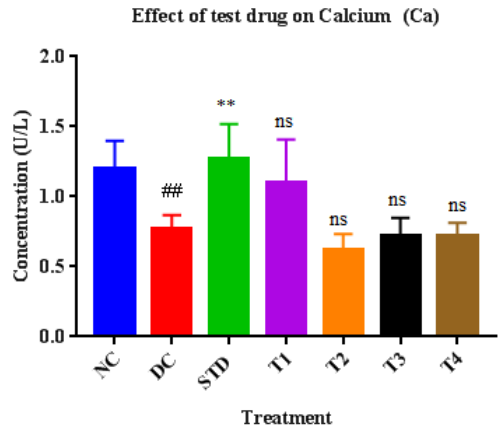


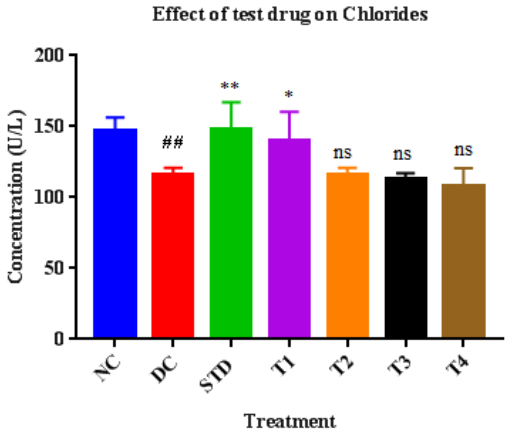


5.39.4. Effect test drug on electrolytes parameters (n-6). Data is represented as mean ± SD. ####P<0.0001, ### P<0.001, ## P<0.01, #P<0.05 as compared to normal Control, ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, ns= nonsignificant.

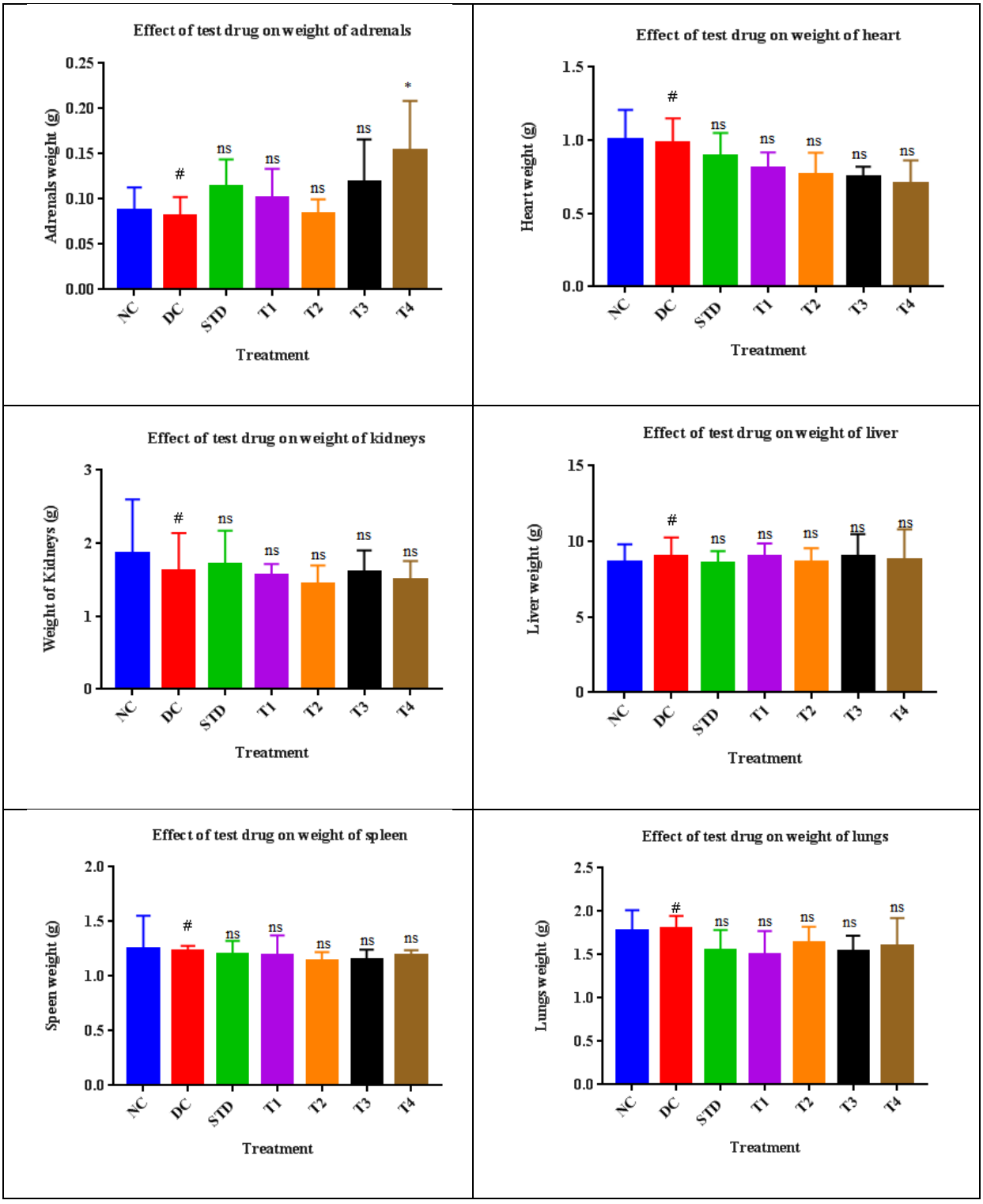


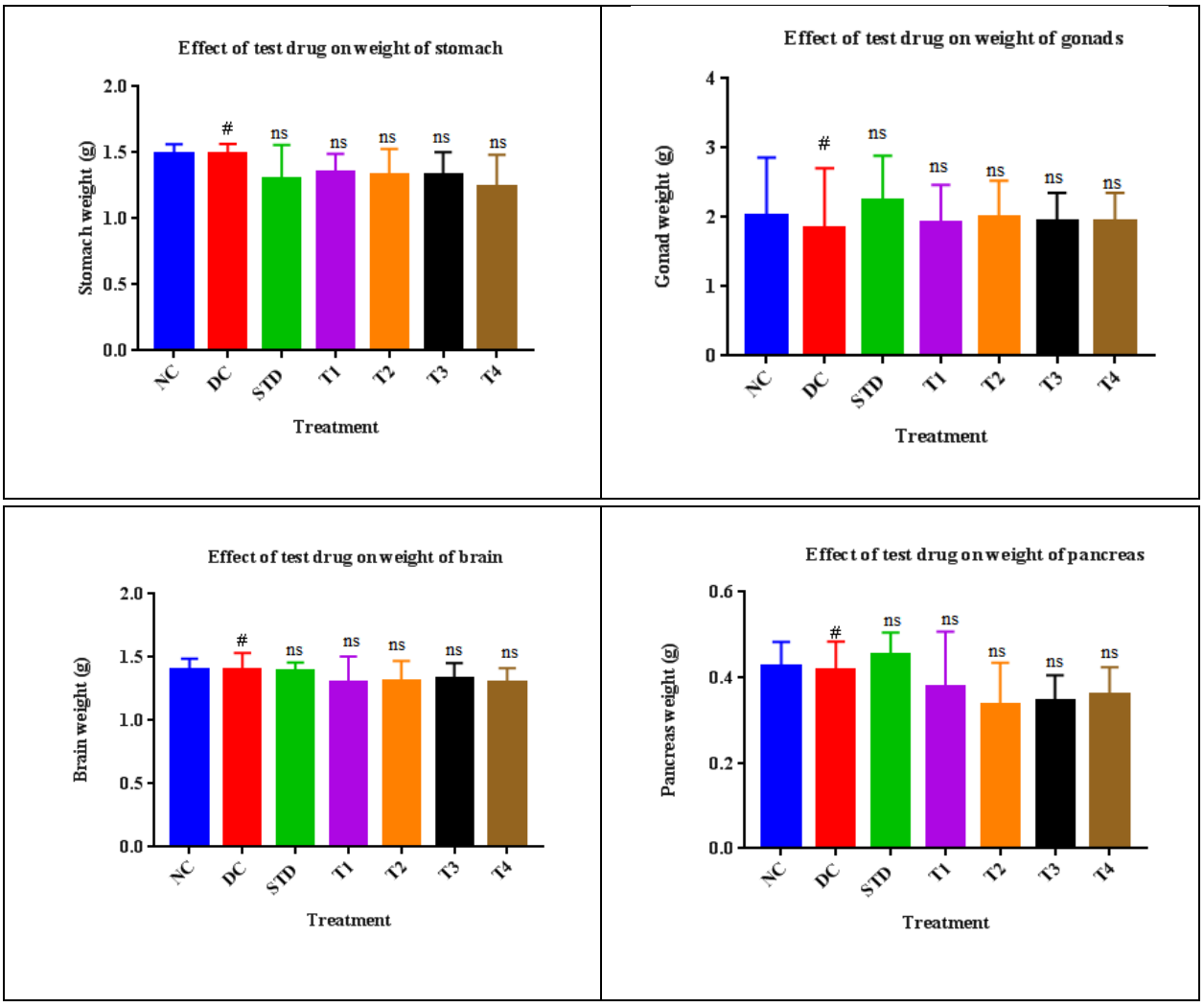






5.39.5. Effect test drug on organ weight (n-6). Data is represented as mean ± SD. ####P<0.0001, ### P<0.001, ## P<0.01, #P<0.05 as compared to normal Control, ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, ns= nonsignificant




Effect of test drug on weight of stomach
Stomach weight (g)
2.0
1.5
1.0
0.5
0.0
#
ns
ns
ns
ns
ns
NC
DC
STD
T1
T2
T3
T4
Treatment
Effect of test drug on weight of gonads
Gonad weight (g)
4
3
2
1
0
#
ns
ns
ns
ns
ns
NC
DC
STD
T1
T2
T3
T4
Treatment
Effect of test drug on weight of brain
Brain weight (g)
2.0
1.5
1.0
0.5
0.0
#
ns
ns
ns
ns
ns
NC
DC
STD
T1
T2
T3
T4
Treatment
Effect of test drug on weight of pancreas
Pancreas weight (g)
0.6
0.4
0.2
0.0
#
ns
ns
ns
ns
ns
NC
DC
STD
T1
T2
T3
T4
Treatment

5.40 Reports of EXPERIMENT OF ANALGESIC ACTIVITY:

Following tests were conducted on (Swiss Albino female mice) to test analgesic activity as per chapter 4.12:-

1)Writhing assay/Acetic acid induced writhing method (As per 4.12.d-2) [217-218]:

2) **Hot plate test(As per 4.12.d-3) [219-222]**

3)**Formalin test(As per 4.12d-1)** .[215-216]

EXPERIMENT FOR ANALGESIC ACTIVITY:-

5.40.1 Effect of test drug on body weight (g) (Writhing)

Group	Animal No.	Marking	Sex	G
Disease Control	**1**	**1**	**F**	29.0
	2	**2**	**F**	34.0
	3	**3**	**F**	31.0
	4	**1**	**F**	36.0
	5	**2**	**F**	30.0
	6	**3**	**F**	35.0
	MEAN			32.5
	SD			2.9
STD- Diclofenac (10mg/Kg)	**7**	**1**	**F**	40.5
	8	**2**	**F**	32.0
	9	**3**	**F**	38.0
	10	**1**	**F**	47.0
	11	**2**	**F**	37.0
	12	**3**	**F**	32.5
	MEAN			37.8
	SD			5.6
T *1 Hinguleshvara Rasa (8.13 mg/kg*)	**13**	**1**	**F**	36.0
	14	**2**	**F**	31.0
	15	**3**	**F**	29.0
	16	**1**	**F**	32.0
	17	**2**	**F**	34.0
	18	**3**	**F**	35.0
	MEAN			32.8
	SD			2.6

Group	Animal No.	Marking	Sex	g
T 2- *Shuddha Hingula(*Red Cinnabar :HgS)*(*16.25 mg/kg)	**19**	**1**	**F**	49.0
	20	**2**	**F**	40.0
	21	**3**	**F**	37.0
	22	**1**	**F**	35.0
	23	**2**	**F**	37.0
	24	**3**	**F**	50.0
	MEAN			**41.3**
	SD			**6.5**
T 3- *Pippali(Piper longum* Linn)(97.50 mg/kg)	**25**	**1**	**F**	39.0
	26	**2**	**F**	32.0
	27	**3**	**F**	35.0
	28	**1**	**F**	45.0
	29	**2**	**F**	39.0
	30	**3**	**F**	39.0
	MEAN			**38.2**
	SD			**4.4**
T 4- *Shuddha Vatsanabh (Aconitum balfourii Stapf) (2.03 mg/kg*)	**31**	**1**	**F**	42.0
	32	**2**	**F**	38.0
	33	**3**	**F**	34.0
	34	**1**	**F**	39.0
	35	**2**	**F**	34.0
	36	**3**	**F**	39.5
	MEAN			**37.8**
	SD			**3.2**

5.40.2. Effect of test drug on body weight (g) (Hot Plate)

Group	Animal No.	Marking	Sex	g
Disease control	**1**	**1**	**F**	45.0
	2	**2**	**F**	38.0
	3	**3**	**F**	37.0
	4	**1**	**F**	42.0
	5	**2**	**F**	58.0
	6	**3**	**F**	51.0
	MEAN			45.2
	SD			8.1
STD-Pentazocine (1.4 mg/kg)	**7**	**1**	**F**	51.0
	8	**2**	**F**	39.0
	9	**3**	**F**	41.0
	10	**1**	**F**	42.0
	11	**2**	**F**	52.0
	12	**3**	**F**	57.0
	MEAN			47.0
	SD			7.3
T 1 *Hinguleshvara Rasa* **(8.13 mg/kg)**	**13**	**1**	**F**	49.0
	14	**2**	**F**	43.0
	15	**3**	**F**	39.0
	16	**1**	**F**	51.0
	17	**2**	**F**	56.0
	18	**3**	**F**	42.0
	MEAN			46.7
	SD			6.4

Group	Animal No.	Marking	Sex	g
T 2- *Shuddha Hingula(*Red Cinnabar :HgS) (16.25 mg/kg)	**19**	**1**	**F**	42.0
	20	**2**	**F**	46.0
	21	**3**	**F**	35.0
	22	**1**	**F**	46.0
	23	**2**	**F**	43.0
	24	**3**	**F**	51.0
	MEAN			43.8
	SD			5.3
T 3- *Pippali(Piper longum* Linn)((97.50 mg/kg)	**25**	**1**	**F**	46.0
	26	**2**	**F**	51.0
	27	**3**	**F**	56.0
	28	**1**	**F**	48.0
	29	**2**	**F**	42.0
	30	**3**	**F**	49.0
	MEAN			48.7
	SD			4.7
T 4- *Shuddha Vatsanabh(Aconitum balfourii* Linn) ((2.03 mg/kg)	**31**	**1**	**F**	49.0
	32	**2**	**F**	51.0
	33	**3**	**F**	52.0
	34	**1**	**F**	39.0
	35	**2**	**F**	48.0
	36	**3**	**F**	51.0
	MEAN			48.3
	SD			4.8

5.40.3. Effect of test drug on body weight (g) (Formalin test)

Group	Animal No.	Marking	Sex	g
DC	**1**	**1**	**F**	46.0
	2	**2**	**F**	41.0
	3	**3**	**F**	37.0
	4	**1**	**F**	41.0
	5	**2**	**F**	52.0
	6	**3**	**F**	54.0
	MEAN			**45.2**
	SD			**6.7**
STD- Diclofenac (10mg/Kg)	**7**	**1**	**F**	46.0
	8	**2**	**F**	44.0
	9	**3**	**F**	38.0
	10	**1**	**F**	51.0
	11	**2**	**F**	57.0
	12	**3**	**F**	53.0
	MEAN			**48.2**
	SD			**6.9**
T 1 *Hinguleshvara Rasa* **(8.13 mg/kg)**	**13**	**1**	**F**	48.0
	14	**2**	**F**	52.0
	15	**3**	**F**	48.0
	16	**1**	**F**	47.0
	17	**2**	**F**	49.0
	18	**3**	**F**	38.0
	MEAN			**47.0**
	SD			**4.7**

Group	Animal No.	Marking	Sex	g
T 2- *Shuddha Hingula(*Red Cinnabar :HgS) (16.25 mg/kg)	**19**	**1**	**F**	43.0
	20	**2**	**F**	33.0
	21	**3**	**F**	44.0
	22	**1**	**F**	45.0
	23	**2**	**F**	44.0
	24	**3**	**F**	44.0
	MEAN			**42.2**
	SD			**4.5**
T 3- *Pippali* (97.50 mg/kg)	**25**	**1**	**F**	40.0
	26	**2**	**F**	47.0
	27	**3**	**F**	38.0
	28	**1**	**F**	52.0
	29	**2**	**F**	45.0
	30	**3**	**F**	48.0
	MEAN			**45.0**
	SD			**5.2**
T 4- *Shuddha Vatsanabh(Aconitum balfourii* Linn) ((2.03 mg/kg)	**31**	**1**	**F**	52.0
	32	**2**	**F**	48.0
	33	**3**	**F**	57.0
	34	**1**	**F**	42.0
	35	**2**	**F**	44.0
	36	**3**	**F**	54.0
	MEAN			**49.5**
	SD			**5.9**

5.40.4. Effect of test drug on Acetic Acid induced Writhing

Group	Animal No.	Onset timing	No. of writhing
Disease Control DC	**1**	4:00	14.0
	2	**3:00**	29.0
	3	**5:00**	36.0
	4	**1:20**	31.0
	5	**1:20**	50.0
	6	**1:21**	44.0
	Mean		**34.0**
	SD		**11.5**
STD- Diclofenac (10mg/Kg)	**7**	**2:51**	11.0
	8	**2:59**	9.0
	9	**2:58**	19.0
	10	**3:33**	16.0
	11	**3:33**	16.0
	12	**3:33**	30.0
	Mean		**16.8**
	SD		**6.8**
T 1 *Hinguleshvara Rasa* **(8.13 mg/kg)**	**13**	**4:06**	31
	14	**4:07**	21
	15	**4:05**	46
	16	**4:30**	31
	17	**4:30**	22
	18	**4:31**	27
	Mean		**29.7**
	SD		**8.3**

Group	Animal No.	Onset timing	No. of writhing
Test-2 *Shuddha Hingula(*Red Cinnabar :HgS)16.25 mg/kg)	**19**	**5:08**	17
	20	**5:09**	18
	21	**5:08**	14
	22	**5:31**	20
	23	**5:31**	22
	24	**5:32**	23
	Mean		**19.0**
	SD		**3.1**
Test-3 *Pippali(Piper longum* Linn)(97.50 mg/kg)	**25**	**11:35**	26
	26	**11:40**	62
	27	**11:44**	39
	28	**12:07**	37
	29	**12:05**	33
	30	**12:05**	70
	Mean		44.5
	SD		15.9
Test-4 (*Shuddha Vatsanabh(Aconitum balfourii* Linn) (-2.03 mg/kg)	**31**	**1:22**	52
	32	**1:21**	38
	33	**1:21**	27
	34	**1:47**	36
	35	**1:47**	33
	36	**1:51**	14
	Mean		33.3
	SD		11.5

Mean of Acetic Acid induced Writhing

Group	No. of writhing
Disease Control DC	**34.0**
Diclofenac (10mg/Kg)	**16.8**
T 1 (*Hinguleshvara Rasa* **-8.13 mg/kg)**	**29.7**
T 2 *Shuddha Hingula (*Red Cinnabar :HgS)- 16.25 mg/kg)	**19.0**
T 3 *Pippali (Piper longum* Linn)(97.50 mg/kg)	**44.5**
T 4 (Shuddha *Vatsanabh (Aconitum balfourii* Linn) (- 2.03 mg/kg)	**33.3**

5.40.5. Effect of test drug on hot plate analgesia

Group	Animal No.	Reaction in Sec			
		30 min	**60min**	**90min**	**120min**
Disease Control DC	**1**	4	5	3	4
	2	6	6	3	5
	3	6	6	4	6
	4	5	7	4	4
	5	5	5	4	5
	6	4	6	2	4
	Mean	5.0	5.8	3.3	4.7
	SD	0.9	0.8	0.8	0.8
STD- Pentazocine (1.4 mg/kg)	**7**	8	-	10	-
	8	8	10	10	-
	9	9	13	-	-
	10	8	-	11	-
	11	9	-	9	11
	12	10	-	10	11
	Mean	8.7	11.5	10.0	11.0
	SD	0.8	2.1	0.7	0.0
T 1 *Hinguleshvara Rasa* **(8.13 mg/kg)**	**13**	-	-	-	-
	14	10	-	-	11
	15	-	-	-	-
	16	12	-	13	12
	17	8	12	12	12
	18	8	9	10	10
	Mean	9.5	10.5	11.7	11.3
	SD	1.9	2.1	1.5	1.0

Group	Animal No.	Reaction in Sec			
		30 min	**60min**	**90min**	**120min**
Test-2 *Shuddha Hingula(*Red Cinnabar :HgS)*a-16.2*5 mg/kg)	**19**	-	10	9	12
	20	-	9	-	-
	21	-	-	11	-
	22	-	-	12	-
	23	-	13	9	-
	24	-	-	10	-
	Mean	0.0	10.7	10.2	12.0
	SD	0.0	2.1	1.3	0.0
Test-*3 (Pimpali Piper longum Linn 97.50* mg/kg)	**25**	9	-	-	6
	26	11	-	10	8
	27	8	-	-	-
	28	9	13	5	8
	29	12	10	8	4
	30	-	-	4	-
	Mean	8.2	11.5	6.8	6.5
	SD	4.3	2.1	2.8	2.3
Test-4 Shuddha *Vatsanabh(Aconitum balfourii* Linn) (-2.03 mg/kg)	**31**	-	10	-	-
	32	9	-	-	-
	33	-	-	-	-
	34	8	12	11	12
	35	-	9	10	-
	36	-	10	8	-
	Mean	8.5	10.3	9.7	12.0
	SD	0.7	1.3	1.5	0.0

Mean of effect of test drug on hot plate analgesia

Group	Reaction in Sec			
	30 min	**60min**	**90min**	**120min**
Disease Control DC	5.0	5.8	3.3	4.7
STD- Pentazocine (1.4 mg/kg)	8.7	11.5	10.0	11.0
T 1 *Hinguleshvara Rasa* **(8.13 mg/kg)**	9.5	10.5	11.7	11.3
T 2 ***Shuddha Hingula(*Red Cinnabar :HgS) (16.25 mg/kg)**	00	10.7	10.2	12.0
T 3 ***Pippali(Piper longum*** **Linn)((97.50 mg/kg)**	8.2	11.5	6.8	6.5
T 4 ***Shuddha Vatsanabh(Aconitum balfourii*** **Linn) ((2.03 mg/kg)**	8.5	10.3	9.7	12.0

5.40.6. Effect of test drug on formalin induced licking

Group	Animal No.	No. of times of licking	
		0-10 mins	40-50 mins
Disease Control DC	**1**	9	12
	2	9	15
	3	10	2
	4	15	15
	5	10	14
	6	12	3
	Mean	10.8	10.2
	SD	2.1	5.5
STD- Diclofenac (10mg/Kg)	**7**	15	1
	8	1	1
	9	-	-
	10	6	4
	11	16	8
	12	-	-
	Mean	9.5	3.5
	SD	7.2	3.3
T 1 *Hinguleshvara Rasa* **(8.13 mg/kg)**	**13**	18	-
	14	-	-
	15	11	-
	16	7	-
	17	-	-
	18	14	-
	Mean	12.5	0.0
	SD	4.7	0.0

Group	Animal No.	No. of times of licking	
		0-10 mins	**40-50 mins**
Test-2 *Shuddha Hingula (*Red Cinnabar :HgS) - 16.25 mg/kg)	**19**	16	2
	20	-	-
	21	1	-
	22	18	-
	23	-	-
	24	12	-
	Mean	11.8	2.0
	SD	7.6	0.0
Test-3 *Pippali(Piper longum* Linn)(97.50 mg/kg)	**25**	1	-
	26	13	-
	27	20	3
	28	7	-
	29	-	-
	30	8	-
	Mean	9.8	3.0
	SD	7.1	0.0
Test-4 (*Shuddha Vatsanabh(Aconitum balfourii* Linn) (- 2.03 mg/kg)	**31**	-	-
	32	-	-
	33	4	-
	34	15	4
	35	-	-
	36	10	2
	Mean	9.7	3.0
	SD	5.5	1.4

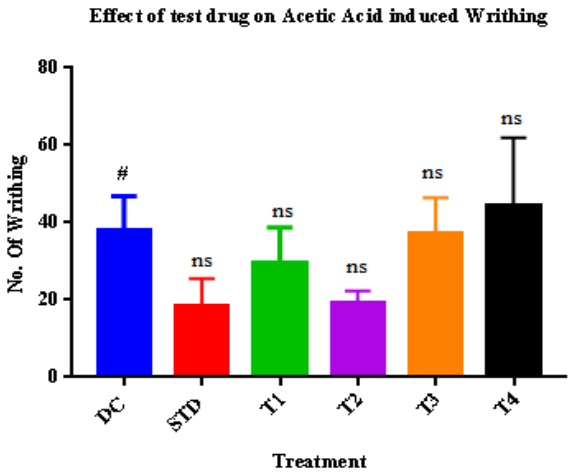
Mean of effect of test drug on formalin induced licking

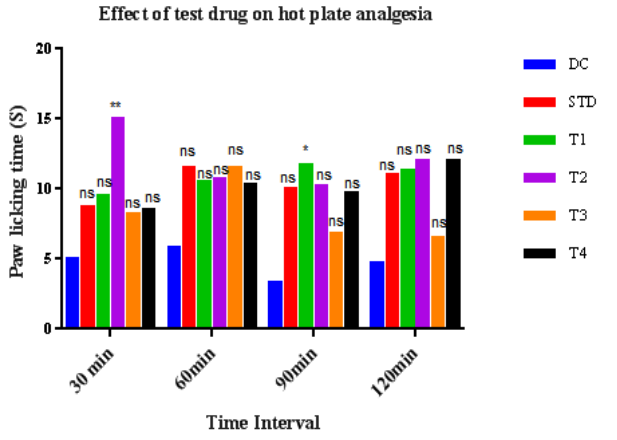
Group	No. of times of licking	
	0-10 min	**40-50mins**
Disease Control DC	10.8	10.2
STD- Diclofenac (10mg/Kg)	9.5	3.5
T 1 – *Hinguleshvara Rasa* **(8.13 mg/kg)**	12.5	0.0
T2- ***Shuddha Hingula(*****Red Cinnabar :HgS) (16.25 mg/kg)**	11.8	2.0
T3- ***Pippali(Piper longum*** **Linn) (97.50 mg/kg)**	9.8	3.0
T4- ***Shuddha Vatsanabh(Aconitum balfourii*** **Linn) (2.03 mg/kg)**	9.7	3.0

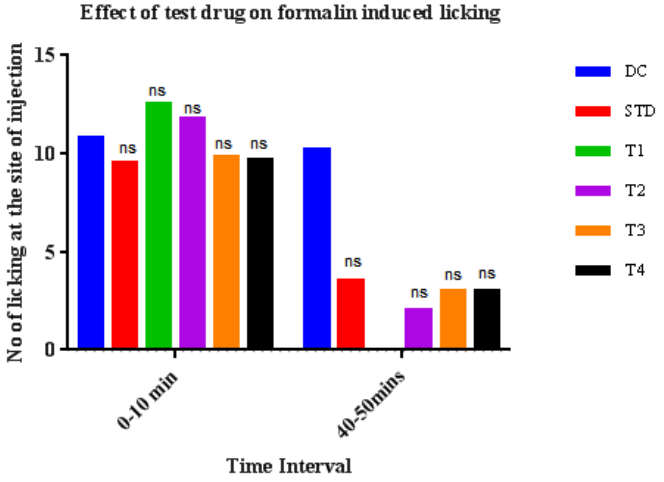
5.41Statistical Analysis:

Part B: EXPERIMENT FOR ANALGESIC ACTIVITY:-

5.41 Analgesic activity of test drug (n-6). Data is represented as mean ± SD. ####P<0.0001, ### P<0.001, ## P<0.01, #P<0.05 as compared to normal Control, ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, ns=

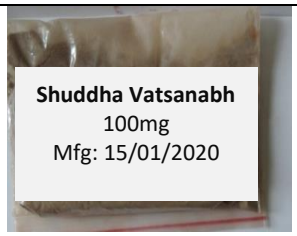
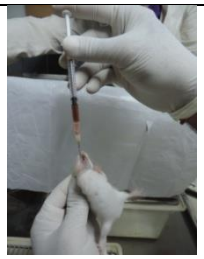
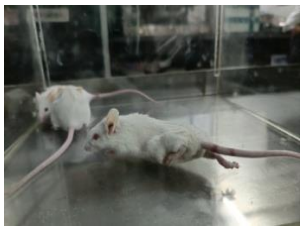






nonsignificant

5.42. Photos of Antipyretic (5.38) and Analgesic(5.40) study of *Hinguleshvara Rasa* , *Shuddha Vatsanabh (Aconitum balfourii* Stapf*), Shuddha Hingula (*Red Cinnabar :HgS), *Pippali (Piper longum* Linn)

			
Hinguleshvara Rasa	***Shuddha Hingula*** **(Red Cinnabar:HgS)**	***Shuddha Vatsanabh*** ***(Aconitum balfourii*** **Stapf*),***	***Pippali (Piper longum*** **Linn)**
			
Diclofinac injection	**Pentazocine injection**	**Paracetamol injection**	**Oral Dosing**
			
Rectal temperature	**Acetic acid induced writhing**	**Formalin induced licking at the site of injection**	**Hot Plate**

6. DISCUSSION

Hinguleshvara Rasa is well known Ayurvedic herbomineral drug widely used for its antipyretic action. Review of literature revealed that *Hinguleshvara Rasa* described in various classical texts of *Rasashastra* using different ingredients. It is labeled as *Bruhat vatgajankush, Bruhad Hinguleshvara et*c. This drug as formulation is studied by various researchers for its different therapeutic activities. However comparative study of *Hinguleshvara Rasa* as a formulation with its individual ingredients is not studied yet. In the present study *Hinguleshvara Rasa* described in *Bhaishajya Ratnavali* is Prepared.[223]As described in research methodology this formulation has minimum ingredients namely *Shuddha Hingula* (Red Cinnabar: HgS).

Shuddha Vatsanabh (Aconitum balfourii Stapf*) & Pippali (Piper longum* Linn. Out of these three ingredients, *Shuddha Hingula* (Red Cinnabar: HgS) *and Shuddha Vatsnabh* ***(Aconitum balfourii* Stapf***)* are known for their *Jvaraghna* (antipyretic) Activity.[224-225] In the present study *Hinguleshvara Rasa* is compared with its all threed ingredients for antipyretic as well as analgesic activity.

For this study, as described in chapter 4 research methodology, *Hinguleshvara Rasa* is prepared by mixing all its three ingredients in equal quantity.

Shodhana is peculiar *Sanskaras* (processing)in *Rasashastra* performed to make drug therapeutically biocompatible.

For *Hingula* (Red Cinnabar: HgS) *Shodhana*, *Ashuddha Hingula* (Red Cinnabar: HgS) was procured from local market. This *Hingula* (Red Cinnabar: HgS) was processed 7 times with *Ardrak swaras* (Ginger Juice *Zingiber officinale* Roscoe) to make it therapeutically biocompatible.[226] As described in research methodology(4.7),*Hingula Shodhana* was performed as per reference of *Rasartnasamuchhaya (*adhyay 3/136*).* Initially 690 gms of *Ashuddha Hingula* was subjected to wet grinding with Ginger juice (*Zinziber officinale* Rosc) for 7 times.

At the end of *shodhana* as mentioned in analytical results 547 gms of *Shuddha Hingula* obtained.

In the present study

The weight of both Hingula and Vatsanabh was observed reduced during the process of Shodhan. The reduction of weight in Hingula might have resulted due to loss of material during manual transfer of material from one container to another.

Analytical Results of *Hingula* (Red Cinnabar: HgS)

6.1.Disscussin on Organoleptic Characters of *Hingula* (Red Cinnabar: HgS)

From results(**5.1)** *Ashuddh Hingula* (Red Cinnabar: HgS) is bricky red colour with metallic lusture. It is odourless while *Shuddha Hingula*(Red Cinnabar: HgS) has odour similar to Ginger juice.

6.2.Disscussion on Physico chemical Analysis of *Hingula* (Red Cinnabar: HgS)

From results(**5.2)** All these analysis reveals that Moisture content (as per method 4.1)of *Shodhit Hingula* (Red Cinnabar: HgS) is more than that of raw *Hingula* (Red Cinnabar: HgS). There is no difference in sulphur content of *Hingula* (Red Cinnabar: HgS) before and after *shodhana*. Mercury content became less after *Shodhana.*

6.3.Disscussion on Microbial load(as per method 4.5.i) of *Hingula* (Red Cinnabar: HgS) before and after *Shodhana*

From results(**5.3)** Microbial load**:**

Viable count as well as fungal count was observed to be Nil(5.3) in all samples of *Ashuddha Hingula* A, *Shuddha Hingula* B.

6.4. Disscussion on Physicochemical Results (as per methods 4.2)of *Ardrak Swarasa* (Juice of *Zinziber officinale* Rosc)

Ardrak (Ginger/*Zingiber officinale* Rosc) was subjected to physicochemical analysis (5.4) and estimation of heavy metals like Cadmium, Lead, and Arsenic &Mercury was performed.

From analysis it was revealed that PH of *Ardrak swarasa* (Ginger juice/juice of *Zingiber officinale* Rosc) is 6.94, which is on the border line of acidic nature.

pH is a unit of measure which describes the degree of acidity or alkalinity of a solution. The negative logarithm of Hydrogen ion activity pH=-log[H+]

pH is measurement of both acidity and alkalinity even though by definition it is a selective measurement of hydrogen ion activity. The pH value of a substance is directly related to the ratio of hydrogen ion [H+] and hydroxyl ion [OH^-] concentrations. If the [H+] concentration is greater than [OH^-], the material is acidic i.e. the pH value is less than 7. If the [OH^-] concentration is greater than the [H+], the material is basic, with pH greater than7.pH is measured on a scale of 0 to 14.

In present study pH of *Ardrak swarasa* (Ginger juice/juice of *Zingiber officinale* Rosc) was found 6.94, it indicated its weak acidic nature.

Moisture content @110^oC is 45.77 %.

Determination of moisture content is one of the important parameter in analysis of crude drugs. Moisture is an inevitable component of crude drug which must be eliminated as far as practicable. Moisture content can affect quality, efficacy, shelf life as well as utility of the drug. Therefore, it must be monitored carefully. This test is directly associated with the process of drying. Drying facilitates the preservation of crude drug, fixation of their constituents, reduction in their weight and lastly subsequent comminution i.e. grinding into powder. Process of drying plays important role in the quality as well as purity of the drug. Moisture requirements for active growth of some of common molds and bacteria that may be found in or on the drugs are relatively low. Therefore, the drying process should reduce the moisture content of the drug below this critical or threshold level. Temperatures as well as duration of drying are important factors associated with this test[**227**]

Weighed sample of crude drug is dried at 100^0C and weighed periodically until no more than 0.25 %is lost in one hour's drying. The total weight loss is expressed as a % of the initial weight of the sample. The residual moisture (if any) which cannot be driven off in this way is called 'bound 'water.

Moisture content limits are stated in the pharmacopoeial monographs on many drugs.
In present study (5.4) moisture content of Ardrak **(***Zingiber officinale* Rosc) is 45.77 %.In this study fresh (wet) *Zingiber officinale* Rosc was used for wet grinding of *Hingula* (Red Cinnabar: HgS).

Medicinal raw materials can be contaminated with arsenic and heavy metals which can be attributed to many causes including environmental pollution and traces of pesticide in plant materials. These elements even in trace amounts are dangerous. So their quantitative estimation is mandatory.

In present study *Ardrak swaras (juice of Zingiber officinale* Rosc) was used for *shodhana* of *Hingula* (Red Cinnabar: HgS).

Heavy metals like Cadmium (Cd), Arsenic (As), and Mercury(Hg)were absent while (Lead) Pb was present in this swarasa. During preparation of *Ardrak swaras* (juice of *Zingiber officinale* Rosc) it was washed with water thoroughly. But its outer skin was not peeled off. This might be the reason of Lead (Pb) contamination observed as its presence in the *Ardrak swaras* (juice of *Zingiber officinale* Rosc)

Specific gravity of a liquid is the weight of a given volume of the liquid at 25°C unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air.

Specific gravity of *Ardrak swaras* (juice of *Zingiber officinale* Rosc) is 1.0296 gm / ml.

6.5 Discussion on TLC (as per method4.2f)Report of analysis of the *Ardrak Swarasa* (Juice of *Zinziber officinale* Rosc):

From the results(**5.5)** it was found that @365two spots having different Rf value and colour obtained.in iodine chamber only one yellow colour spot having Rf 0.76 was observed.

***Pippali (Piper longum* Linn):**

Pippali ***(Piper longum* Linn**) is second ingredient of *Hinguleshvara Rasa*. In *Ayurvedi*c pharmaceutics *Nava* (Fresh)and *Purana* (Old) dravyas is an unique concept practiced for selection of raw materials. Few drugs are said to be used in *Purana* (old) stage. E.g. *Guda* (Jaggery), *Guggulu* (*Commiphera mukul), Vidanga (Embelica ribes*), *Pippali (Piper longum* Linn). More than one year old drug from its plucking period is known as *purana* dravyas**.**[228] *Pippali (Piper longum* Linn) is recommended to be used as *Purana*. Considering this concept total seven samples of *Pippali (Piper longum Linn*) was collected directly from site of cultivation during its plucking period i.e. Winter season. Since winter 2014 to winter 2019 were considered as old samples of *Pippali (Piper longum* Linn) and sample of winter 2021 was considered as new samples. All these samples were subjected to physicochemical, Phrmacognostical analysis. Estimation of total alkaloid and Piperine content of each sample was also performed. From results of above studies sample of winter 2016 was selected for preparation of *Hinguleshvara Rasa.* It is observed that Piperine content of *Pippali (Piper longum* Linn) less than one year old /fresh is very less whereas piperine content of *Purana/* old samples is more. As per the guidance of pharmacognosist and on the basis of result of Pharmacognosy and phytochemical findings Sample C was selected for preparation of *Hinguleshvara Rasa*. In this sample 'C' of *Pippali (Piper longum* Linn),(plucked in winter 2016) percentage of piperine and total alkaloids were found high as compared to other samples of *Pippali (Piper longum* Linn). Following test were performed on ***Pippali (Piper longum* Linn** and their interpretation is as follows:

6.6 Disscussin on Microbial Load of *Pippali (piper longum* Linn)

Microbial load**:**[229-230]

Viable count as well as fungal count was observed to be Nil(5.6) in all samples of ***Pippali (Piper longum* Linn)** (method applied As per 4.5i)

6.7 Disscussin on Physicochemical Analysis (as per methods 4.5c to4.5 h) of *Pippali (piper longum* Linn)are as follows:

6.7.1Foreign Matter **:[231]**

Foreign Matter is the parts of the organ/organs other than those parts of drugs mentioned in the definition and description of drug are known as foreign organic matters. They may be insects, moulds, earthy materials animal excreta etc. Each and every drug has its own limit. According to Ayurvedic pharmacopoeia of India Foreign matter of *Pippali (Piper longum* Linn) should be not more than 2%. Considering the definition, it is clear that the drug having less foreign matter having greater purity. In present study Foreign matter (from results5.7)of *Pippali (Piper longum* Linn) of batch C, is 0.7%. which is within the standards of API Ayurvedic Pharmacopoeia of India.

Foreign matter of sample 'C' is 0.7%(as found in analytical result 5.7) which was selected for present study.

As per previous two studies foreign matter of *Pippali (Piper longum* Linn) is 1.62+_0.12 and 0% respectively**[232-233].**

6.7.2Moisture Content/loss on Drying:[234]

The moisture content of drug will be responsible for decomposition of crude drugs either producing chemical change or microbial growth. So the moisture content of drug should be determined and controlled. The moisture content of drug is determined by heating drug at 105^0c, in an oven to constant weight. Loss On Drying is determined by heating the sample below its melting point in an oven and it includes all volatile matter including water content and solvents. Loss On Drying is an unspecific analytical technique removing not only water but all other volatile impurities like alcohol etc. from the sample. Degree of drying depends upon 1) temperature & 2) drying time.

In present study Loss on Drying of *Pippali (Piper longum* Linn) fruits (as per analytical result 5.7) of sample 'C' was 11.09% which was used for the preparation of *Hinguleshvara Rasa*.

Moisture Content/loss on Drying of *Pippali (Piper longum* Linn) fruits were not mentioned in Ayurvedic pharmacopoeia of India. In previous two studies it was found 15.70+_0.051 and 10.88 respectively.[235-236]

6.7.3Total Ash:[237 *Pippali (Piper longum* Linn)

The determination of ash is useful for detecting low grade products, exhausted drugs and excess of sandy or earthy matter. Different type of Ash values are used in detection of crude drugs like Total ash, Acid insoluble Ash, Water soluble ash, Sulphated ash. Total Ash is useful in detecting crude drugs that are mixed with various mineral substances like sand, soil, calcium oxalate, chalk powder or other drugs with different inorganic contents to improve their appearance.

The maximum temperature used for total ash should be not more than 450^0C because alkali chlorides that may be volatile in higher temperature would be lost.

In present study total ash of Sample 'C' of *Pippali (Piper longum* Linn) is2.53.(As mentioned 5.7)According to Ayurvedic pharmacopoeia of India Total Ash of *Pippali (Piper longum* Linn) should not be more than 7%.. In previous two studies it was found 8.3+_0.015 and 4.88 respectively.[238-239]

6.7.4Acid Insoluble Ash[240]**:**Acid insoluble ash means the ash insoluble in dilute hydrochloric acid. It is often of more value than the total Ash. A higher limit of acid insoluble ash is incorporated especially in cases where silica may be present or when the calcium oxalate content of the drug is very high.

Following are the known health hazards of calcium oxalate.

It causes eye irritation, dermatitis, It causes irritation of digestive track. Its inhalation causes respiratory tract irritation. Chronic effect is kidney injury.

Similarly crystalline silica particles causes multiple diseases including silicosis an incurable lung disease that lead to disability and death. Respirable crystalline silica also causes lung cancer, chronic obstructive pulmonary diseases(COPD) and kidney diseases. Acid insoluble ash of

batche 'C' of Pippali (*Piper longum* Linn) was 0.43 (as mentioned in 5.7) which is within range of values given by Ayurvedic pharmacopoeia of India.

It is indicative of less or no silica or calcium oxalate present in old and new samples of *Pippali (Piper longum* Linn) .

According to Ayurvedic pharmacopoeia of India Acid insoluble ash of *Pippali (Piper longum* Linn) should be not more than 0.5%.In previous two studies it was found 1.16+_0.06 and 0.09 respectively.[241-242]

6.7.5Extractive Value:

This matter determines the amount of active constituents in a given amount of medicinal plant material when extracted with solvents. It is employed for that material for which no chemical or biological assay method exist. As mentioned in Indian pharmacopoeia 1996, the determination of water soluble and alcohol soluble extractives is used as a means of evaluating crude drugs which are not readily estimated by other means. Alcohol soluble Extractive of C batch of *Piper longum* Linn is 19.97%. Water soluble extractive of C batch of *Piper longum* Linn is 40.30%

According to Ayurvedic pharmacopoeia of India Alcohol soluble Extractive of *Pippali (Piper longum* Linn) should be not less than 5%. In previous study it was found 17%[243-244]

According to Ayurvedic pharmacopoeia of India Water soluble extractive of *Pippali (Piper longum* Linn) should be not less than 7%. In previous study it was found 9.92%[245-246]

Alcohol soluble and water soluble Extractive(as per result 5.7) of sample 'C' of *Pippali (Piper longum* Linn)is 19.97% and 40.30% respectively which was used for preparation of *Hinguleshvara Rasa.* All these results are found to be in range given by Ayurvedic Pharmacopoeia of India.

6.8Disscussin on TLC of *Pippali* (*Piper longum* Linn)[247]:

Thin layer chromatography (TLC)[247]:

From Thin layer chromatography(TLC) results (as per result 5.8)of *Piper longum* Linn batch C,it was observed that @ 245 total 6 spots(0.10,0.13,0.26,0.40,0.65,0.71all Yellow),@ 365,three spots (0.36,0.6,0.50(all Blue),and after derivatization with Dragendorff reagent 2 spots (0.26,0.40 all orange) were observed.

6.9Disscussin on Phytochemical Analysis of *Piper longum* Linn are as follows:

Phytochemical analysis includes estimation of total alkaloid as well as estimation of Piperine content from fruits of *Piper longum* Linn sample C

6.9.1Total Alkaloid of *Pippali (Piper longum* Linn):[248]

Alkaloid constitutes a major class of chemical group present in plant drugs. Alkaloids may be described as naturally occurring organic substances, having a cyclic nitrogenous nucleus exhibiting basic properties and having a pronounced physiological action. All alkaloids contain at least one nitrogen atom and in the majority of cases, the compound is basic. This means that salt formation can occur in the presence of acid. This fundamental property of alkaloids is used in their extraction. Total alkaloid content of Pippali (Piper longum Linn) is highest in batch C (plucked in 2016) and it is (0.48%) is also high among all samples (As mentioned in 5.10).

6.9.2Estimation of Piperine content from fruits of *Piper longum* Linn sample C

Piperine % of Pippali Piper longum Linn is highest in batch C (plucked in 2016) and it is 0.72%[249]

It is observed that batch 'C' of *Pippali* (*Piper longum* Linn.) fruits has higher % of Piperine (0.72) as well as its total alkaloid content (0.48%) is also high among all samples (As mentioned in 5.10).

6.10 Disscussin on Pharmacognosy(as per methods4.5a) of fruits of *Pippali (Piper longum* Linn), (As per 5.12 analytical results):

Raw drugs play very important role in the Ayurvedic formulations. Authenticated raw drugs will give very good clinical results. Quality of the raw drugs depends on the Cultivation, collection,

age, storage. In *Ayurveda* many drugs are required purification, processing, drying etc . A drug stored over a period of one year from the day of its collection or production, is classified as *Purana dravyas*. On the basis of period of storage from the day of harvesting, collection or production, the drugs are grouped as *Nava*(fresh) and *Purana* (old) dravya.

Present study was carried out to study the effect of post-harvest storage period on seven samples of fruits of *Pippali* (*Piper longum* Linn.) which were collected in winter of years 2014,2015,2016,2017, 2018 and 2019 respectively. These samples were considered as Purana/Old Sample of Piper longum Linn. Sample plucked in winter 2021 was considered as Fresh/Nava(New) sample .

Pharmacognosy and HPLC study were conducted and on that basis sample C of (Piper longum Linn fruits were selected for experimental study. Total alkaloid percentage and % of Piperine was found high in batch C among all seven batches of (Piper longum Linn fruits

6.11 Disscussion on Organoleptic characters of fruits of *Pippali (Piper longum* Linn)are as follows:

Sample C plucked in winter 2016 possesses Pungent odour, black colour, gave strong tingling sensation on taste.(as per results 5.13)

6.12 Discussion on Physicochemical as well as microbial results(as per5.14) of *Gomutra* (cow urine) are as follows:

Physico chemical analytical study of fresh cow urine (5.14)revealed that pH of all samples of cow urine ranges between 7 to 8.It means Cow urine is slightly alkaline in nature. Specific gravity of all samples

of cow urine is about 1.01 to 1.03.Total solid contents(as per method 4.5j) ranges from 9.08to 10.8%.

The observed values of microbial load of Gomutra (Cow urine) were found less than$10x10^{3,}$which are within limits as per Ayurvedic Pharmacopoeia of India **[251-252]**.

6.13 Disscussion on *Vatsanabh/Aconite(Aconitum balfourii* Stapf):

6.13.1 *Shodhana* of *Vatsanabh*[250] (*Aconitum balfourii* Stapf) *Vatsanabh shodhana in* cow urine was performed in three batches and each batch contain 1200 gms of *Vatsanabh* before *shodhana*. **After s*hodhan*a approximately 800 gms of *Vatsanabh* was obtained from(786,784 and 802 gms) each batch.**

The reduction in weight of Vatsanabh might have resulted from loss of material during washing.

6.13.2 Disscussion on physicochemical analysis (methods applied as per 4.4) of *Aconitum balfourii* Stapf (before and after *shodhana*) are as follows:

6.13.2.a Foreign matter(As per 4.4c)of ***Aconitum balfourii* Stapf** was nil. Considering the definition ,it is clear that the drug having less foreign matter having greater purity. In present study Foreign matter of all samples of ***Aconitum balfourii* Stapf (Before and after *shodhana)*** was nil..

From above result we can conclude that foreign matter limits for present samples of ***Aconitum balfourii* Stapf (before and after shodhana)** was nil. In Ayurvedic Pharmacopoea of India monograph of *Aconitum chasmanthum* Stapf is given. Foreign matter of *Aconitum chasmanthum* Stapf should not be more than 2% as per Ayurvedic Pharmacopoeia of India.

6.13.2.b. Estimation of Loss on drying(As per 4.4d) of samples of *Aconitum balfourii* Stapf (before and after *shodhana*):[253]

Loss on drying of *Ashuddha* ***Aconitum balfourii* Stapf (as per results 5.16)was 11.48,** Loss on drying of *Shuddha* ***Aconitum balfourii* Stapf A, B, and C was12.41,12.38,12.12 respectively.** Loss on drying of *Shuddha* composite ***Aconitum balfourii* Stapf (A+B+C) was 12.33.** Observed values of Loss on drying of *Aconitum balfourii* Stapf was increased after *shodhana* process. During *shodhana* pieces of ***Aconitum balfourii* Stapf were dipped in** *Gomutra***(**Cow urine) for three days in *teevra Atapa* (scorching sun heat).

In one of the previous study % of moisture content[254] of *Aconitum ferox* Wall was9.64 before *shodhana,*and after *shodhana* with *Gomutra* cow urine it was(12.70) which showed increase in % moisture content after *shodhana.*

6.13.2.c Determination of Total Ash (As per 4.4e)content of *Vatsanabh* (*Aconitum balfourii* Stapf) :

Total ash content**(as per results 5.16)** of *Ashuddha Vatsanabh* (*Aconitum balfourii* Stapf) was 1.53, *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) sample A,B,C was 2.20, 2.96,1.99 and composite (A+B+C) was 1.98.If values of total ash are less then purity of sample is considered to be more. But here therapeutic purification of *Ashuddha Vatsanabh* (*Aconitum balfourii* Stapf) was performed in *Gomutra* (cow urine). During *shodhana* ,pieces of ***Aconitum balfourii* Stapf were dipped in** *Gomutra* **(**Cow urine) for three days in *teevra Atapa* (scorching sun heat). This may be the reason for getting values of total ash content are more in *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) sample A,B,C and composite (A+B+C) than *Ashuddha Vatsanabh* (*Aconitum balfourii* Stapf).

In Ayurvedic Pharmacopoea of India monograph of *Aconitum chasmanthum* Stapf is given. **Total Ash content** of *Aconitum chasmanthum* Stapf[255] should not be more than 5.5% as per Ayurvedic Pharmacopoeia of India.

In one of the previous study[256] % of **Total Ash** content of *Aconitum ferox* Wall was 4.93before *shodhana,* and after *shodhana* with *Gomutra* cow urine it was(5.11) which showed increase in % **Total Ash** content after *shodhana.*

6.13.2.d. Determination of Acid insoluble Ash(As per methods 4.4f)

Acid insoluble Ash(as per results 5.16) of *Ashuddha Vatsanabh* (*Aconitum balfourii* Stapf) was 0.48and *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) sample A,B,C was 0.48,0.47,0.46 respectively and composite (A+B+C) was 0.47.

In Ayurvedic Pharmacopoea of India monograph of *Aconitum Chasmanthum* Stapf is given. **Acid insoluble Ash** of *Aconitum Chasmanthum* Stapf should not be more than 2% as per Ayurvedic Pharmacopoeia of India.[257]

In one of the previous study[258] % of **Acid insoluble Ash** content of *Aconitum ferox* Wall was 1.24 before *shodhana* and after *shodhana* with *Gomutra* cow urine it was(1.38.) which showed increase in % moisture content after *shodhana.* In present study no difference in % of **Acid insoluble Ash of** *Ashuddha Vatsanabh* (*Aconitum balfourii* Stapf) and *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) was found.

6.13.2.e. Alcohol soluble Extractive(As per methods of 4.4g) *Vatsanabh* (*Aconitum balfourii* Stapf):

Alcohol soluble Extractive (as per results 5.16)of *Ashuddha Vatsanabh* (*Aconitum balfourii* Stapf) was 6.31and *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) sample A, B, C was 11.04,10.40,9.46, respectively and composite (A+B+C) was 11.91.

In present study % of Alcohol soluble extractives of *Vatsanabh* (*Aconitum balfourii* Stapf) was increased after therapeutic purification in *Gomutra* cow urine. In Ayurvedic Pharmacopoea of India monograph of *Aconitum Chasmanthum* Stapf is given. **% of Alcohol soluble extractives** of *Aconitum Chasmanthum* Stapf should not be less than 8% as per Ayurvedic Pharmacopoeia of India[259]

In one of the previous study **% [260] of Alcohol soluble extractives** of *Aconitum ferox* Wall was 4.88 before *shodhana*, and after *shodhana* with *Gomutra* cow urine it was(3.43) which showed decrease in % **Alcohol soluble extractives** after *shodhana.*

6.13.2.f. Water soluble Extractive (As per method 4.4h)*Vatsanabh* (*Aconitum balfourii* Stapf):

Water soluble Extractive of(as per results 5.16) *Ashuddha Vatsanabh* (*Aconitum balfourii* Stapf) was62.33 and *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) sample A,B,C was 65.61,67.84,67.03,respectively and composite (A+B+C) was 67.41

In present study % of Water soluble extractives (as per results 5.16)of *Vatsanabh* (*Aconitum balfourii* Stapf) was increased after therapeutic purification in *Gomutra* cow urine. In Ayurvedic Pharmacopoea of India monograph of *Aconitum Chasmanthum* Stapf is given. **% of Water soluble extractives** of *Aconitum Chasmanthum* Stapf should not be less than 24% as per Ayurvedic Pharmacopoeia of India.[261]

In one of the previous study **% of water soluble extractives** of *Aconitum ferox* Wall was 17.71 before *shodhana* and after *shodhana* with *Gomutra* cow urine it was(13.85) which showed decrease in % **water soluble extractives** after *shodhana*.[262]
According to species values may be changing.

6.13.3.Disscussion on Microbial load(as per methods 4.5i) of *Vatsanabh*

Microbial Load of ***Aconitum balfourii*** **Stapf (Before and after shodhana)(As per results 5.17)**:

were found less than$10x10^{3,}$which are within limits as per Ayurvedic Pharmacopoeia of India [252].

These samples are taken for further study.

6.13.4. Disscussion on TLC of *Vatsanabh (Aconitum balfourii* Stapf*) :*

In TLC of *Ashuddha Vatsanabh(as per results* **5.18 &5.19)** (*Aconitum balfourii* Stapf total 8 number of spots were observed(@254,365&after spray) and in *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) sample total number of spots observed in A,(3,3,3) B(3,3,3),C and composite (A+B+C) was (3,1,1) respectively@254,@365 and after spray with Dragendorff reagent.

From above observed values it was clear that if each spot corresponds to each constituent, then number of constituents of *Ashuddha Vatsanabh* (*Aconitum balfourii* Stapf were more which were decreased after *shodhana.*

It represents that unwanted constituents were get eliminated after *shodhana* process with cow urine.

In Ayurvedic Pharmacopoeia of India TLC monograph of (Ashuddha) *Aconitum Chasmanthum* Stapf is given. Findings are as follows:0.10, 0.20 ,0.39, 0.56, 0.74,0.96 and all spots are yellow.

Dragendorff reagent :0.39,0.96 all spots are orange.

6.13.5. Disscussion on Organoleptic characters (as per results 5.20):

Roots of Ashuddha *Vatsanabh* ***Aconitum balfourii*** **stapf was dark brown in colour After *shodhana* with cow urine its colour was light brown. *Shuddha Vatsanabh*** *Aconitum balfourii* Stapf had peculiar odour of cow urine. ***Shuddha Vatsanabh*** *Aconitum balfourii* Stapf was smooth to touch as compared to raw or roots of **Ashuddha *Vatsanabh*** *Aconitum balfourii* **stapf**

6.13.6 . Disscussion on Phytochemical Analysis(as per methods 4.6) of *Vatsanabh* *(Aconitum balfourii* Stapf)

As per thr results(5. 21 Estimation of Aconitine contents)Percentage of Aconitine in *Vatsanabh* (*Aconitum balfourii* Stapf before shodhana was found0.027. After shodhana in Gomutra(Cow urine)it should get reduced to fulfill the purpose of *shodhana*. In present study sample A,B,C and composite (A+B+C) of *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf) was found 0.00133,0.00547,0.00358 and 0.00363. From above observation it was revealed that Aconitine % of *Vatsanabh* (*Aconitum balfourii* Stapf) after *shodhana* get reduced which proves of efficacy of *shodhana* procedure described in *Rasashastra.*

Chromatogram of standard *Aconitine* has shown peak at R_f at 29.309. All the other samples (samples *Ashudha Vatsanabh* & A,B,C) has shown peak at same R_f ie 29.866, 29.328, 30.201, 29.556 respectively. However the highest area is shown by Ashudha *Vatsanabh* while it get decreased in sample B, sample C, and sample A respectively.

As per the results 5. 21.2.Estimation of Total Alkaloid contents ,it was found that total alkaloid% of *Vatsanabh* (*Aconitum balfourii* Stapf before *Shodhana* was found less and after *shodhana* it was increased in two batches of *Shuddha Vatsanabh* (*Aconitum balfourii* Stapf A, C and in composite (A+B+C). In batch B there was no difference in % of total alkaloid was observed. From above results it can be conclude that few alkaloids from Gomutra (Cow urine) exerts

synergetic effects so the % of total alkaloids either remains as it is or increased at the same time % of Aconitine was decreased (Antagonistic action) . This *shodhana* of Aconitum balfourii Stapf in cow urine helped to prove *Sanshlesh & Vishlesh* concept of Ayurveda Pharmaceutics.

6.13.6. Disscussion on Pharmacognosy

As per results 5.23*Vatsanabha* comes under *Visha varga,* requires purification. Purification increases the quality of the raw drugs and also efficacy of the drug. The study reveals that the normal *Vatsanabha* was Dark brown in colour whereas after *Shodhana sample* was light brown in colour and also the odour is also changed whereas *Shodhit* samples had cow's urine smell and normal was unpleasant smell and causes slight giddiness..

Dried tuberous root less shriveled. Circular scars of detached lateral rootlets observed.

Shape- Conical shaped tuberous root attached to aehrial stem, wider at the top upto 1.6 cm, tapering towards the lower end.

Length- Upto 8 cm, Thickness- up to 1.5 cm

Colour-

External- Dark brown greish, Internal- yellowish white

Fracture- very hard

T.S. passing through the centre of tuberous root is circular somewhat undulating in outline showed outermost 5-7 rows of suberized dark brown colored cells of metaderm followed by 10-15 layers of cortex. followed by 10-15 layers of cortex.

Presence of solitary as well as grouped scattered angulared oval and rounded pitted and non pitted stone cells outside of the endodermis. Mostly cortex parenchyma cells are embedded with few isolated stone cells and simple and compound starch grains. Cambium is continuous and xylem is complete 'V' shaped with base thickening. Parenchyma cells of the root are not heavily loaded with simple and compound starch grains as compare to other species.

Thus on the basis of above observations its confirmed that the given sample belongs to the root of *Aconitum balfourii* Homles Ex stapf (Family- Ranunculaceae).

6.14 *Hinguleshvara Rasa* :

Total quantity obtained=736 gms

6.14.1 Organoleptic character *of Hinguleshvara Rasa*

Hinguleshvara Rasa is Brownish dull red in colour, pungent in taste and has typical odour of its constituent. It is smooth to touch. It does not float on water.

6.14.2 Physicochemical Analysis of *Hinguleshvara Rasa*

Observed values as per (5.26) of Physicochemical Analysis are not mentioned in Ayurvedic pharmacopoeia of India.In present study Loss on drying(as per 4.1a) is5.91%, Loss on Ignition(as per 4.1d)is 94.16%, Total ash(as per 4.1b)is 5.84, Acid insoluble Ash(as per 4.1c)is 1.06, Water Soluble Ash is 1.45%.

6.14.3 Microbial load(as per methods 4.5i) of *Hinguleshvara Rasa*

Observed values(as per result5.27) of total viable count (160) and total fungal count (40) which are less than $10x10^3$ As per Ayurvedic pharmacopoeia of India[263] these values are within normal limits so this *Hinguleshvara Rasa* was subjected to assess its acute oral toxicity study, antipyretic as well as analgesic activity study.

6.14.4Thin Layer Chromatograph of *Hinguleshvara Rasa* as per results 5.29:

Thin layer chromatography of *Hinguleshvara Rasa* revealed that @254nm total 7 spots (0.14,0.26,0.34,0.40,0.71,0.77,0.96 all Yellow), @365nm total 7 spots (0.14,0.29,0.51, 0.59,0.74,0.81,0.89 all Blue) and after Derivatization with Dragendorff reagent two orange spots were observed. As there is no previous study found, obtained data could not be compared with other results.

6.14.5 Estimation of Aconitine, piperine & Total Alkaloid % of *Hinguleshvara Rasa* (as per results 5.31)

Sr. No.	Parameters	Result
1	%Total Alkaloids (By Gravimetry)	0.48 %
1	% Piperine (ByHPLC)	0.059 %
2	% Aconitine (ByHPLC)	0.0022 %

Key-Word:%-Percentage;ppm –Parts per million

No previous study was found on *Hinguleshvara Rasa* to compare these findings.

6.15 Characterization of of *Ashuddha Hingula* (Impure Red Cinnabar: HgS), *Shuddha Hingula* (Red Cinnabar processed with ginger juice).,*Hinguleshvara Rasa* prepared by ball mill and *Hinguleshvara Rasa* prepared by trituration (manual grinding).

The present study was conducted on *Hinguleshvara Rasa* mentioned in *Bhaishajya Ratnavali Jvarachikitsa5*/483) All findings related to characterization, pharmacological activities observed in this study are related to this selected formulation of *Hinguleshvara Rasa* mentioned in *Bhaishajya Ratnavali Jvarachikitsa5*/483. These observations are not applicable for *Hinguleshvara Rasa* prepared using different ingredients and different compositions as mentioned in different texts and classics.

6.15.a.Dicussion on particle size Analysis(as per method 4.3b) as per results 5.33:

Prior to *shodhana Ashuddha Hingula* (Impure Red Cinnabar: HgS) was grounded finely. The grounded *Ashuddha Hingula* (Impure Red Cinnabar: HgS) was composed of 45%of particles within the range of 0 to 2.5 micron, whereas 38% of particles were within the range of 2.5 to 5 micron. 94% of particles were of the size less than 10micron.

Ashuddha Hingula (Impure Red Cinnabar: HgS) so ground containing 94% of particles below the size of 10 micron was subjected to *shodhana* process by grinding in Ginger juice. It was repeatedly ground to produce a product containing 96% of particles below the size of 10 microns.57% particles in this product were below 2.5 microns. *Shuddha Hingula* (Red Cinnabar:

HgS processed with Ginger juice) so produced was used to prepare *Hinguleshvara Rasa*. *Hinguleshvara Rasa* was prepared by manual grinding as well as grinding with ball mill. *Hinguleshvara Rasa* produced by manual grinding contained 94% of particles below the size of 10 microns. In this product 45.5%particles were of the size of below 2.5 micron whereas 38% particles were found in the range of 2.5 to5 microns.

Hinguleshvara Rasa prepared by Ball Mill contained 57% particles below 2.5 microns size whereas 25% particles were found within the range of 2.5 to 5 microns. 96% particles of this *Hinguleshvara Rasa* were of the size below 10 microns.

The observation indicate that grinding with ball mill produces fine particles in more numbers as compared to manual grinding.

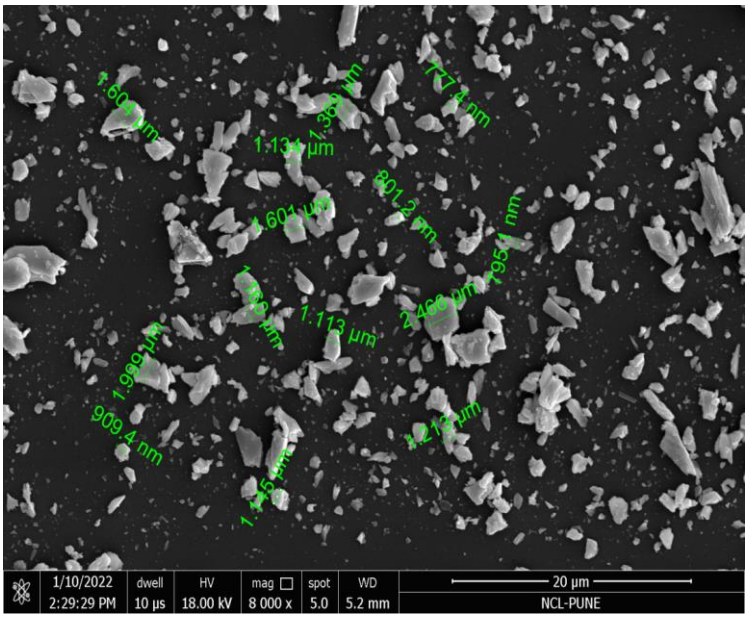
(In *Hinguleshvara Rasa prepared* by Ball Mill 57% particles within the range of 0 to 2.5 microns where as in *Hinguleshvara Rasa* prepared by trituration contain 45%particle within this range of below 2.5 micron.

6.15.b.Dicussion on Field Emmission Scanning Electron Microscopy (FESEM) (as per method4.3a),(**as per results 5.34)**
of *Ashuddha Hingula* (Impure Red Cinnabar HgS), *Shuddha Hingula* (Red Cinnabar processed with ginger juice). *Hinguleshvara Rasa* prepared by ball mill and *Hinguleshvara Rasa* prepared by manual grinding(trituration) are as follows:
The Scale of 20 micrometer was used for measuring the particle size of all the samples in SEM image.

6.15.b-1. SEM of *Ashuddha Hingula* (Impure Red Cinnabar: HgS):- (as per results 5.34.1)
The SEM image of *Ashuddha Hingula* (Impure Red Cinnabar: HgS) shows particles of various sizes and shapes. Particles of irregular shapes are observed in the field. Triangular, cylindrical, pentagonal, hexagonal, particles can be seen in the image. SEM image of particles appear compact agglumerated. Size of the particles of *Ashuddha Hingula* (Impure Red Cinnabar: HgS) from image observed at 8000 magnification from SEM was as follows:

The smallest particle size observed is 777.4nanometer whereas largest particle size is 2.466 micrometer. And Average particle size is 1.291 micrometer

SEM of *Ashuddha Hingula*(Impure Red Cinnabar: HgS):

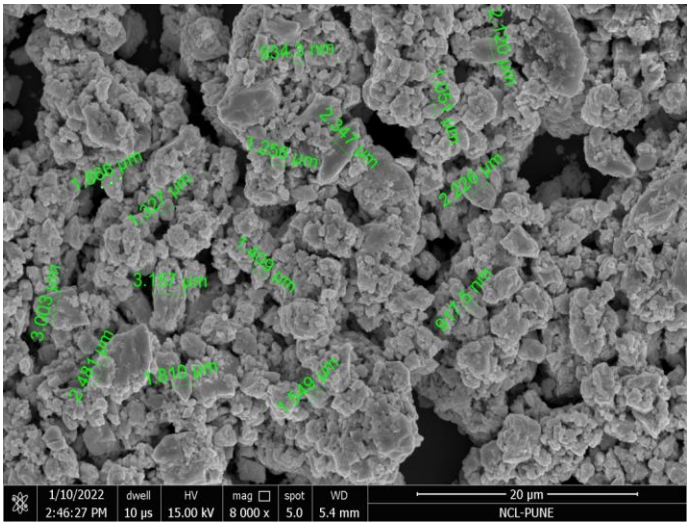


6.15.b-2. SEM of *Shuddha Hingula* (Red Cinnabar: HgS processed with Ginger juice):- (as per results 5.34.2)

Size of the particles of ***Shuddha Hingula*** **(Red Cinnabar: HgS processed with Ginger juice)** from image observed at 8000 magnification from SEM was as follows:

The smallest particle size observed is 0.917micrometer whereas largest particle size is 3.157micrometer. And Average particle size is 1.8302micrometer.

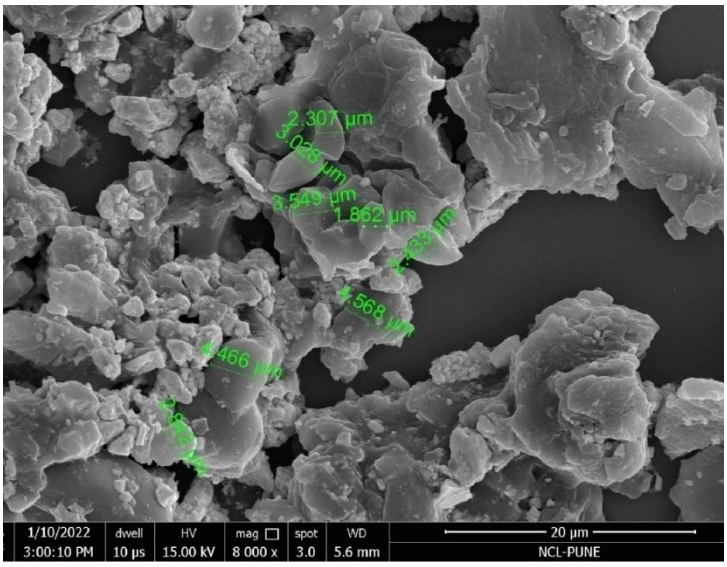
SEM of *Shuddha Hingula* (Red Cinnabar: HgS processed with Ginger juice):-



6.15.b-3. SEM of *Hinguleshvara Rasa prepared* by Ball Mill(as per results 5.34.3):

Size of the particles of ***Hinguleshvara Rasa prepared*** **by Ball Mill**from image observed at 8000 magnification from SEM was as follows:

The smallest particle size observed is 1.862micrometer whereas largest particle size is4.568micrometer. And Average particle size is 3.131micrometer.

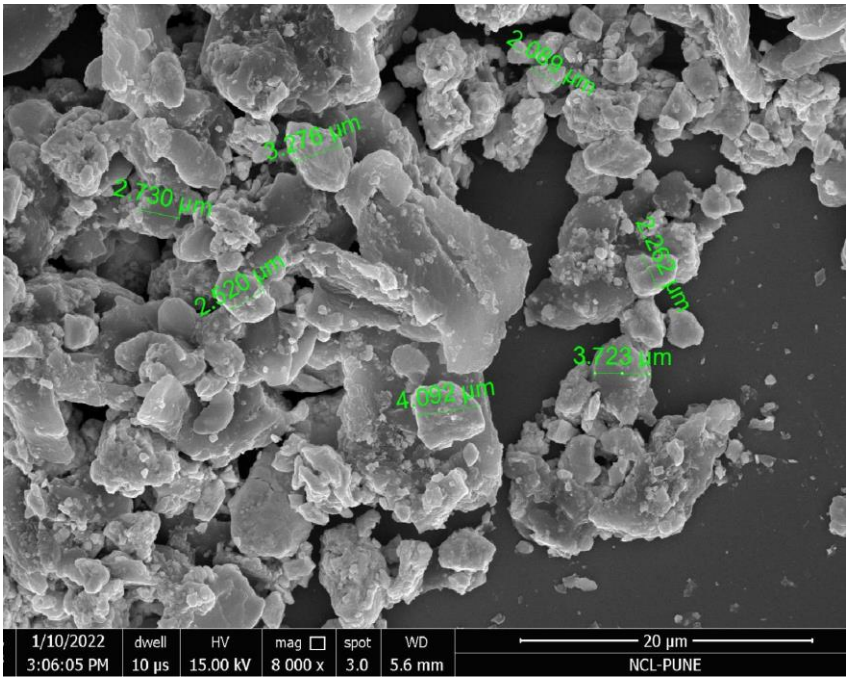


SEM of *Hinguleshvara Rasa prepared* by Ball Mill

6.15.b-4. SEM of *Hinguleshvara Rasa* prepared by Trituration(as per results 5.34.4):

Size of the particles of ***Hinguleshvara Rasa* by prepared by Trituration** from image observed at 8000 magnification from SEM was as follows:

The smallest particle size observed is 2.089micrometer whereas largest particle size is 4.568micrometer. And Average particle size is **4.092**micrometer.



SEM of *Hinguleshvara Rasa* prepared by Trituration

In SEM studies only a small area is scanned, therefore size of the particles falling in the scanned area can only measured. Therefore average particle size of the whole sample can not be measured by SEM technique. However size of the smallest particle can be predicted in this technique.

Measuring the size of the particles using particle size analyzer appears more useful. In this technique multiple areas of the sample are scanned. This technique gives %distribution of size in the range of 0to 2.5 microns,2.5 to 5 microns ,5 to 10 microns and above 10 microns. It is observed that almost all samples contained more than 95% particles below the size of 10 microns.

6.15.c.FTIR Fourier transform infrared spectroscopy(as per methods4.3c), (as per results 5.35): is a technique which is used to obtain infrared spectrum of absorption, emission and photoconductivity of substances. It is used to detect different functional groups. FTIR spectroscopy is a disperse method which means that frequencies. measurements are performed over a broad spectrum instead of narrow band frequencies.

In present study FTIR: Fourier transform infrared spectroscopy of all samples was conducted to identify functional groups. Comparison of FTIR spectra of *Ashuddha Hingula*(Impure Red Cinnabar: HgS) and *Shuddha Hingula* (processed Red Cinnabar :HgS*)*showed some common peaks which refer to the presence of *Hingula* Red Cinnabar: HgS. These common peaks are 149,1437,1507,1540, 1576,1635,1700,1772,2114 . Whereas some additional peaks are observed in FTIR spectra of *Shuddha Hingula* (processed Red Cinnabar :HgS).These peaks belongs to wave no 663,759,823,988 and few others. These peaks are believed to belong to Ardrak swarasa (Ginger juice, juice of Zinziber officinales Rosc), which was used for shodhana of *Ashuddha Hingula* (Impure Red Cinnabar: HgS).

FTIR spectra of *Hinguleshvara Rasa* prepared by manual grinding and by using Ball Mill appear very much similar to each other. This similarity indicates that method of grinding does not produced any significant difference with the product as far as its chemical composition is concern.

Comparison of FTIR spectra of *Shuddha Hingula* and *Hinguleshvara Rasa* shows some common peaks and some different peaks .Peaks refer to wave no 759/761,1419,1540,1617, 1718/1716, 2114/2112,296/2918 are common. They believed to belong to Hingula (Red Cinnabar :HgS).

Additional peaks observed in FTIR spectra of *Hinguleshvara Rasa* are believe to belong to ingredient Pippali (*Piper longum* Linn) and Vatsanabh (Aconitum balfourii Stapf).

6.15.d.X-Ray Diffraction (XRD)(as per method 4.3d) **of *Ashuddha Hingula* (Impure Red Cinnabar: HgS), *Shuddha Hingula* (Red Cinnabar processed with ginger juice). *Hinguleshvara Rasa* prepared by Ball Mill and *Hinguleshvara Rasa* prepared by manual grinding(trituration) are as (as per results 5.36):**

The X-Ray intensities of Ashuddha Hingula are higher than the Shuddha Hingula, Ball Milled Hingula, and Triturated Hingula. The X-Ray peaks were suppressed in the ball milled *Hingula*.HgS is the prominent and common in all four samples.

ANIMAL EXPERIMENT RESULTS:

6.16.Study of acute oral toxicity was (**As per the result 5.37):**
As mentioned in chapter materials and methods(4.10)
Acute oral toxicity of *Hinguleshvara Rasa, Shuddha Vatsanabh (Aconitum balfourii* Stapf*), & Shuddha Hingula* (Red Cinnabar) HgS by OECD 423 guideline

This study was conducted on Swiss Albino mice (female mice 06-08 wks age,25-30 gm weight)
.
Study was conducted at Apt Testing and research Pvt Ltd.

(**As per the result 5.37):**The test material **"*Hinguleshvara Rasa, Shuddha Hingula* (Red Cinnabar:HgS) *and Shuddha Vatsanabh (Aconitum balfourii* Stapf** "did not cause mortality in the treated female mice at the dose of 300 mg/kg. The test *material* ***Hinguleshvara Rasa and Shuddha Hingula*** **(Red Cinnabar:HgS)** did not cause mortality in the treated female mice at the dose of 2000 mg/kg.While the test material "***Shuddha Vatsanabh (Aconitum balfourii* Stapf)***"* at the dose of 2000 mg/kg caused death of three animals in 24hrs after the dosing. The body weight gain by the treated female mice was not found to be affected during 14 days observation period.

In Histopathology analysis no abnormalities were detected in the tissues of Liver, Kidney, Heart and Gastric Mucosa of all three groups at dose 300 mg/Kg.

In Histopathology analysis of tissues of Liver, Kidney, Heart and Gastric Mucosa in group *Hinguleshvara Rasa and Shuddha Hingula* (Red Cinnabar:HgS), no abnormalities were detected at dose 2000mg/Kg.

It can be concluded, that Globally Harmonized Classification System (GHS) for classification of chemicals which cause acute Toxicity (OECD 423) series on testing and assessment, Number 33; Harmonized Integrated Classification System for Human Health and Environmental Hazard of Chemical Substances and Mixtures [ENV/JM/MONO (2001)6], the test materials **"*Hinguleshvara Rasa and Shuddha Hingula* (Red Cinnabar:HgS)"** has been classified in GHS Category 5, 5000 mg/kg body weight for the obligatory labelling requirement for oral toxicity. While the test materials **"*Shuddha Vatsanabh (Aconitum balfourii* Stapf)*"*"**has been classified in GHS Category 3, >50-300 mg/kg body weight for the obligatory labelling requirement for oral toxicity.

As described in review of literature, in previous study it was observed that acute and sub-acute toxicity study of *Ashuddha* and *Shuddha Hingula* reported that *Ashuddha Hingula* is proved to be having toxic effect while *Shuddha Hingula* at 250 mg is non toxic safe drug.[264]

Similarly in review of literature, one previous study on *Pippali(Piper longum* Linn) concluded that at the dose of 3750mg/kg at five times higher dose than therapeutic equivalent dose, there was no mortality in acute toxicity study. And in sub chronic toxicity study even at three time higher dose than therapeutic equivalent dose i.e. 2250mg/kg no serious toxicity was observed on different parameters studied.[265]

In a sighting study ,300mg/kg of cow urine –treated extracted (suspension of chloroform extract after shodhana ayurvedically treated A.ferox) was given and monitored. No death occurred till 14th day. Another animal was dosed at 2000mg/kg of cow urine treated extract ,no death occurred till the 10th day but animal died at 11th day ,which indicated delayed toxicity.[266]

In present study no death of mice occurred at 300 mg /kg of cow urine treated *Vatsanabh (Aconitum balfourii* Stapf), but death of mice occurred on 3rd day at 2000mg/kg cow urine treated *Vatsanabh (Aconitum balfourii* Stapf).

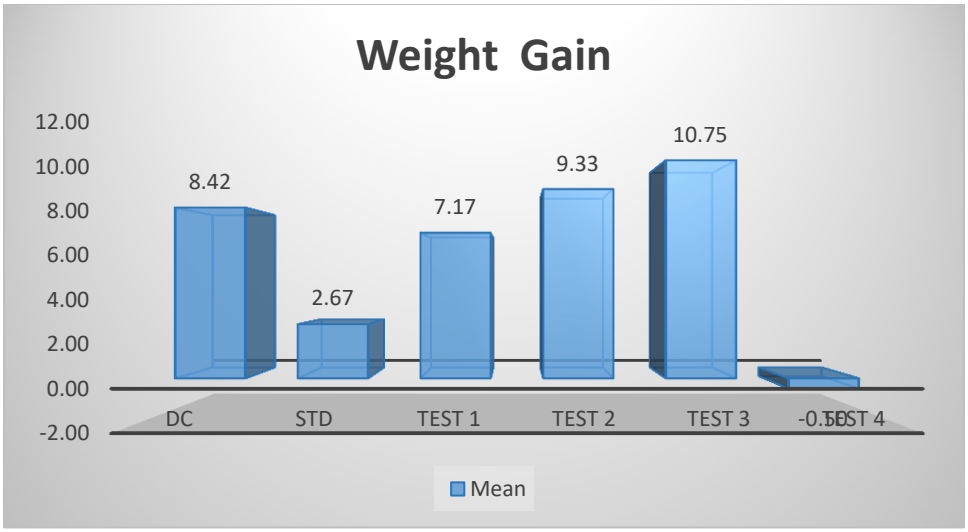
As per results of antipyretic experiment (5.38):

6.17 Weight gain after antipyretic study (as per results 5.38.1)

Weight Gain	N	Mean	SD	SE	F-Value	P-Value
DC-Brewer's Yeast	6	8.42	9.69	3.95	1.511	0.216
STD- Brewer's Yeast+ Pracetamol	6	2.67	10.67	4.36		
Brewer's Yeast +Hinguleshvara RasaT1	6	7.17	3.25	1.33		
Brewer's Yeast + Shuddha HingulaT2	6	9.33	9.18	3.75		
Brewer's Yeast + Shuddha VatsanabhT3	6	10.75	7.01	2.86		
Brewers Yeast +PimpaliT4	6	-0.50	9.73	3.97		
Total	36	6.31	8.93	1.49		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among six groups.



6.18.Following ingestion of brewer's yeast rise in temperature was observed in all groups except the Paracetamol (Std) group. It indicates Paracetamol prevented rise in temperature.

Initial Temprature:

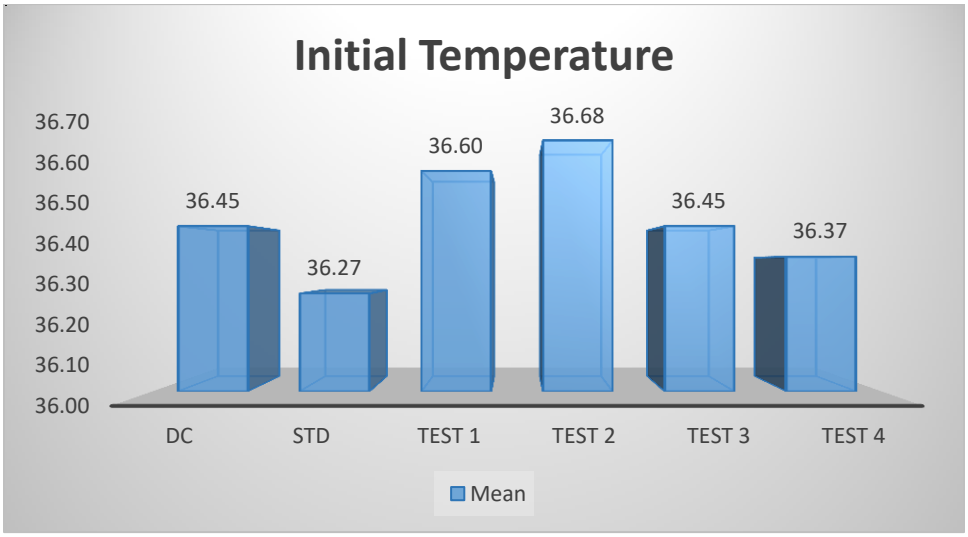
	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	36.45	0.42	0.17	0.271	0.9255637
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	6	36.27	1.24	0.51		
Brewer's Yeast +*Hinguleshvara Rasa* (5.63 mg/kg)T1	6	36.60	0.57	0.23		
Brewer's Yeast +Hingula (Red Cinnabar:HgS) (11.25 mg/Kg)T2	6	36.68	0.56	0.23		
Brewer's Yeast + *Shuddha Vatsanabh* (*Aconitum balfourii*	6	36.45	0.29	0.12		

Stapf) (1.40 mg/Kg)T3						
Brewers Yeast+ Pimpali *(Piper longum* Linn*)* (67.5 mg/Kg)T4	6	36.37	0.80	0.33		
Total	36	36.47	0.68	0.11		

*>0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that there is no significant difference among six groups.

The result indicates that there is no significant difference in antipyretic effect of standard drug paracetamol and other test drugs.



6.18.1 Effect of test drug on Brewer's Yeast induced pyrexia after 18 Hrs:

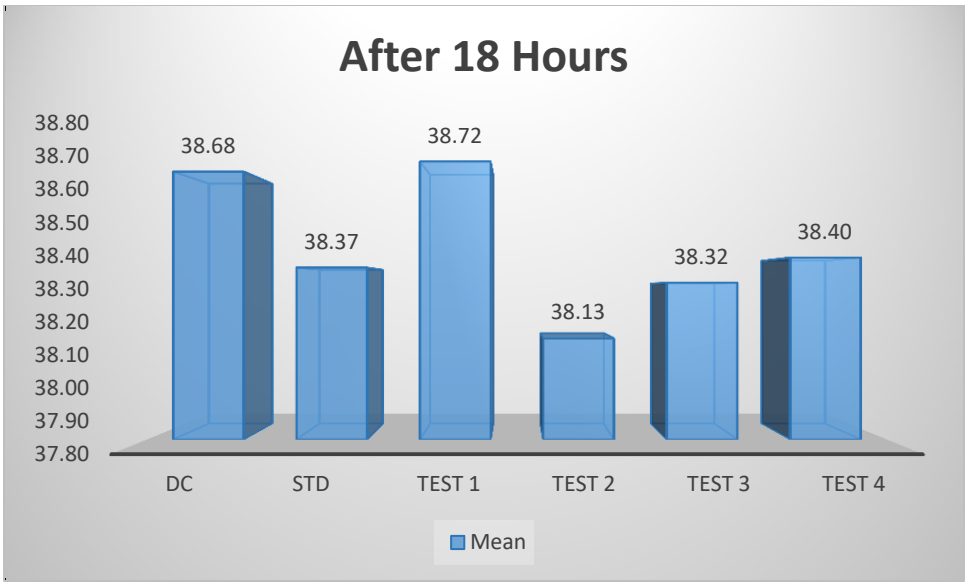
After 18 Hrs	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	38.68	0.44	0.18	1.286	0.2959486
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	6	38.37	0.71	0.29		
Brewer's Yeast +*Hinguleshvara Rasa* (5.63 mg/kg)T1	6	38.72	0.57	0.23		
Brewer's Yeast +Hingula (Red Cinnabar:HgS) (11.25 mg/Kg)T2	6	38.13	0.36	0.15		
Brewer's Yeast + *Shuddha Vatsanabh* (*Aconitum balfourii Stapf*) (1.40 mg/Kg)T3	6	38.32	0.34	0.14		
Brewers Yeast+ Pimpali *(Piper longum* Linn*)* (67.5 mg/Kg)T4	6	38.40	0.38	0.15		
Total	36	38.44	0.49	0.08		

*>0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that there is no significant difference among six groups.

No difference in temperature and action of drugs after 18 hrs in all groups.

The result indicates that there is no significant difference in antipyretic effect of standard drug paracetamol and other test drugs.



6.18.2 Effect of test drug on Brewer's Yeast induced pyrexia after 30 mins:

30 min	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	38.83	0.69	0.28	5.496	0.0010460*
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	6	37.70	0.52	0.21		
Brewer's Yeast +*Hinguleshvara Rasa* (5.63 mg/kg)T1	6	39.10	0.58	0.24		
Brewer's Yeast +Hingula (Red Cinnabar:HgS) (11.25 mg/Kg)T2	6	38.25	0.36	0.15		
Brewer's Yeast + *Shuddha Vatsanabh* (*Aconitum balfourii Stapf*) (1.40 mg/Kg)T3	6	38.47	0.29	0.12		
Brewers Yeast+ Pimpali	6	37.50	1.12	0.46		

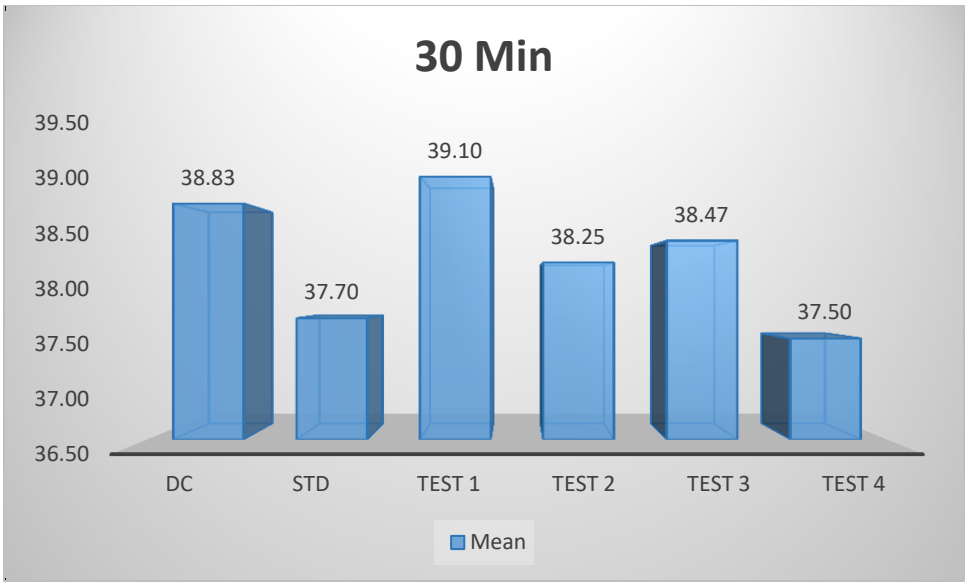
(Piper longum Linn*)* (67.5 mg/Kg)T4						
Total	36	38.31	0.84	0.14		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among six groups. The result indicates that there is no significant difference in antipyretic effect of standard drug paracetamol and other test drugs.

Further we can observe that, mean value for **Brewer's Yeast + *Hinguleshvara Rasa* (5.63 mg/kg)T1** group is maximum (39.10)while mean value for **Brewers Yeast + Pimpali (*Piper longum* Linn) (67.5 mg/Kg)T4** group is minimum (37.5) among six groups. It indicates after 30 mintues Pippali (*Piper longum* Linn) shows good antipyretic activity than other drugs.

Mean value of Standard drug paracetamol is (37.70) which is near to mean value of Pippali (*Piper longum* Linn) group.



6.18.3 Effect of test drug on Brewer's Yeast induced pyrexia after 1hr:

1 hr	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	38.87	0.39	0.16	7.806	0.0000838*
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	6	37.12	0.45	0.19		
Brewer's Yeast +*Hinguleshvara Rasa* (5.63 mg/kg)T1	6	39.33	0.67	0.27		
Brewer's Yeast +Hingula (Red Cinnabar:HgS) (11.25 mg/Kg)T2	6	38.53	0.60	0.24		
Brewer's Yeast + *Shuddha Vatsanabh* (*Aconitum balfourii Stapf*) (1.40 mg/Kg)T3	6	38.35	0.83	0.34		
Brewers Yeast+ Pimpali *(Piper longum* Linn*)* (67.5 mg/Kg)T4	6	37.48	1.18	0.48		
Total	36	38.28	1.03	0.17		

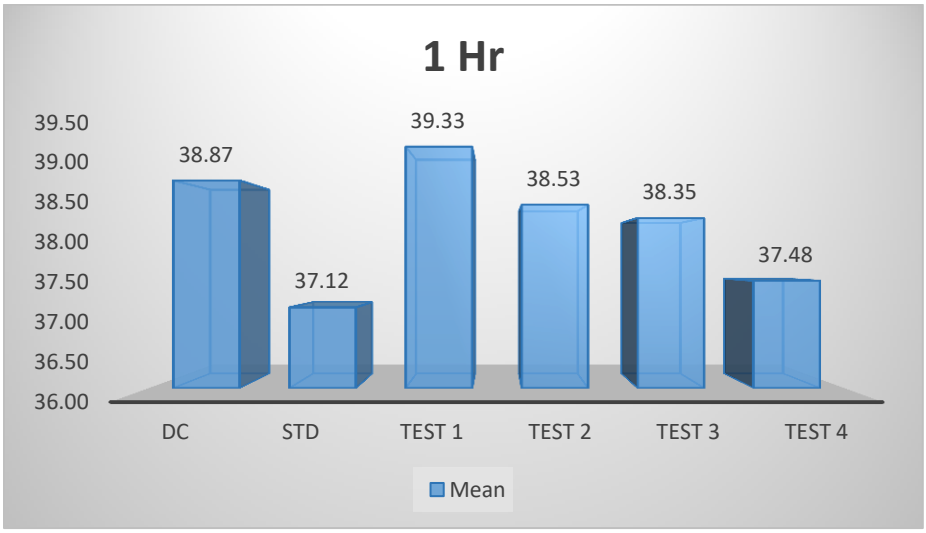
*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that there is significant difference among six groups.

The result indicates that there is statistically significant difference in antipyretic effect of standard drug paracetamol and other test drugs. Paracetamol has more antipyretic effect compared to test drugs at the end of one hour after administration of drug.

Further we can observe that, mean value for **Brewer's Yeast +*Hinguleshvara Rasa* (5.63 mg/kg) T1** group is maximum (39.33) while mean value for standard control group is minimum (37.12) among six groups.

It shows Paracetamol prevented rise in temperature. Other test drugs has no effect after one hour.



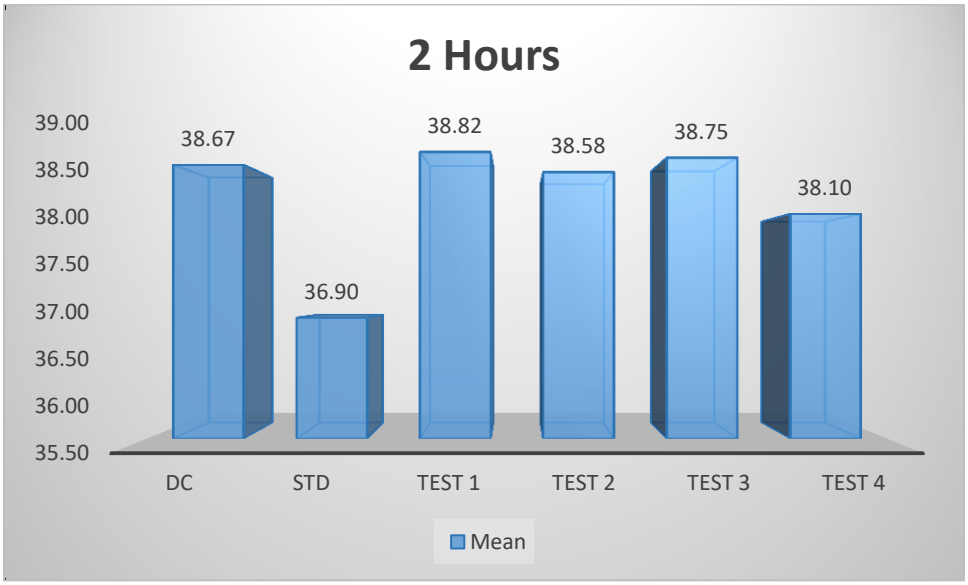
6.18.4 Effect of test drug on Brewer's Yeast induced pyrexia after 2hrs:

2 hr	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	38.67	0.56	0.23	10.166	0.0000091*
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	6	36.90	0.64	0.26		
Brewer's Yeast +*Hinguleshvara Rasa* (5.63 mg/kg)T1	6	38.82	0.67	0.27		
Brewer's Yeast +Hingula (Red Cinnabar:HgS) (11.25 mg/Kg)T2	6	38.58	0.66	0.27		
Brewer's Yeast + *Shuddha Vatsanabh* (*Aconitum balfourii Stapf*) (1.40 mg/Kg)T3	6	38.75	0.36	0.15		
Brewers Yeast+ Pimpali *(Piper longum* Linn*)* (67.5 mg/Kg)T4	6	38.10	0.41	0.17		
Total	36	38.30	0.86	0.14		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that there is significant difference among six groups. Further we can observe that, mean value for **Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/kg) T1** and **Brewer's Yeast + Vatsanabh (1.40 mg/Kg) T3** group is maximum while mean value for standard control group is minimum among six groups. It shows Paracetamol prevented rise in temperature. Other test drugs has no effect after two hour.

The standard drug Paracetamol has statistically significant antipyretic effect as compared to all test drugs at the end of two hours.



6.18.5 Effect of test drug on Brewer's Yeast induced pyrexia after 4 hrs:

4 hr	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	38.15	0.86	0.35	3.873	0.0079404*
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	6	36.85	0.93	0.38		
Brewer's Yeast +*Hinguleshvara Rasa* (5.63 mg/kg)T1	6	38.52	0.34	0.14		
Brewer's Yeast +Hingula (Red Cinnabar:HgS) (11.25 mg/Kg)T2	6	38.12	0.58	0.24		
Brewer's Yeast + *Shuddha Vatsanabh* (*Aconitum balfourii Stapf*) (1.40 mg/Kg)T3	6	38.02	0.46	0.19		
Brewers Yeast+ Pimpali *(Piper longum* Linn*)* (67.5 mg/Kg)T4	6	37.70	0.89	0.36		

Total	36	37.89	0.85	0.14		

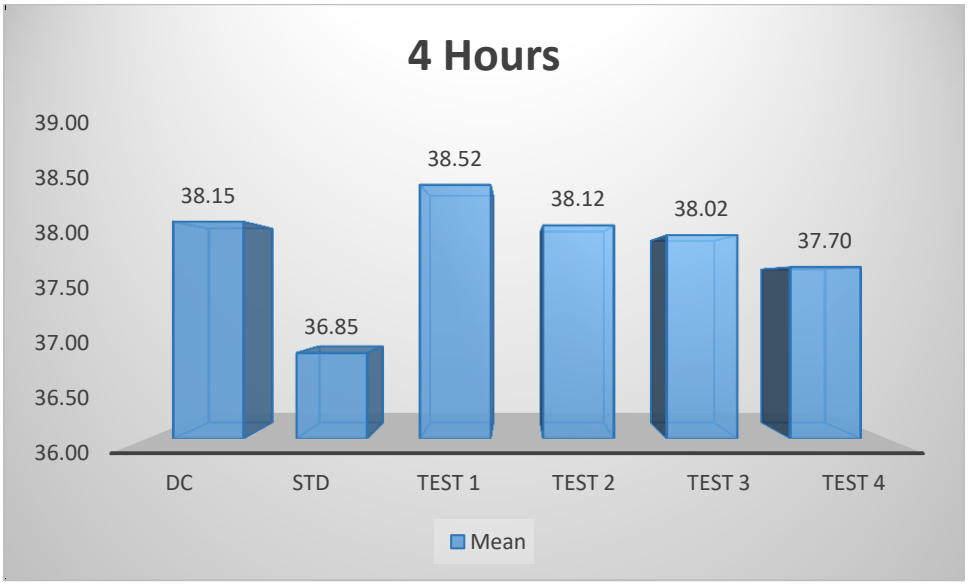
*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that there is significant difference among six groups.

Further we can observe that, mean value for **Brewer's Yeast + *Hinguleshvara Rasa* (5.63 mg/kg)T1** group is maximum (38.52)while mean value for standard control group is minimum(36.85) among six groups.

It shows Paracetamol prevented rise in temperature. Other test drugs has no effect after four hour.

Paracetamol has more antipyretic effect compared to test drugs at the end of four hours after administration of drug.



6.18.6 Effect of test drug on Brewer's Yeast induced pyrexia after 6 Hrs:

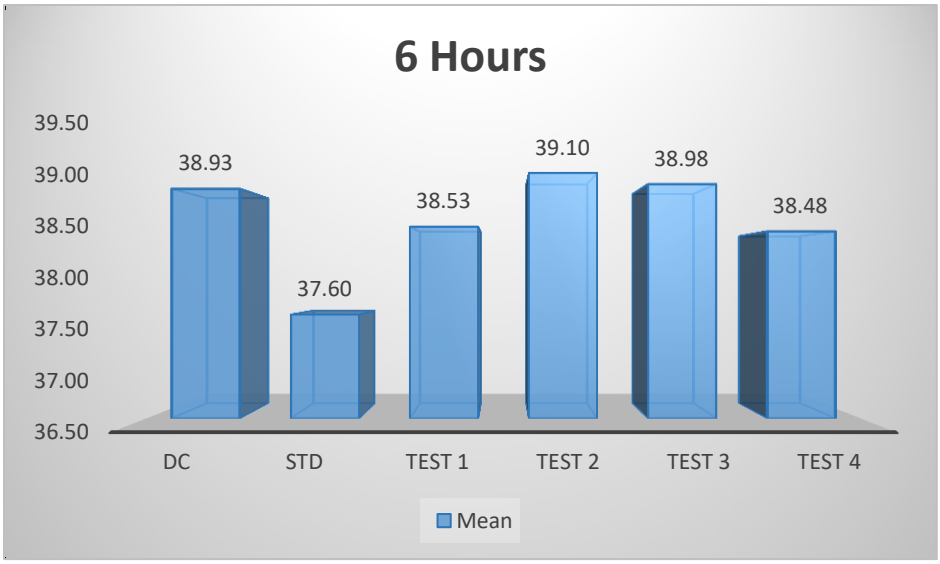
6 hr	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	38.93	0.74	0.30	3.901	0.0076551*
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	6	37.60	1.02	0.41		
Brewer's Yeast +*Hinguleshvara Rasa* (5.63 mg/kg)T1	6	38.53	0.55	0.22		
Brewer's Yeast +Hingula (Red Cinnabar:HgS) (11.25 mg/Kg)T2	6	39.10	0.82	0.33		
Brewer's Yeast + *Shuddha Vatsanabh* (*Aconitum balfourii Stapf*) (1.40 mg/Kg)T3	6	38.98	0.32	0.13		
Brewers Yeast+ Pimpali *(Piper longum* Linn*)* (67.5 mg/Kg)T4	6	38.48	0.41	0.17		
Total	36	38.61	0.81	0.14		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among six groups.

Further we can observe that, mean value for **Brewer's Yeast + *Shuddha Hingula* (Red Cinnabar: HgS) (11.25 mg/Kg) T2** group is maximum (39.10) while mean value for standard control group is minimum (37.6)among six groups.

It shows Paracetamol prevented rise in temperature. Other test drugs has no effect after six hour. Paracetamol has more antipyretic effect compared to test drugs at the end of six hours after administration of drug.

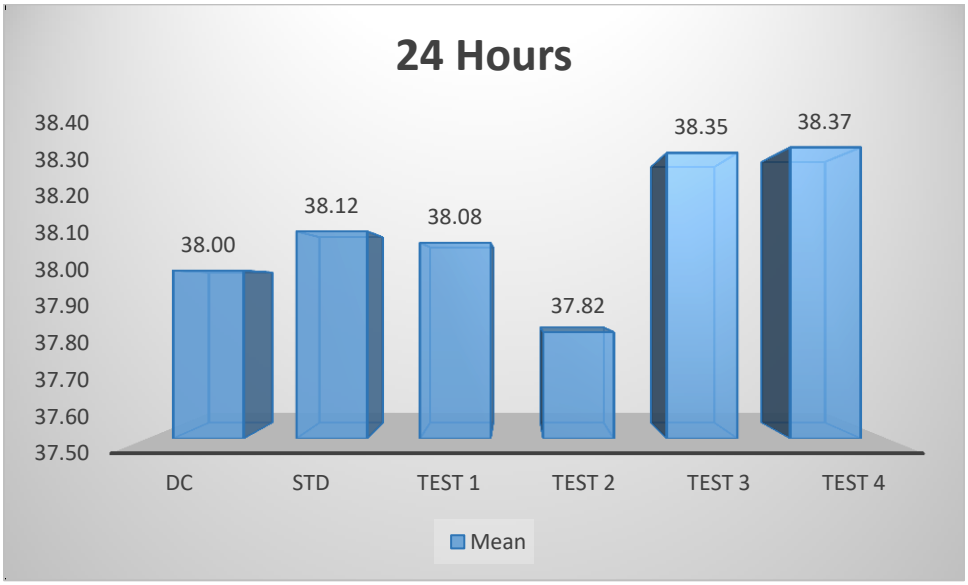


6.18.7 Effect of test drug on Brewer's Yeast induced pyrexia after 24 Hrs:

24 hr	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	38.00	0.39	0.16	0.911	0.4871735
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	6	38.12	0.99	0.40		
Brewer's Yeast +*Hinguleshvara Rasa* (5.63 mg/kg)T1	6	38.08	0.29	0.12		
Brewer's Yeast +Hingula (Red Cinnabar:HgS) (11.25 mg/Kg)T2	6	37.82	0.55	0.22		
Brewer's Yeast + *Shuddha Vatsanabh*	6	38.35	0.29	0.12		

(*Aconitum balfourii Stapf*) (1.40 mg/Kg)T3						
Brewers Yeast+ Pimpali *(Piper longum* Linn*)* (67.5 mg/Kg)T4	6	38.37	0.39	0.16		
Total	36	38.12	0.54	0.09		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among six groups. Test drugs are as effective as standard drug Paracetamol in antipyretic activity at the end of twenty-four hours.



No effect after twenty four hours in standard or test drug group was noticed.

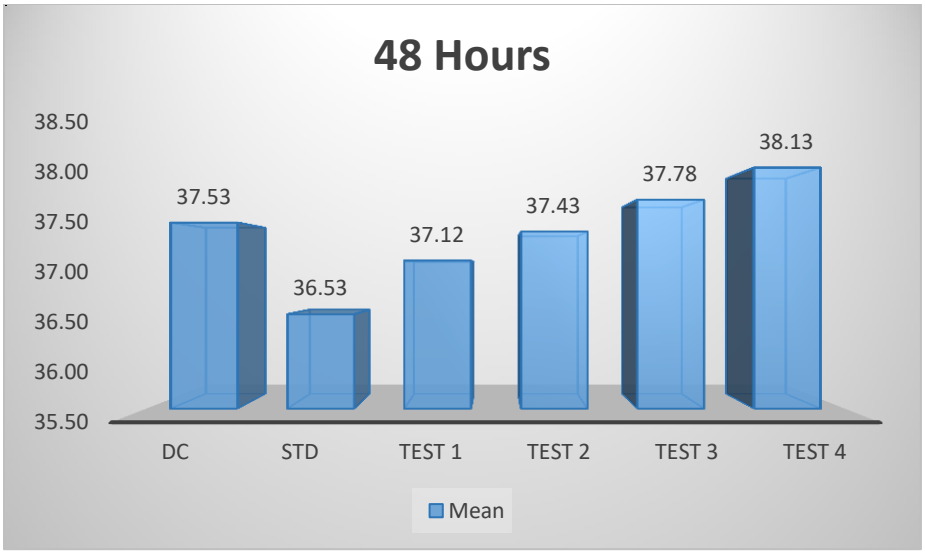
6.18.8 Effect of test drug on Brewer's Yeast induced pyrexia after 48 Hrs:

48 hr	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	37.53	0.91	0.37	4.058	0.0062256*
STD- Brewer's Yeast+ Pracetamol (150mg/kg)	6	36.53	1.04	0.42		
Brewer's Yeast +*Hinguleshvara Rasa* (5.63 mg/kg)T1	6	37.12	0.69	0.28		
Brewer's Yeast +Hingula (Red Cinnabar:HgS) (11.25 mg/Kg)T2	6	37.43	0.44	0.18		
Brewer's Yeast + *Shuddha Vatsanabh* (*Aconitum balfourii Stapf*) (1.40 mg/Kg)T3	6	37.78	0.29	0.12		
Brewers Yeast+ Pimpali *(Piper longum* Linn*)* (67.5 mg/Kg)T4	6	38.13	0.23	0.10		
Total	36	37.42	0.81	0.13		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among six groups.

Paracetamol is more effective in antipyretic activity as compared to test drugs after 48 hours.

Further we can observe that, mean value for **Brewers Yeast + Pimpali (67.5 mg/Kg)T4** group is maximum (38.13)while mean value for standard control group is minimum (36.53)among six groups.

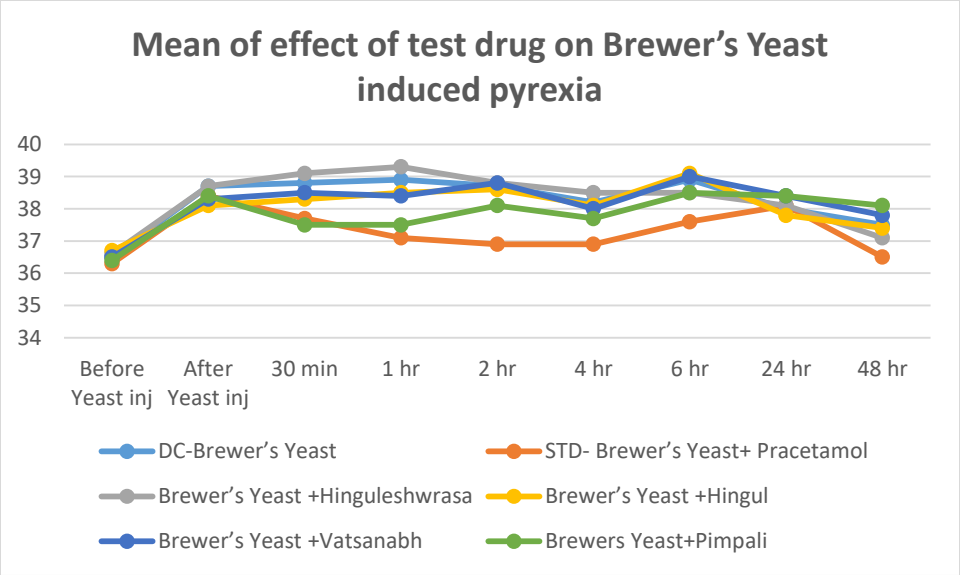


It shows Paracetamol prevented rise in temperature. Other test drugs has no effect after fourty eight hours.

6.18.9.Mean of effect of test drug on Brewer's Yeast induced pyrexia(as per 5.38.2)

Groups	Before Yeast inj	After Yeast inj	After Drug Administration								
			30 min	1 hr	2 hr	4 hr	6 hr	24 hr	48 hr	F-Value	P-Value
DC-Brewer's Yeast	36.5	38.7	38.8	38.9	38.7	38.2	38.9	38	37.5	1.593	0.154
STD- Brewer's Yeast+ Pracetamol	36.3	38.4	37.7	37.1	36.9	36.9	37.6	38.1	36.5	1.321	0.258
Brewer's Yeast + *Hinguleshvara Rasa*	36.6	38.7	39.1	39.3	38.8	38.5	38.5	38.1	37.1	1.325	0.256
Brewer's Yeast + Shuddha Hingula	36.7	38.1	38.3	38.5	38.6	38.1	39.1	37.8	37.4	1.526	0.175
Brewer's Yeast + Shuddha Vatsanabh	36.5	38.3	38.5	38.4	38.8	38	39	38.4	37.8	1.360	0.240
Brewers Yeast+Pimpali	36.4	38.4	37.5	37.5	38.1	37.7	38.5	38.4	38.1	1.281	0.278

Repeated Measures ANOVA Test is carried out to test significance in all groups at different time points. Above table shows that, P-Value for all six groups are greater than **0.05. Hence, we can conclude that, there is no significant change observed in mean at** different time points.



After 18 hrs of injecting Brewer's Yeast in the animals of all the groups except Normal Control, the rectal temperature was increased in all the groups. After oral administration of Standard (Paracetamol) and respective test drugs, no significant reduction in the rectal temperature was observed in all the treated groups.

Based on the above results obtained it can be concluded that the test drugs *Hinguleshvara Rasa* (T1), *Shodhita Hingula* (T2), *Shodhita Vatsanabh* (T3) and Pippali (T4) are having the potent analgesic activity but less anti-pyretic activity.

Overall results indicate that the standard drug Paracetamol is more effective in antipyretic activity than the test drugs *Hinguleshvara Rasa*, specifically in initial period like at the end of one hour, two hours. However in later stage like twenty-four hours and more the antipyretic activity of Paracetamol and *Hinguleshvara Rasa* is almost similar. Still it can be concluded that the antipyretic activity of *Hinguleshvara Rasa* doesn't match with that of standard drug Paracetamol

Actually *Hinguleshvara Rasa* is widely used for its antipyretic activity. In present study antipyretic activity of *Hinguleshvara Rasa* was compared with its all three ingredients namely *Shuddha Hingula* (Red Cinnabar:HgS), *Shuddha Vatsana*bh (*Aconitum balfourii* Stapf) and *Piper longum* Linn. It was found that all these four test drug exerted better analgesic activity than their known antipyretic action. In previous few study of *Hinguleshvara Rasa* research scholars mentioned that *Hinguleshvara Rasa* has good analgesic activity as well as antipyretic activity.

In one study research scholar mentioned that antipyretic activity of *Vatsanabh* when its *shodhana* performed in cow milk was more than when its *shodhana* performed in cow urine [**267**] In present study *Vatsanabh shodhana* was performed in cow urine and not in cow urine. So expected antipyretic activity was not found.

Hinguleshvara Rasa prepared with *bhavana* gave potent antipyretic effect while *Hinguleshvara Rasa* prepared without *bhavana* was exerted weak antipyretic activity**.**[**268]**

In present study as *Bhavana dravya* was not mentioned in classical reference of *Hinguleshvara Rasa* given in Bhaishajya Ratnavali, so *bhavana* were not performed. This is also the reason for not getting expected antipyretic activity of *Hinguleshvara Rasa*

If these earlier two findings compared with present study, it was clear in present study *shodhana of Vatsnabh Aconitum balfourii* Stapf was performed in cow urine and not in cow milk. As there was no dravya mentioned for wet grinding of *Hinguleshvara* in *Bhaishajya Ratnavali, bhavana* were not performed. This is the reason why an expected antipyretic activity of *Hinguleshvara Rasa* was not observed in this study.

6.19 effect on Haematological parameters after antipyretic study (as per results 5.38.3):

6.19.1 Effect on White blood cells

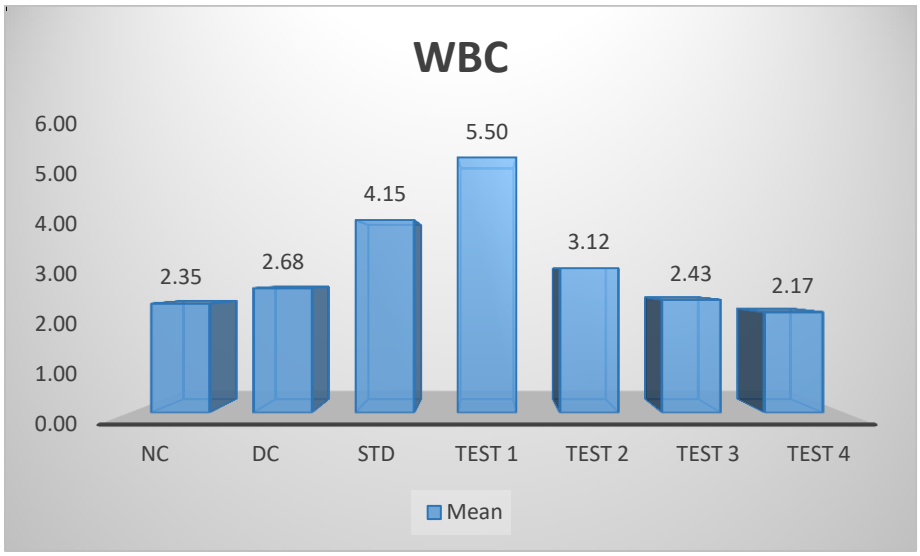
WBC	N	Mean	SD	SE	F-Value	P-Value
NC	6	2.35	0.98	0.40	6.260	0.000*
DC-Brewer's Yeast	6	2.68	0.57	0.23		
STD- Brewer's Yeast+ Pracetamol	6	4.15	2.08	0.85		
Brewer's Yeast +Hinguleshvara Rasa T1	6	5.50	1.19	0.49		
Brewer's Yeast + Shuddha Hingula T2	6	3.12	1.17	0.48		
Brewer's Yeast + Shuddha Vatsanabh T3	6	2.43	0.96	0.39		
Brewers Yeast+Pippali T4	6	2.17	0.77	0.31		
Total	42	3.20	1.58	0.24		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for **Brewer's Yeast + *Hinguleshvara Rasa* T1** group is maximum while mean value for **Brewers Yeast + Pippali (*Piper longum* Linn) T4** group is minimum among seven groups.

White blood cells protects body from infection. In this study pyrexia was produced by Inducing Brewer's yeast. From above table mean value of platelets for **Brewer's Yeast + *Hinguleshvara Rasa* T1** group is more than standard group of paracetamol.



6.19.2. Effect on Lymphocyte

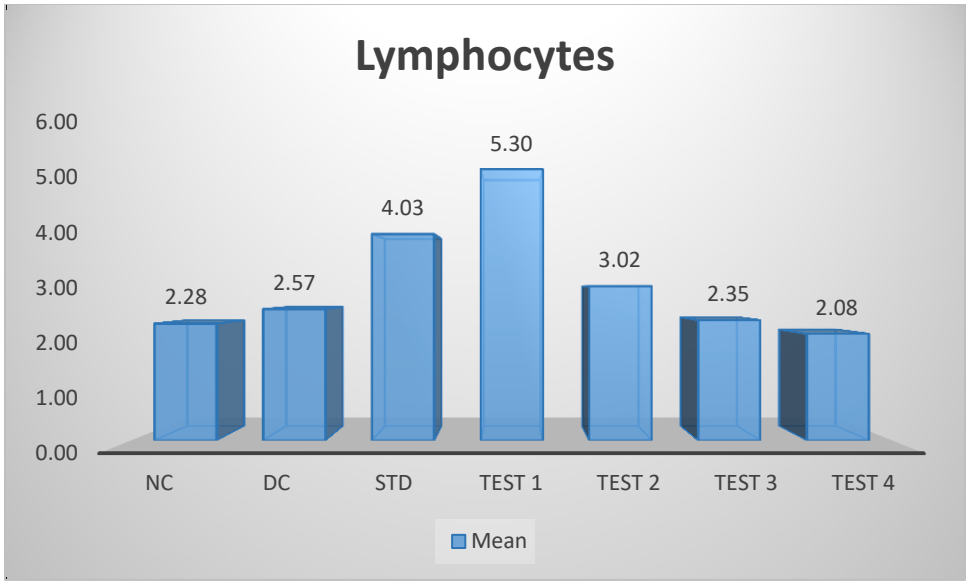
Lymphocytes	N	Mean	SD	SE	F-Value	P-Value
NC	6	2.28	0.96	0.39	6.013	0.000*
DC-Brewer's Yeast	6	2.57	0.57	0.23		
STD- Brewer's Yeast+ Pracetamol	6	4.03	2.04	0.83		
Brewer's Yeast +Hinguleshvara Rasa T1	6	5.30	1.19	0.49		
Brewer's Yeast + Shuddha Hingula T2	6	3.02	1.17	0.48		

Brewer's Yeast + Shuddha Vatsanabh T3	6	2.35	0.93	0.38		
BrewersYeast+ Pippali T4	6	2.08	0.75	0.30		
Total	42	3.09	1.54	0.24		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

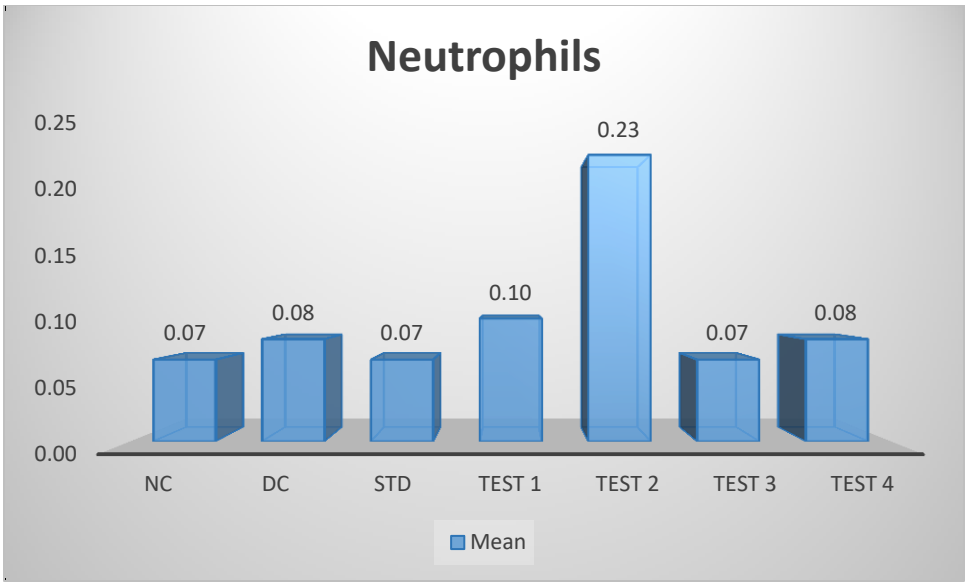
Further we can observe that, mean value for **Brewer's Yeast + *Hinguleshvra Rasa* T1** group is maximum while mean value for **Brewers Yeast+Pippali T4** group is minimum among seven groups. Lymphocytes are of three types namely T cells, Natural killers and B cells . Lymphocyte produces proteins to protect body by fighting against infection.



6.19.3. Effect on Neutrophils

Neutrophils	N	Mean	SD	SE	F-Value	P-Value
NC	6	0.07	0.05	0.02	0.960	0.466
DC-Brewer's Yeast	6	0.08	0.08	0.03		
STD- Brewer's Yeast+ Pracetamol	6	0.07	0.05	0.02		
Brewer's Yeast +Hinguleshvara Rasa T1	6	0.10	0.00	0.00		
Brewer's Yeast + Shuddha Hingula T2	6	0.23	0.38	0.15		
Brewer's Yeast + Shuddha Vatsanabh T3	6	0.07	0.05	0.02		
Brewers Yeast+Pippali T4	6	0.08	0.04	0.02		
Total	42	0.10	0.15	0.02		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among seven groups.



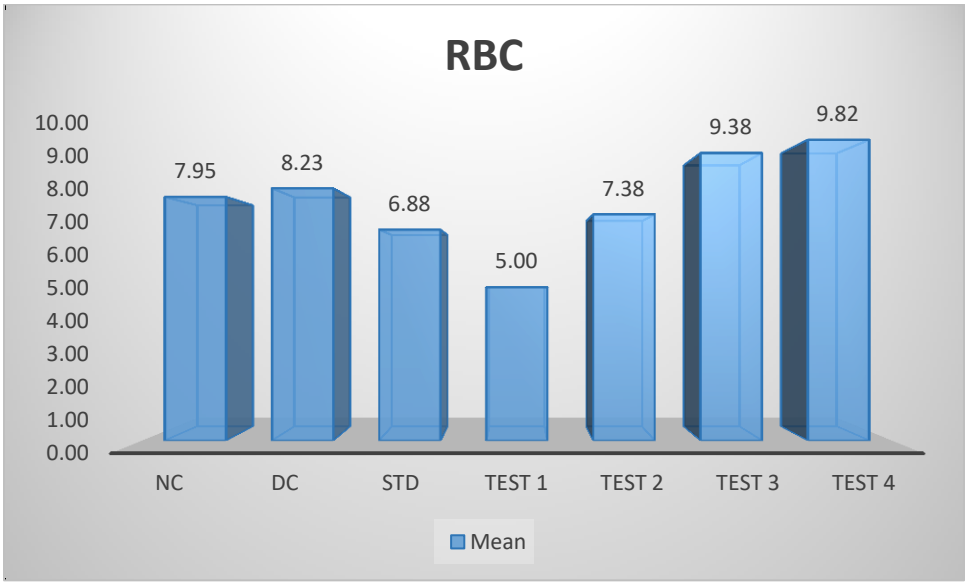
6.19.4. Effect on Red Blood Corspuscle

RBC	N	Mean	SD	SE	F-Value	P-Value
NC	6	7.95	0.93	0.38	6.881	0.000*
DC-Brewer's Yeast	6	8.23	0.39	0.16		
STD- Brewer's Yeast+ Pracetamol	6	6.88	0.68	0.28		
Brewer's Yeast +Hinguleshvara RasaT1	6	5.00	3.12	1.27		
Brewer's Yeast + Shuddha Hingula T2	6	7.38	0.55	0.22		
Brewer's Yeast + Shuddha Vatsanabh T3	6	9.38	1.69	0.69		
Brewers Yeast+Pippali T4	6	9.82	1.24	0.50		
Total	42	7.81	2.06	0.32		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for ***Brewers Yeast+Pippali T4*** group is maximum while mean value for ***Brewer's Yeast + Hinguleshvara Rasa T1*** group is minimum among seven groups.



6.19.5. Effect on Haemoglobin

Hemoglobin	N	Mean	SD	SE	F-Value	P-Value
NC	6	14.73	1.86	0.76	8.056	0.000*
DC-Brewer's Yeast	6	15.48	1.09	0.45		
STD- Brewer's Yeast+ Pracetamol	6	12.50	1.45	0.59		
Brewer's Yeast +Hinguleshvara RasaT1	6	12.62	0.45	0.18		

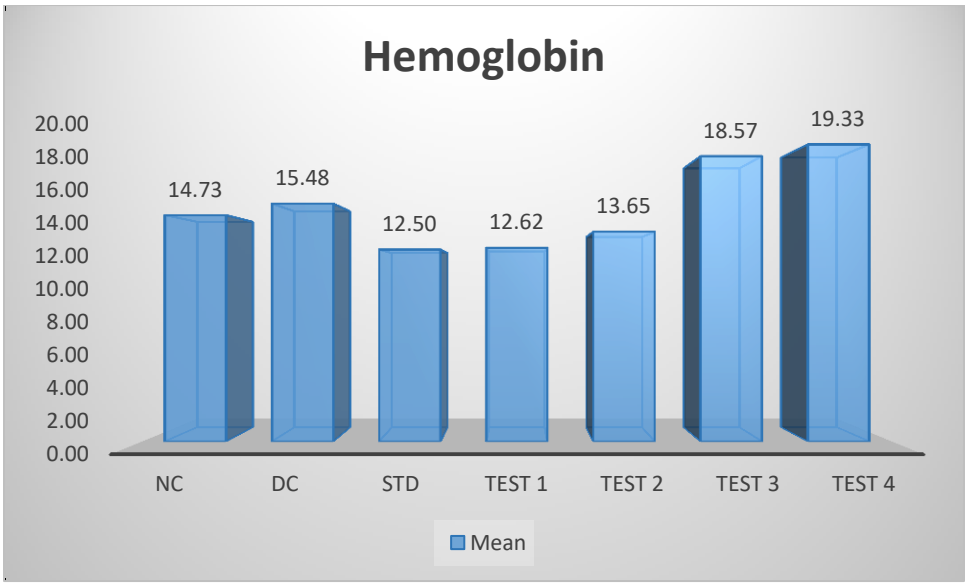
Brewer's Yeast + Shuddha Hingula T2	6	13.65	1.36	0.55		
Brewer's Yeast + Shuddha Vatsanabh T3	6	18.57	4.42	1.81		
Brewers Yeast+Pippali T4	6	19.33	3.28	1.34		
Total	42	15.27	3.37	0.52		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for ***Brewers Yeast+Pippali T4*** group is maximum while mean value for standard control (Paracetamol) group is minimum among seven groups.

Haemoglobin as molecular heat transducer through its oxygenation and deoxygenation cycle.



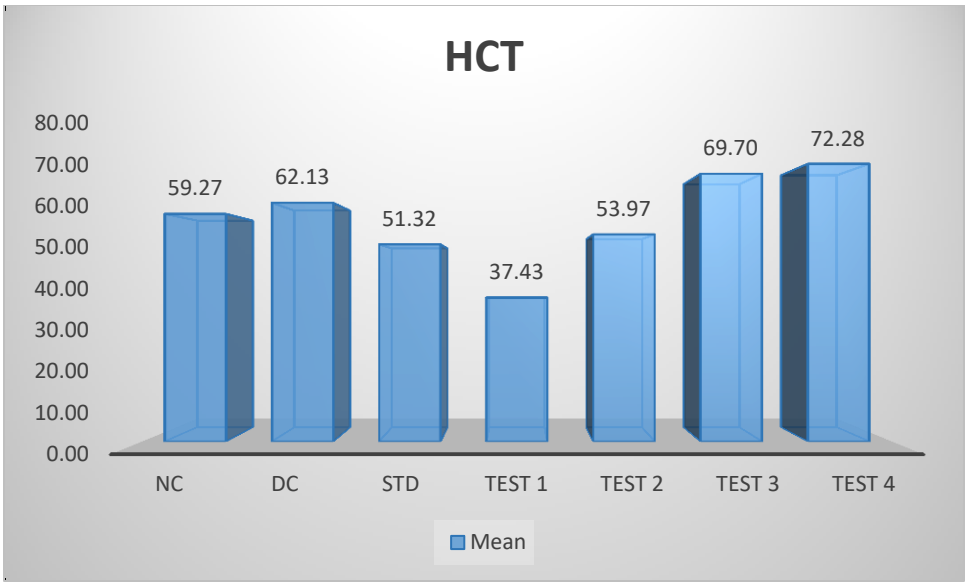
6.19.6 Effect on.Haematocret

HCT	N	Mean	SD	SE	F-Value	P-Value
NC	6	59.27	5.96	2.43	6.169	0.000*
DC-Brewer's Yeast	6	62.13	4.12	1.68		
STD- Brewer's Yeast+ Pracetamol	6	51.32	5.28	2.16		
Brewer's Yeast +Hinguleshvara RasaT1	6	37.43	23.29	9.51		
Brewer's Yeast + Shuddha Hingula T2	6	53.97	4.87	1.99		
Brewer's Yeast + Shuddha Vatsanabh T3	6	69.70	14.06	5.74		
Brewers Yeast+Pippali T4	6	72.28	10.62	4.34		
Total	42	58.01	15.50	2.39		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for ***Brewers Yeast+Pippali T4*** group is maximum while mean value for ***Brewer's Yeast +Hinguleshvara Rasa T1*** group is minimum among seven groups.



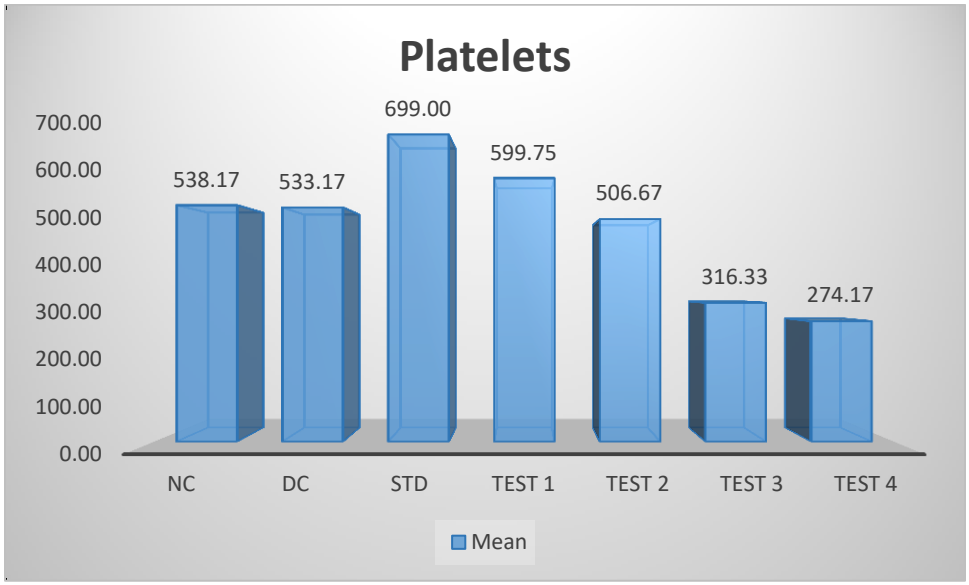
6.19.7 Effect on.Platelets

Platelets	N	Mean	SD	SE	F-Value	P-Value
NC	6	538.17	185.33	75.66	5.290	0.001*
DC-Brewer's Yeast	6	533.17	123.67	50.49		
STD- Brewer's Yeast+ Pracetamol	6	699.00	171.36	69.96		
Brewer's Yeast +Hinguleshvara Rasa T1	6	599.75	227.66	92.94		
Brewer's Yeast + Shuddha Hingula T2	6	506.67	112.32	45.85		
Brewer's Yeast + Shuddha Vatsanabh T3	6	316.33	160.43	65.49		
Brewers Yeast+Pippali T4	6	274.17	108.39	44.25		
Total	42	495.32	205.12	31.65		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for ***STD- Brewer's Yeast+ Pracetamol*** standard control group is maximum while mean value for ***Brewers Yeast+Pippali T4*** group is minimum among seven groups.



In present study for an assessment of antipyretic action of all test drugs, pyrexia induced by Brewer's yeast (Saccharomyces cerevisiae).

During pyrexia body mechanism works in two ways:

1) To reduce body temperature by thermoregulation.

2) To protect body from infection by immune building mechanism.

Haematological parameters like WBC, Lymphocytes play an important role building immune system.

Haematology Parameters: No statistical changes were observed in hematology parameters.

WBC:

Further we can observe that, mean value for(WBC) Brewer's Yeast +*Hinguleshvara Rasa* T1 group is maximum 5.50, while mean value for (WBC) Brewers Yeast+Pippali T4 group is minimum 2.17 among seven groups.

Lymphocyte:

Further we can observe that, mean value for Lymphocytes in Brewer's Yeast + *Hinguleshvra Rasa* T1 group is maximum 5.30,while mean value for Lymphocytes in Brewers Yeast+Pippali T4 group is minimum 2.08 among seven groups. Lymphocytes are of three types namely T cells, Natural killers and B cells . Lymphocyte produces proteins to protect body by fighting against infection. Mean value for Lymphocytes **in *Pracetamol group is* 4.03.**

Neutrophils

There is no significant difference in mean value of Neutrophils among seven groups.

In group of *Hinguleshvara Rasa* value of WBC and Lymphocyte found more than standard group Paracetamol. It can assume that though *Hinguleshvara Rasa* was not exerts antipyretic activity by reducing body temperature but helps to protects body from infection by building immune system.

RBC :

Further we can observe that, mean value for RBC ***in Brewers*** *Yeast+Pippali T4* group is maximum 9.82, while mean value for RBC *inBrewer's Yeast +Hinguleshvara Rasa T1* group is minimum 5.00 among seven groups.

Haemoglobin:

Further we can observe that, mean value for Haemoglobin in

Brewers Yeast+Pippali T4 group is maximum 19.33while mean value for standard control group *Paracetamol* is minimum 12.50among seven groups.

Haemoglobin as molecular heat transducer through its oxygenation and deoxygenation cycle. In group of Pippali value of RBC and Haemoglobin were found more than standard group Paracetamol. It can assume that Pippali helps to protect body from hypoxia during pyrexia.

Haematocret :

Further we can observe that, mean value for Haematocret in *Brewers Yeast+PimpaliT4* group is maximum 72.28, while mean value for Haematocret *in Brewer's Yeast +Hinguleshvara Rasa T1* group is minimum 37.43,among seven groups.

Platelate:

Further we can observe that, mean value for Platelets in ***STD- Brewer's Yeast+ Pracetamol*** **standard control group is maximum 699.00, while mean value for Platelets in *Brewers Yeast+Pippali T4* group is minimum** 274.17 among seven groups.

6.20. Effect on Biochemical parameters after antipyretic study (as per results 5.38.4):

6.20.1. Effect on Total protein

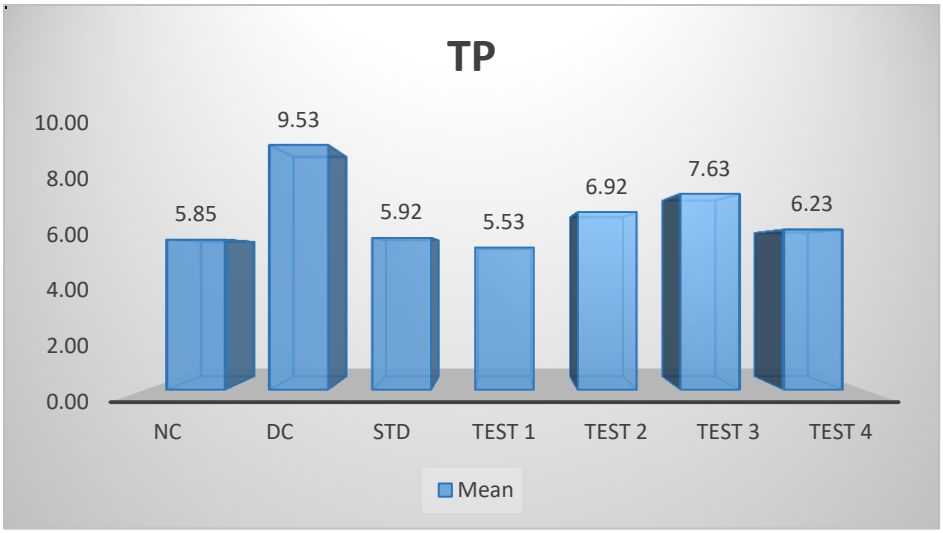
TP	N	Mean	SD	SE	F-Value	P-Value
Normal Contral	6	5.85	0.63	0.26		
Brewer's Yeast (10 mg/Kg, s.c)	6	9.53	3.18	1.30		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	5.92	1.17	0.48		
Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1	6	5.53	0.86	0.35	5.511	0.00042*
Brewer's Yeast+ *Shodhita Hingula* (11.25 mg/Kg)T2	6	6.92	0.55	0.22		
Brewer's Yeast+ Shodhita Vatsanabh (1.40 mg/Kg)T3	6	7.63	1.18	0.48		

Brewer's Yeast+ *Pippali* (67.5 mg/Kg)T4	6	6.23	0.81	0.33		
Total	42	6.80	1.88	0.29		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for disease control group is maximum while mean value for Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg) T1 group is minimum among seven groups.

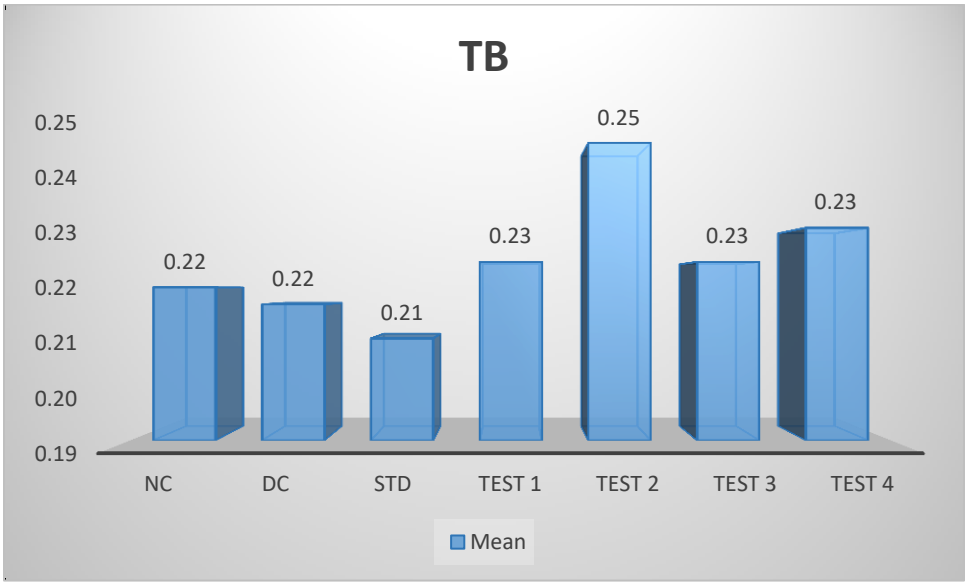


6.20.2. Effect on Total Bilirubin

TB	N	Mean	SD	SE	F-Value	P-Value
Normal Control	6	0.22	0.04	0.02	0.335	0.91364
Brewer's Yeast (10 mg/Kg, s.c)	6	0.22	0.03	0.01		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	0.21	0.07	0.03		

Brewer's Yeast+ *Hinguleshvara Rasa (5.63 mg/Kg)T1*	6	0.23	0.04	0.02		
Brewer's Yeast+ *Shodhita Hingula* (11.25 mg/Kg)T2	6	0.25	0.06	0.03		
Brewer's Yeast+ *Shodhita Vatsanabh* (1.40 mg/Kg)T3	6	0.23	0.06	0.03		
Brewer's Yeast+ *Pippali*(67.5 mg/Kg)T4	6	0.23	0.04	0.02		
Total	42	0.23	0.05	0.01		

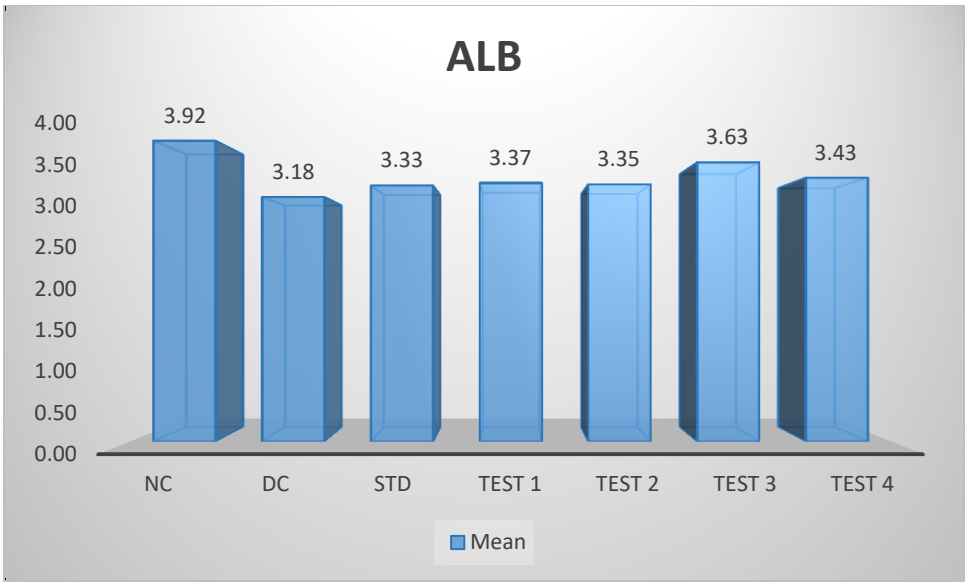
One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among seven groups.



6.20.3. Effect on Total Albumin

ALB	N	Mean	SD	SE	F-Value	P-Value
Normal Control	6	3.92	0.69	0.28	0.523	0.78659
Brewer's Yeast (10 mg/Kg, s.c)	6	3.18	0.73	0.30		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	3.33	0.96	0.39		
Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1	6	3.37	0.71	0.29		
Brewer's Yeast+ *ShodhitaHingula* (11.25 mg/Kg)T2	6	3.35	0.69	0.28		
Brewer's Yeast+ ShodhitaVatsanabh (1.40 mg/Kg)T3	6	3.63	1.19	0.49		
Brewer's Yeast+ *Pippali* (67.5 mg/Kg)T4	6	3.43	0.63	0.26		
Total	42	3.46	0.79	0.12		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among seven groups.

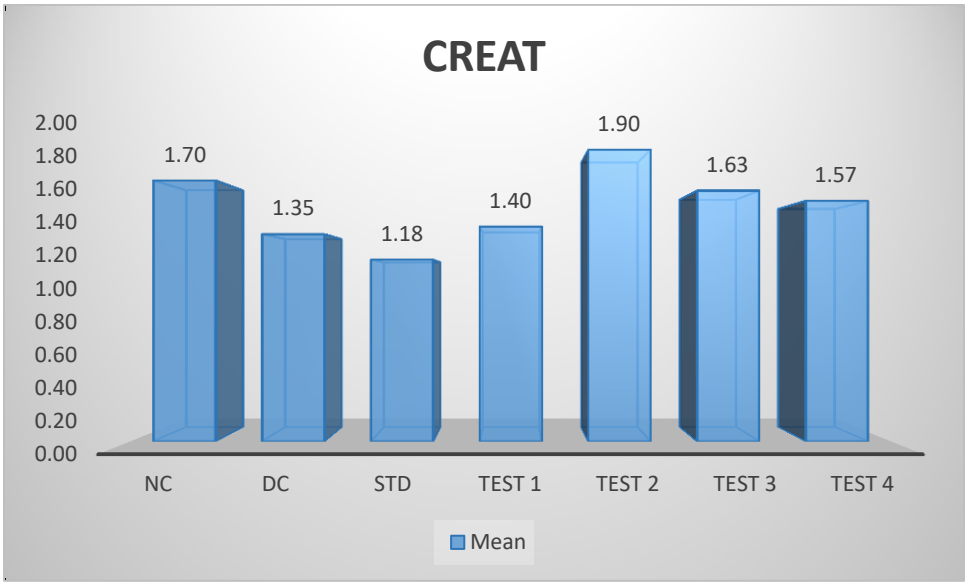


6.20.4. Effect on Total Creatinine

Creat	N	Mean	SD	SE	F-Value	P-Value
Normal Control	6	1.70	0.24	0.10	2.351	0.05177
Brewer's Yeast (10 mg/Kg, s.c)	6	1.35	0.62	0.25		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	1.18	0.31	0.12		
Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1	6	1.40	0.29	0.12		
Brewer's Yeast+ *ShodhitaHingula* (11.25 mg/Kg)T2	6	1.90	0.30	0.12		
Brewer's Yeast+ ShodhitaVatsanabh (1.40 mg/Kg)T3	6	1.63	0.21	0.08		
Brewer's Yeast+ *Pippali* (67.5	6	1.57	0.53	0.22		

mg/Kg)T4						
Total	42	1.53	0.42	0.06		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among seven groups.



6.20.5. Effect on Alkaline phosphatase

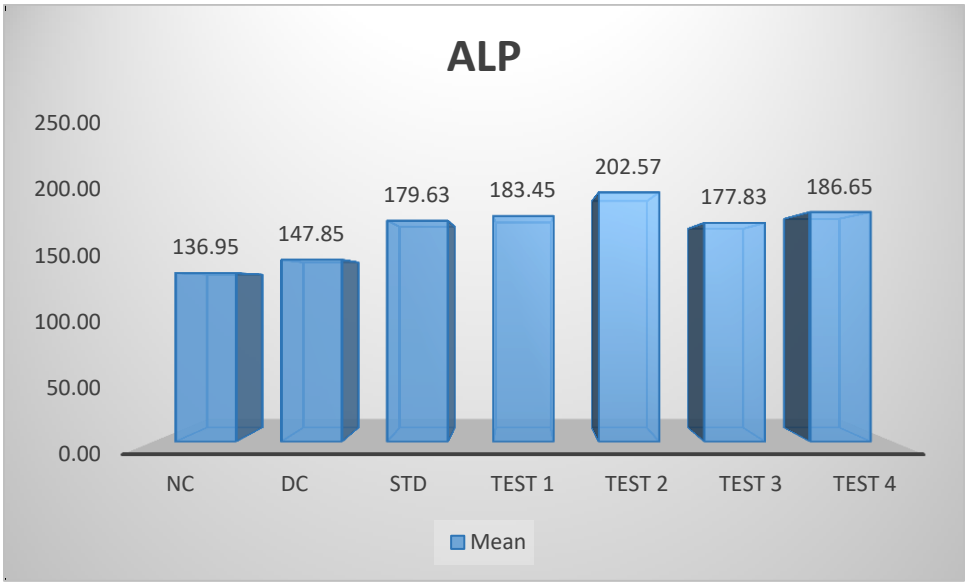
ALP	N	Mean	SD	SE	F-Value	P-Value
Normal Control	6	136.95	29.90	12.21	4.234	0.00263*
Brewer's Yeast (10 mg/Kg, s.c)	6	147.85	15.56	6.35		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	179.63	28.39	11.59		
Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1	6	183.45	19.94	8.14		
Brewer's Yeast+ *Shodhita Hingula*	6	202.57	19.90	8.13		

(11.25 mg/Kg)T2						
Brewer's Yeast+ Shodhita Vatsanabh (1.40 mg/Kg)T3	6	177.83	41.78	17.06		
Brewer's Yeast+ *Pippali*(67.5 mg/Kg)T4	6	186.65	27.47	11.22		
Total	42	173.56	33.20	5.12		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

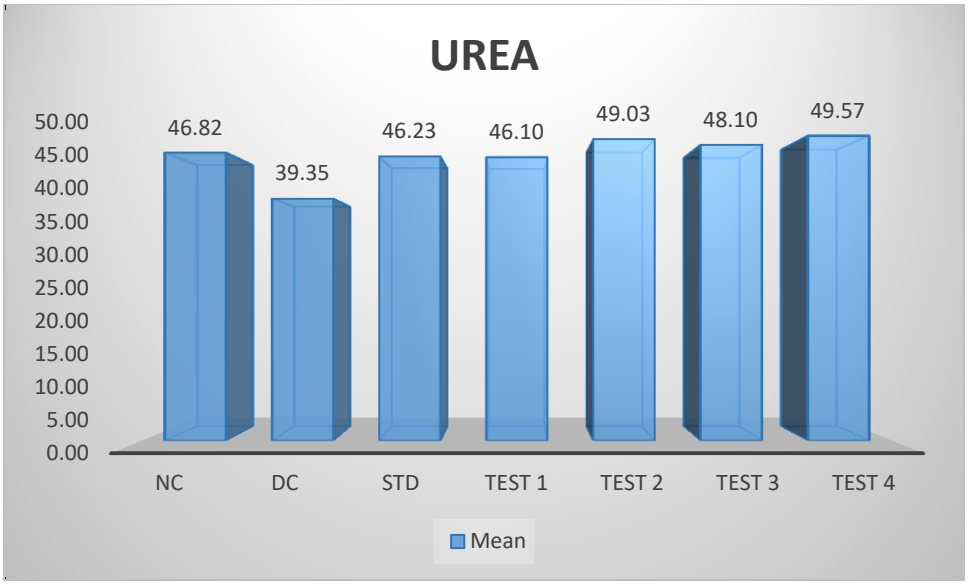
Further we can observe that, mean value for Brewer's Yeast+ *Shodhita Hingula* (11.25 mg/Kg)T2 group is maximum while mean value for normal control group is minimum among seven groups.



6.20.6. Effect on Urea

Urea	N	Mean	SD	SE	F-Value	P-Value
Normal Control	6	46.82	9.24	3.77	0.817	0.56444
Brewer's Yeast (10 mg/Kg, s.c)	6	39.35	9.08	3.71		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	46.23	11.25	4.59		
Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1	6	46.10	12.42	5.07		
Brewer's Yeast+ *Shodhita Hingula* (11.25 mg/Kg)T2	6	49.03	8.05	3.28		
Brewer's Yeast+ Shodhita Vatsanabh (1.40 mg/Kg)T3	6	48.10	6.91	2.82		
Brewer's Yeast+ *Pippali* (67.5 mg/Kg)T4	6	49.57	6.11	2.49		
Total	42	46.46	9.12	1.41		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among seven groups.



6.20.7 Effect on Asparate transaminase

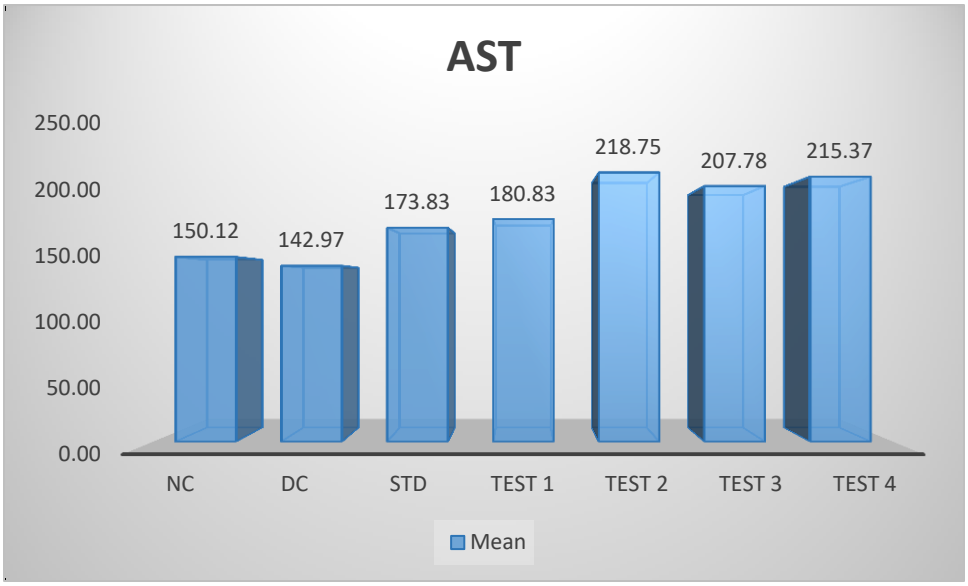
AST	N	Mean	SD	SE	F-Value	P-Value
Normal Control	6	150.12	17.38	7.09	5.479	0.00044*
Brewer's Yeast (10 mg/Kg, s.c)	6	142.97	28.96	11.82		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	173.83	34.53	14.10		
Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1	6	180.83	47.94	19.57		
Brewer's Yeast+ *Shodhita Hingula* (11.25 mg/Kg)T2	6	218.75	7.08	2.89		
Brewer's Yeast+ Shodhita Vatsanabh (1.40 mg/Kg)T3	6	207.78	48.11	19.64		
Brewer's Yeast+ *Pippali* (67.5	6	215.37	17.00	6.94		

mg/Kg)T4						
Total	42	184.24	41.51	6.40		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for Brewer's Yeast+ *Shodhita Hingula* test 2 group is maximum while mean value for disease control group is minimum among seven groups.



6.20.8. Effect on Alanine amino transferase

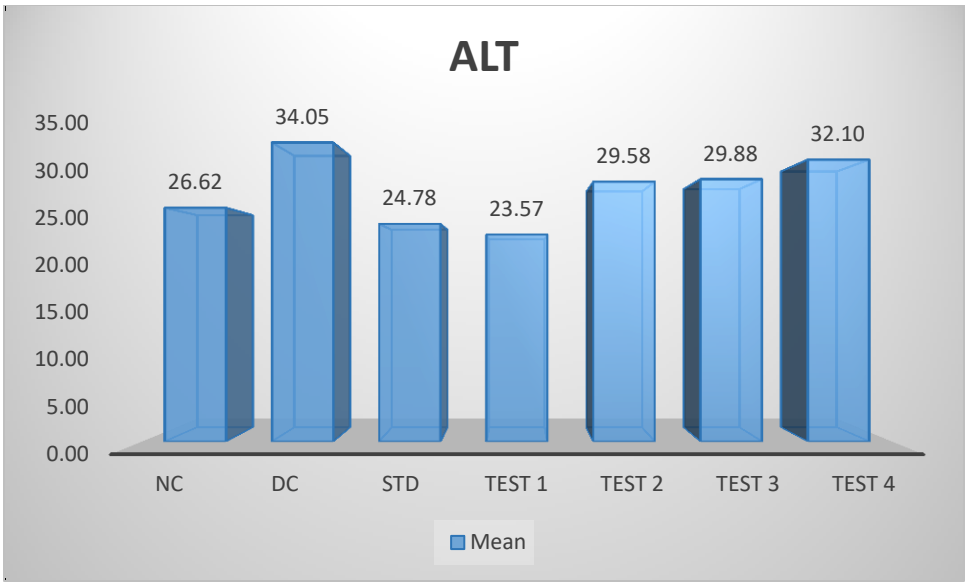
ALT	N	Mean	SD	SE	F-Value	P-Value
Normal Control	6	26.62	6.33	2.59	3.398	0.00953*
Brewer's Yeast (10 mg/Kg, s.c)	6	34.05	5.82	2.38		
Brewer's Yeast+ Paracetamol	6	24.78	6.07	2.48		

(150 mg/kg)						
Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1	6	23.57	3.87	1.58		
Brewer's Yeast+ *Shodhita Hingula* (11.25 mg/Kg)T2	6	29.58	1.95	0.80		
Brewer's Yeast+ Shodhita Vatsanabh (1.40 mg/Kg)T3	6	29.88	4.84	1.97		
Brewer's Yeast+ *Pippali* (67.5 mg/Kg)T4	6	32.10	5.38	2.20		
Total	42	28.65	5.93	0.91		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for disease control group is maximum while mean value for Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg) T1 test 1 group is minimum among seven groups.



6.20. Effect on Blood biochemistry after antipyretic study :

No statistical changes were observed in Total proteins, Total bilirubin, ALP, Creatinine, ALT and Urea. While AST levels were found to be increased in *Shuddha Hingula*T2 and *Pippali (Piper longum* Linn)T4 group as compared to diseased control.

Total protein

Further we can observe that, mean value for **Total protein in**

disease control group is maximum 9.53, while mean value for **Total protein in** Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1 group is minimum5.53 among seven groups.

Total Bilirubin

There is no significant difference for total Bilirubin among seven groups.

Total Albumin

There is no significant difference for Albumin among seven groups.

Total Creatinine

There is no significant difference for Creatinin among seven groups.

Alkaline phosphatage

Further we can observe that, mean value for **Alkaline phosphatage**

ALPin Brewer's Yeast+ *Shodhita Hingula* (11.25 mg/Kg)T2 group is maximum202.57 while mean value for **Alkaline phosphatage**

ALP in normal control group is minimum136.95 among seven groups.

Elevated level of ALP in paracetamol group and Shuddha Hingula group indicates that all the drugs are metabolized in liver therefore causing its inflammation.

This inflammation get releaved following withdrawl of drug. Whereas such elevation of ALP or other liver enzyme is not observed in disease control group because brewer's yeast used to induce pyrexia does not significantly affect liver.

Urea

There is no significant difference for Urea among seven groups.

Asparatetransminase

Further we can observe that, mean value for **Asparatetransminase AST in** Brewer's Yeast+ *Shodhita Hingul* test 2 group is maximum 218.75,while mean value for **Asparatetransminase AST in** disease control group is minimum 142.97among seven groups.

Alanine aminotransferase

Further we can observe that, mean value for **Alanine aminotransferase ALT in**

disease control group is maximum 34.05while mean value for **Alanine aminotransferase**

ALT in Brewer's Yeast+ *Hinguleshvara Rasa*

(5.63 mg/Kg)T1test 1 group is minimum 23.57among seven groups

6.21. Effect on Electrolytes After Antipyretic study as per results 5.38.5:

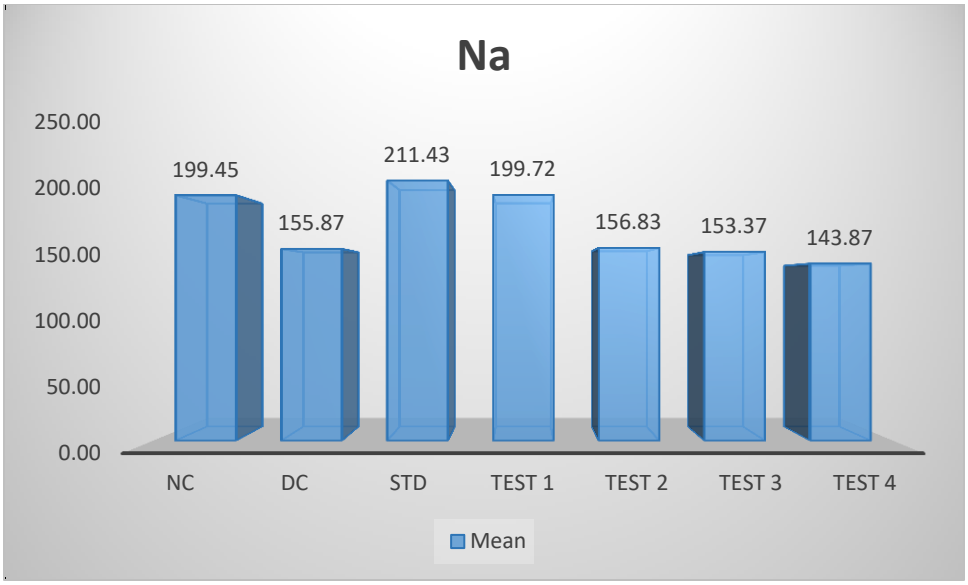
6.21.1. Effect on Sodium

Na	N	Mean	SD	SE	F-Value	P-Value
NC	6	199.45	17.11	6.99	9.826	0.000*
DC-Brewer's Yeast	6	155.87	8.90	3.64		
STD- Brewer's Yeast+ Pracetamol	6	211.43	30.94	12.63		
Brewer's Yeast +Hinguleshvara Rasa T1	6	199.72	37.35	15.25		
Brewer's Yeast + Shuddha Hingula T2	6	156.83	9.11	3.72		
Brewer's Yeast + Shuddha Vatsanabh T3	6	153.37	9.43	3.85		
Brewers Yeast+ Pippali T4	6	143.87	20.70	8.45		
Total	42	174.36	32.99	5.09		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for ***STD- Brewer's Yeast+ Pracetamol*** standard control group is maximum while mean value for ***Brewers Yeast+Pippali T4*** group is minimum among seven groups.



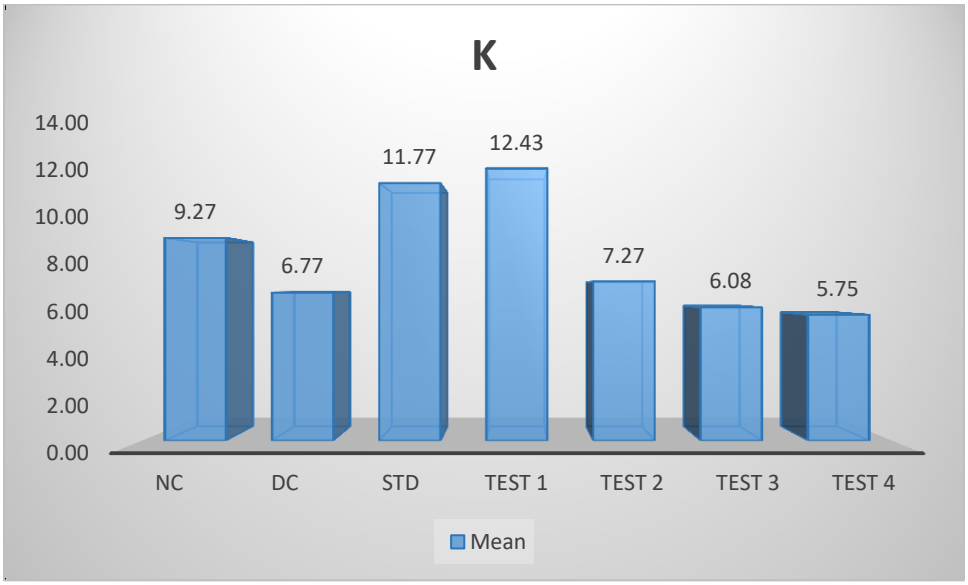
6.21.2. Effect on Potassium

K	N	Mean	SD	SE	F-Value	P-Value
NC	6	9.27	1.43	0.58	21.186	0.000*
DC-Brewer's Yeast	6	6.77	0.49	0.20		
STD- Brewer's Yeast+ Pracetamol	6	11.77	0.69	0.28		
Brewer's Yeast +Hinguleshvara Rasa T1	6	12.43	2.38	0.97		
Brewer's Yeast + Shuddha Hingula T2	6	7.27	2.24	0.91		
Brewer's Yeast + Shuddha Vatsanabh T3	6	6.08	0.56	0.23		
Brewers Yeast+PippaliT4	6	5.75	1.01	0.41		
Total	42	8.48	2.89	0.45		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for ***Brewer's Yeast +Hinguleshvara Rasa T1*** group is maximum while mean value for ***Brewers Yeast+Pippali T4*** group is minimum among seven groups.



6.21.3. Effect on Calcium

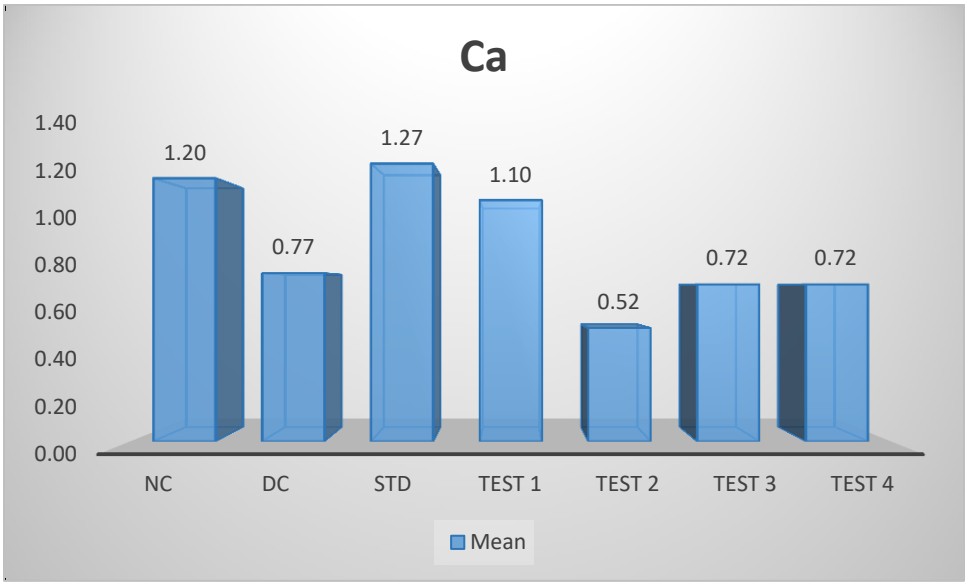
Ca	N	Mean	SD	SE	F-Value	P-Value
NC	6	1.20	0.20	0.08	11.053	0.000*
DC-Brewer's Yeast	6	0.77	0.10	0.04		
STD- Brewer's Yeast+ Pracetamol	6	1.27	0.25	0.10		
Brewer's Yeast +Hinguleshvara Rasa T1	6	1.10	0.31	0.13		
Brewer's Yeast +	6	0.52	0.28	0.11		

Shuddha Hingula T2						
Brewer's Yeast + Shuddha Vatsanabh T3	6	0.72	0.13	0.05		
Brewers Yeast+ Pippali T4	6	0.72	0.10	0.04		
Total	42	0.90	0.33	0.05		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for ***STD- Brewer's Yeast+ Pracetamol*** standard control group is maximum while mean value for ***Brewer's Yeast + Shuddha Hingula T2*** group is minimum among seven groups.

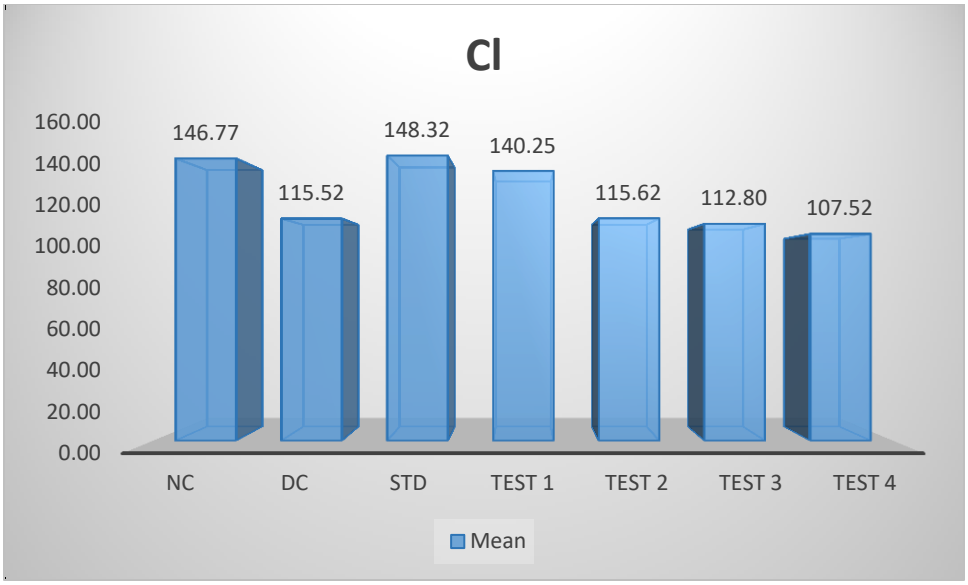


6.21.4. Effect on Chlorine

Cl	N	Mean	SD	SE	F-Value	P-Value
NC	6	146.77	9.68	3.95	11.831	0.000*
DC-Brewer's Yeast	6	115.52	5.46	2.23		
STD- Brewer's Yeast+ Paracetamol	6	148.32	18.83	7.69		
Brewer's Yeast +Hinguleshvara Rasa T1	6	140.25	20.21	8.25		
Brewer's Yeast + Shuddha Hingula T2	6	115.62	5.23	2.14		
Brewer's Yeast + Shuddha Vatsanabh T3	6	112.80	4.29	1.75		
Brewers Yeast+ Pippali T4	6	107.52	13.02	5.32		
Total	42	126.68	20.17	3.11		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for ***Brewer's Yeast + Hinguleshvara Rasa T1*** group is maximum while mean value for ***Brewers Yeast + Pippali T4*** group is minimum among seven groups.



6.21. Effect on Electrolytes After Antipyretic study:

Pyrexia increases risk of fluid and electrolyte disturbances. Medication during pyrexia causes excessive urination and profuse sweating physical exertion also cause electrolyte imbalance.

Sodium

Further we can observe that, mean value for Sodium Na in ***STD- Brewer's Yeast+ Pracetamol*** standard control group is maximum 211.43, while mean value for ***Brewers Yeast+Pippali T4*** group is minimum143.87 among seven groups.

Potassium

Further we can observe that, mean value for **Potassium K in** ***Brewer's Yeast +Hinguleshvara RasaT1*** group is maximum 12.43,while mean value for **Potassium K in *Brewers Yeast+Pippali T4*** group is minimum5.75. among seven groups.

Calcium

Further we can observe that, mean value for **Calcium Ca in *STD- Brewer's Yeast+ Pracetamol*** standard control group is maximum 1.27, while mean value for **Calcium Ca in *Brewer's Yeast +Hingula T2*** group is minimum 0.52among seven groups.

Chlorine

Further we can observe that, mean value for **Chlorine**Cl in ***Brewer's Yeast + Paracetamol*** standard group is maximum148.32, while mean value for **Chlorine** Cl in ***Brewers Yeast+Pippali T4*** group is minimum107.52 among seven groups.

6.22. Effect on Organ weight after antipyretic study as per results 5.38.6:

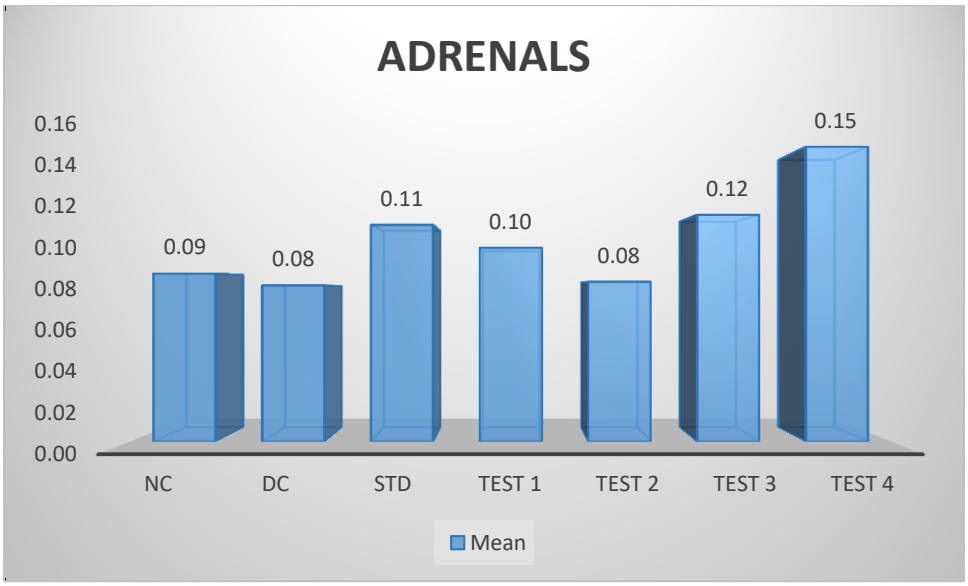
6.22.1. Effect on Adrenals:

Adrenals	N	Mean	SD	SE	F-Value	P-Value
Normal Control	6	0.09	0.02	0.01	3.266	0.01175*
Brewer's Yeast (10 mg/Kg, s.c)	6	0.08	0.02	0.01		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	0.11	0.03	0.01		
Brewer's Yeast+ *Hinguleshvara Rasa*(5.63 mg/Kg)T1	6	0.10	0.03	0.01		
Brewer's Yeast+*Shodhita Hingula*(11.25 mg/Kg)T2	6	0.08	0.02	0.01		
Brewer's Yeast+Shodhita Vatsanabh (1.40 mg/Kg)T3	6	0.12	0.05	0.02		
Brewer's Yeast+ *Pippali* (67.5 mg/Kg)T4	6	0.15	0.05	0.02		
Total	42	0.11	0.04	0.01		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for Brewer's Yeast+ *Pippali* (test 4 group is maximum while mean value for Brewer's Yeast+*Shodhita Hingula*(11.25 mg/Kg)T2 test 2 group is minimum among seven groups.



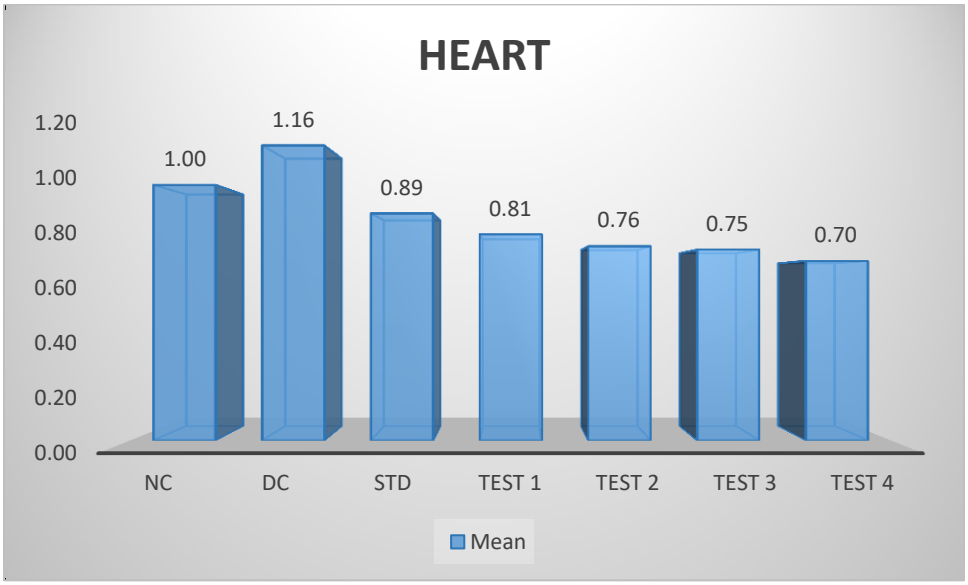
6.22.2 Effect on Heart

Heart	N	Mean	SD	SE	F-Value	P-Value
Normal Control	6	1.00	0.21	0.08	7.031	0.00006*
Brewer's Yeast (10 mg/Kg, s.c)	6	1.16	0.15	0.06		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	0.89	0.16	0.07		
Brewer's Yeast+ *Hinguleshvara Rasa*(5.63 mg/Kg)T1	6	0.81	0.11	0.04		
Brewer's Yeast+*Shodhita Hingula* (11.25mg/Kg)T2	6	0.76	0.15	0.06		
Brewer's Yeast+Shodhita Vatsanabh(1.40 mg/Kg)T3	6	0.75	0.07	0.03		
Brewer's Yeast+ *Pippali* (67.5 mg/Kg)T4	6	0.70	0.16	0.06		
Total	42	0.87	0.21	0.03		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for disease control group is maximum while mean value for Brewer's Yeast+ *Pippali* (67.5 mg/Kg)T4test 4 group is minimum among seven groups.

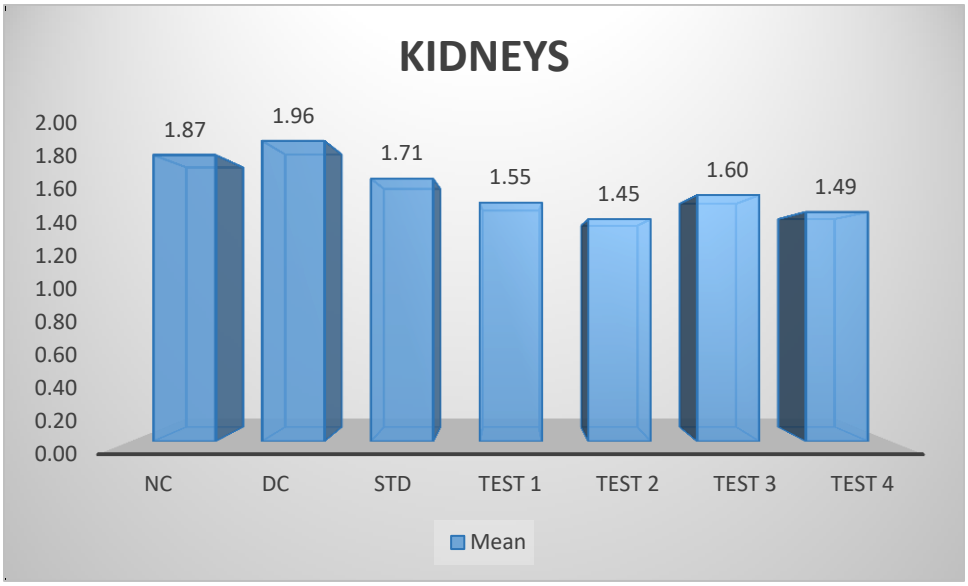


6.22.3. Effect on Kidneys

Kidneys	N	Mean	SD	SE	F-Value	P-Value
Normal Control	6	1.87	0.74	0.30	1.348	0.26269
Brewer's Yeast (10 mg/Kg, s.c)	6	1.96	0.37	0.15		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	1.71	0.46	0.19		
Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1	6	1.55	0.16	0.07		
Brewer's Yeast+*Shodhita Hingula* (11.25 mg/Kg)T2	6	1.45	0.25	0.10		

Brewer's Yeast+Shodhita Vatsanabh (1.40 mg/Kg)T3	6	1.60	0.30	0.12		
Brewer's Yeast+ *Pippali* (67.5mg/Kg)T4	6	1.49	0.26	0.11		
Total	42	1.66	0.41	0.06		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among seven groups.

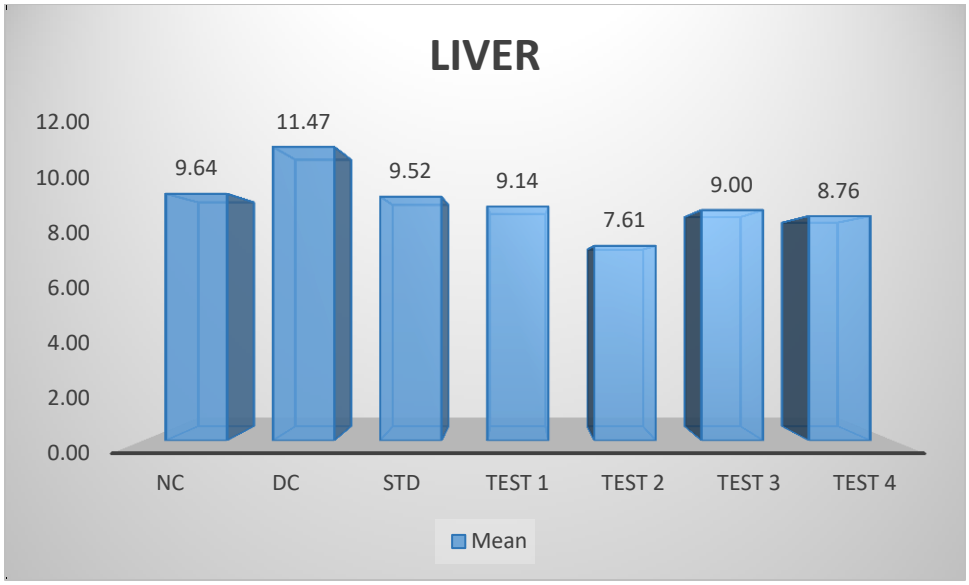


6.22.4. Effect on Liver

Liver	N	Mean	SD	SE	F-Value	P-Value
Normal Control	6	9.64	3.00	1.23		
Brewer's Yeast (10 mg/Kg, s.c)	6	11.47	1.92	0.78		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	9.52	1.92	0.78	1.540	0.19426
Brewer's Yeast+ *Hinguleshvara Rasa*(5.63	6	9.14	1.15	0.47		

mg/Kg)T1						
Brewer's Yeast+*Shodhita Hingula*(11.25 mg/Kg)T2	6	7.61	3.59	1.47		
Brewer's Yeast+Shodhita Vatsanabh (1.40mg/Kg)T3	6	9.00	1.48	0.60		
Brewer's Yeast+ *Pippali* (67.5mg/Kg)T4	6	8.76	2.04	0.83		
Total	42	9.30	2.39	0.37		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among seven groups.



6.22.5. Effect on Spleen

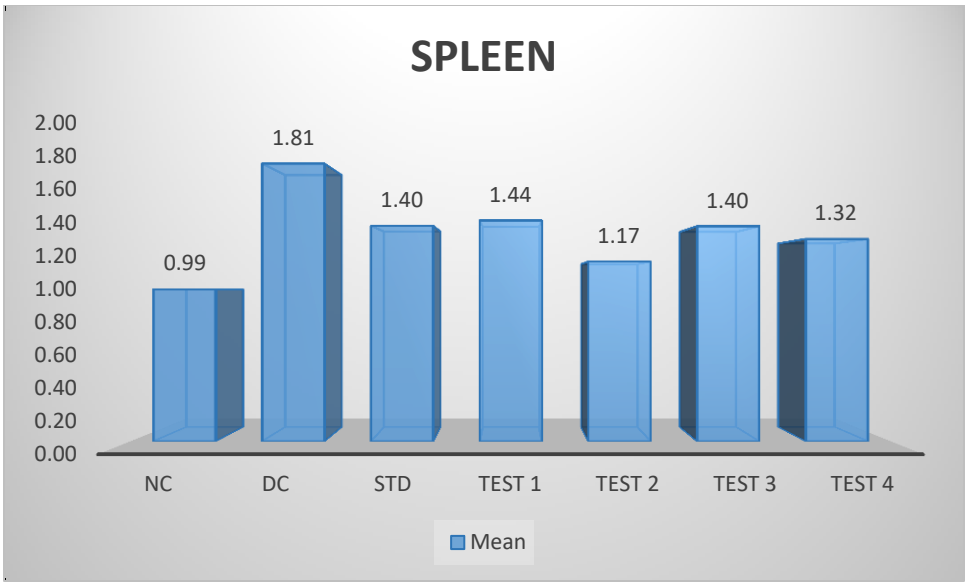
Spleen	N	Mean	SD	SE	F-Value	P-Value
NC	6	0.99	0.21	0.08	5.031	0.00082*
Brewer's Yeast (10 mg/Kg, s.c)	6	1.81	0.12	0.05		
Brewer's Yeast+ Paracetamol	6	1.40	0.22	0.09		

(150 mg/kg)						
Brewer's Yeast+ *Hinguleshvara Rasa*(5.63 mg/Kg)T1	6	1.44	0.53	0.22		
Brewer's Yeast+*Shodhita Hingula* (11.25mg/Kg)T2	6	1.17	0.15	0.06		
Brewer's Yeast+Shodhita Vatsanabh (1.40mg/Kg)T3	6	1.40	0.28	0.12		
Brewer's Yeast+ *Pippali* (67.5mg/Kg)T4	6	1.32	0.22	0.09		
Total	42	1.36	0.35	0.05		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1 group is maximum while mean value for normal control group is minimum among seven groups.

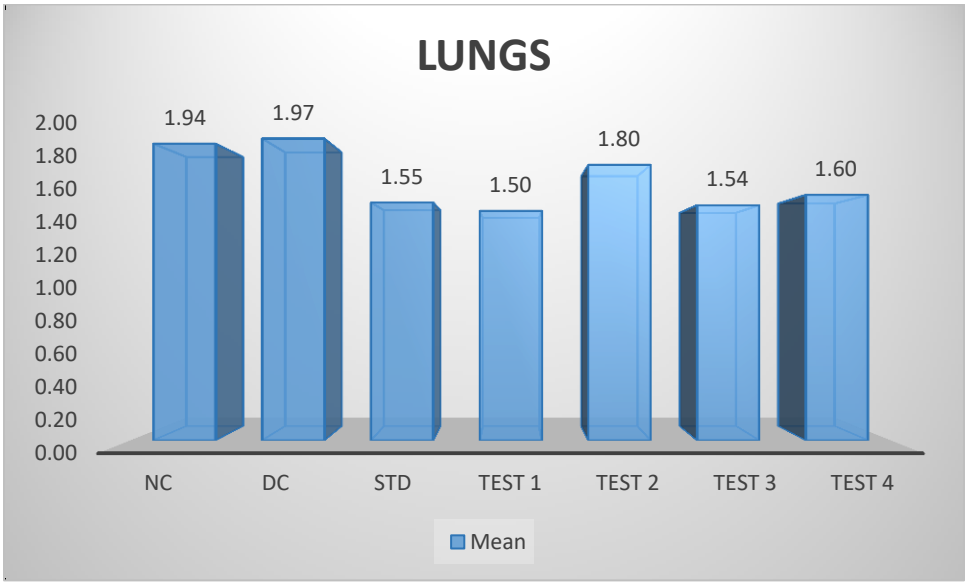


6.22.6. Effect on Lungs

Lungs	N	Mean	SD	SE	F-Value	P-Value
NC	6	1.94	0.36	0.15	2.053	0.08443
Brewer's Yeast (10 mg/Kg, s.c)	6	1.97	0.39	0.16		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	1.55	0.23	0.09		
Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1	6	1.50	0.27	0.11		
Brewer's Yeast+ *Shodhita Hingula* (11.25 mg/Kg)T2	6	1.80	0.52	0.21		
Brewer's Yeast+ Shodhita Vatsanabh (1.40 mg/Kg)T3	6	1.54	0.18	0.07		

Brewer's Yeast+ *Pippali* (67.5 mg/Kg)T4	6	1.60	0.32	0.13		
Total	42	1.70	0.37	0.06		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among seven groups.

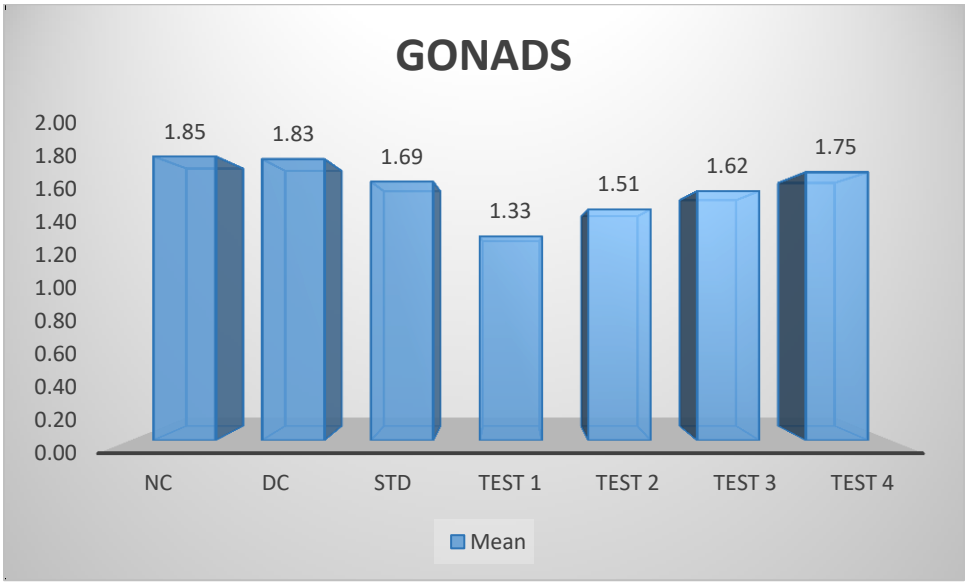


6.22.7. Effect on Gonads

Gonads	N	Mean	SD	SE	F-Value	P-Value
NC	6	1.85	1.26	0.52	0.161	0.98535
Brewer's Yeast (10 mg/Kg, s.c)	6	1.83	1.37	0.56		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	1.69	1.23	0.50		
Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1	6	1.33	1.00	0.41		

Brewer's Yeast+*Shodhita Hingula* (11.25mg/Kg)T2	6	1.51	1.06	0.43		
Brewer's Yeast+Shodhita Vatsanabh (1.40 mg/Kg)T3	6	1.62	1.12	0.46		
Brewer's Yeast+ *Pippali* (67.5mg/Kg)T4	6	1.75	0.84	0.34		
Total	42	1.65	1.07	0.16		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among seven groups.



6.22.8. Effect on Brain

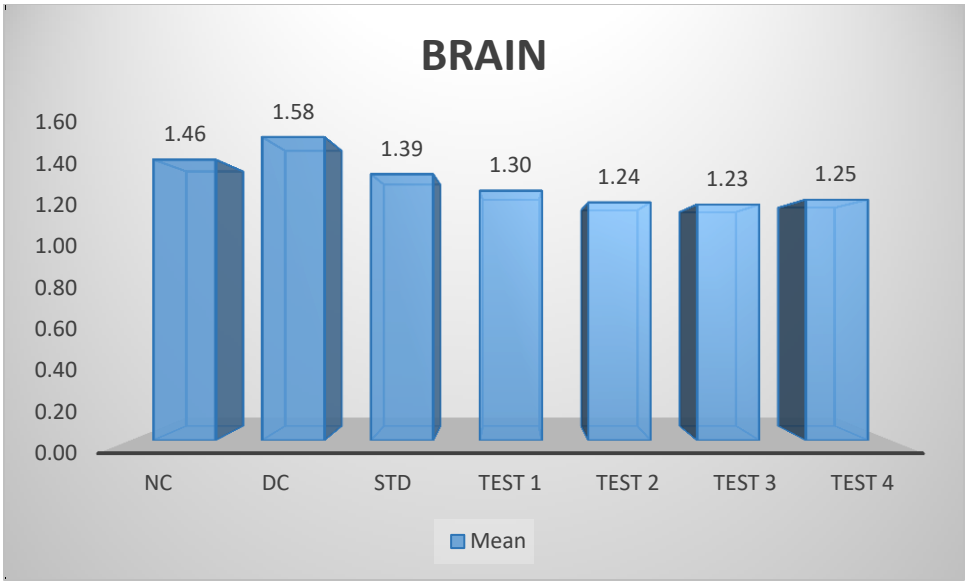
Brain	N	Mean	SD	SE	F-Value	P-Value
NC	6	1.46	0.20	0.08	3.605	0.00689*
Brewer's Yeast (10	6	1.58	0.11	0.04		

mg/Kg, s.c)						
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	1.39	0.07	0.03		
Brewer's Yeast+ *Hinguleshvara Rasa*(5.63 mg/Kg)T1	6	1.30	0.21	0.08		
Brewer's Yeast+*Shodhita Hingula* (11.25mg/Kg)T2	6	1.24	0.23	0.09		
Brewer's Yeast+ Shodhita Vatsanabh (1.40 mg/Kg)T3	6	1.23	0.20	0.08		
Brewer's Yeast+ *Pippali* (67.5 mg/Kg)T4	6	1.25	0.13	0.05		
Total	42	1.35	0.20	0.03		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for disease control group is maximum while mean value for Brewer's Yeast+ Shodhita Vatsanabh (1.40 mg/Kg) T3 group is minimum among seven groups.

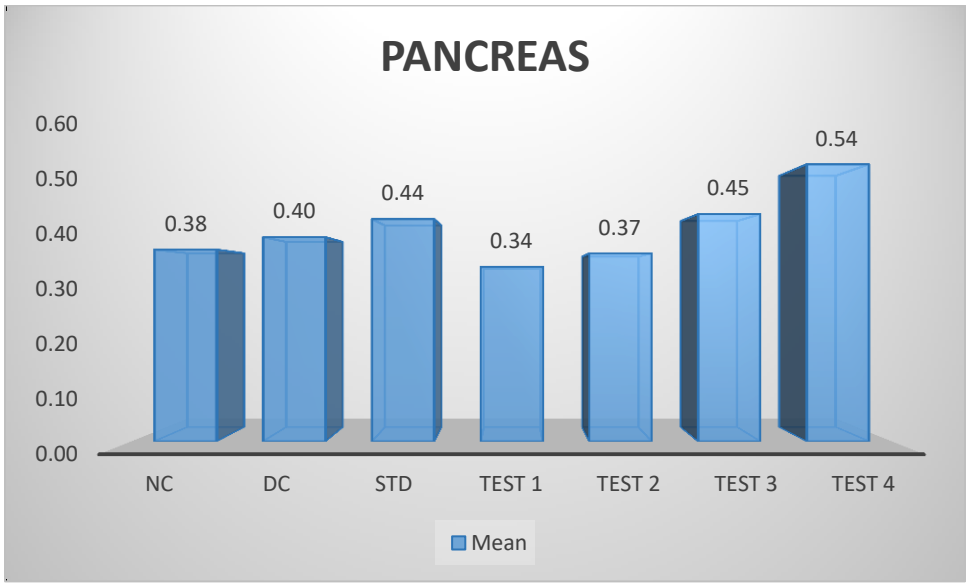


6.22.9. Effect on Pancreas

Pancreas	N	Mean	SD	SE	F-Value	P-Value
NC	6	0.38	0.10	0.04	1.023	0.42698
Brewer's Yeast (10 mg/Kg, s.c)	6	0.40	0.09	0.04		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	0.44	0.09	0.04		
Brewer's Yeast+ *Hinguleshvara Rasa* (5.63 mg/Kg)T1	6	0.34	0.18	0.07		
Brewer's Yeast+ *Shodhita Hingula* (11.25 mg/Kg)T2	6	0.37	0.17	0.07		
Brewer's Yeast+ Shodhita Vatsanabh (1.40 mg/Kg)T3	6	0.45	0.24	0.10		

Brewer's Yeast+ *Pippali* (67.5 mg/Kg)T4	6	0.54	0.19	0.08		
Total	42	0.42	0.16	0.03		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among seven groups.



6.22.10. Effect on Stomach

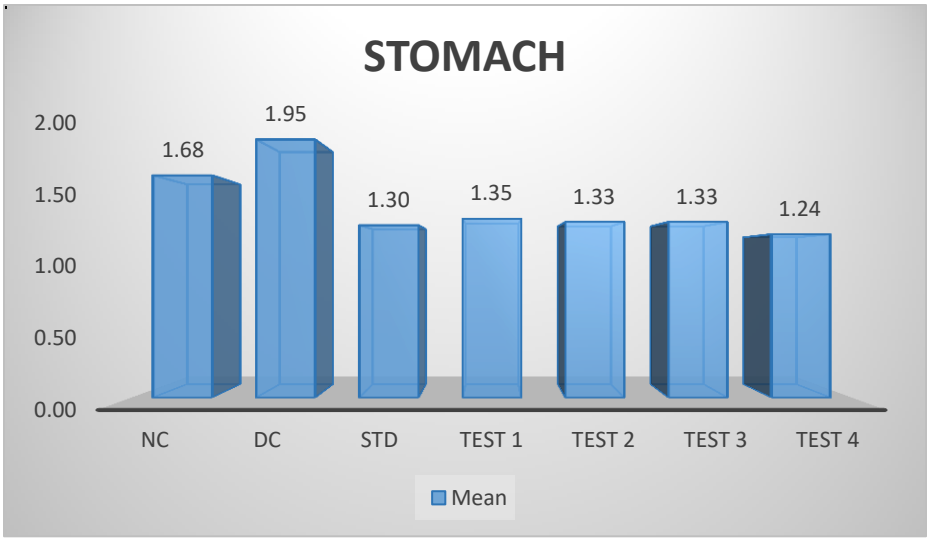
Stomach	N	Mean	SD	SE	F-Value	P-Value
NC	6	1.68	0.25	0.10	5.977	0.00022*
Brewer's Yeast (10 mg/Kg, s.c)	6	1.95	0.45	0.18		
Brewer's Yeast+ Paracetamol (150 mg/kg)	6	1.30	0.25	0.10		
Brewer's Yeast+ *Hinguleshvara Rasa*	6	1.35	0.14	0.06		

(5.63 mg/Kg)T1						
Brewer's Yeast+ *Shodhita Hingula* (11.25 mg/Kg)T2	6	1.33	0.20	0.08		
Brewer's Yeast+ Shodhita Vatsanabh (1.40 mg/Kg)T3	6	1.33	0.17	0.07		
Brewer's Yeast+ *Pippali* (67.5mg/Kg)T4	6	1.24	0.25	0.10		
Total	42	1.45	0.34	0.05		

*<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among seven groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among seven groups.

Further we can observe that, mean value for disease control group is maximum while mean value for Brewer's Yeast+ *Pippali* (67.5 mg/Kg) T4 group is minimum among seven groups.



There was no significant difference observed in body weight and organ weight of all groups.

In histopathology analysis of Liver and Kidney tissues, minimal (+1) degenerative changes were observed in diseased control group as compared to Normal Control, STD (Paracetamol), *Hinguleshvara Rasa* T1, *Shuddha Hingula (*Red Cinnabar:HgS) T2, *Vatsanabh (Aconitum*

Balfourii Stapf) T3 and *(Pippali Piper longum* Linn)T4 group. While no abnormalities were detected in the tissues of heart and gastric mucosa in all the groups from the results described in analytical results. 5.38.7 to 5.38.10

6.23. EXPERIMENT OF ANALGESIC ACTIVITY (as per result 5.40)

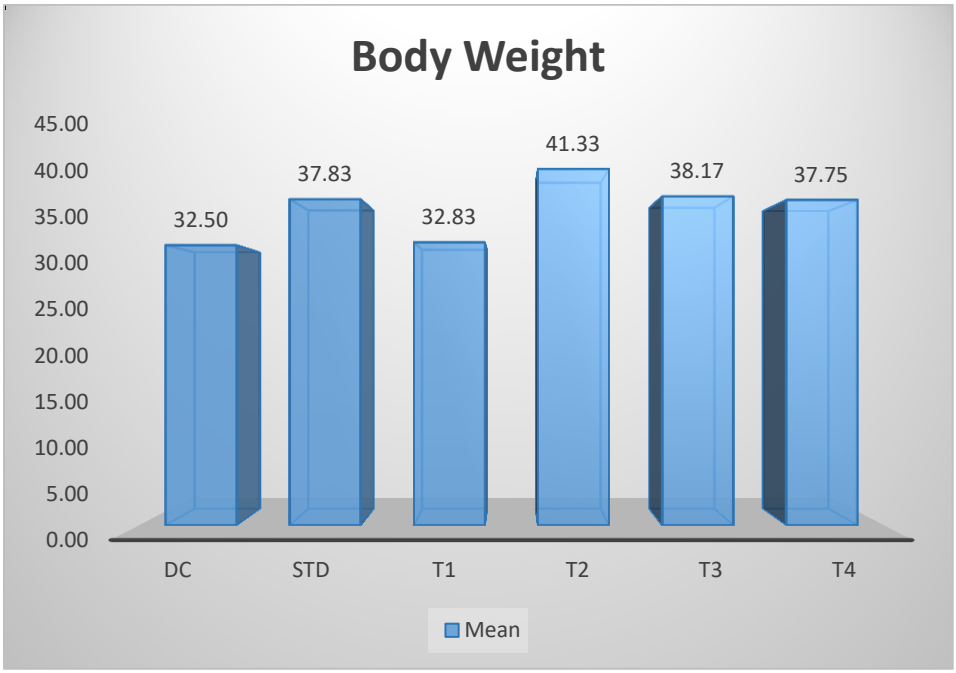
6.23.1.Effect of test drug on body weight (g) (Writhing) as per results 5.40.1:

Body Weight	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	32.50	2.88	1.18	3.565	0.012*
Diclofenac (10mg/Kg)	6	37.83	5.56	2.27		
T 1 –*Hinguleshvara Rasa* (8.13 mg/kg)	6	32.83	2.64	1.08		
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	6	41.33	6.53	2.67		
T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	6	38.17	4.40	1.80		
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	6	37.75	3.19	1.30		
Total	36	36.74	5.19	0.87		

***P<0.05**

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among six groups.

Further we can observe that, mean value for **T2- *Shuddha Hingula* (Red Cinnabar :HgS)** group is maximum while mean value for *Hinguleshvara Rasa* T1 group is minimum among six groups.

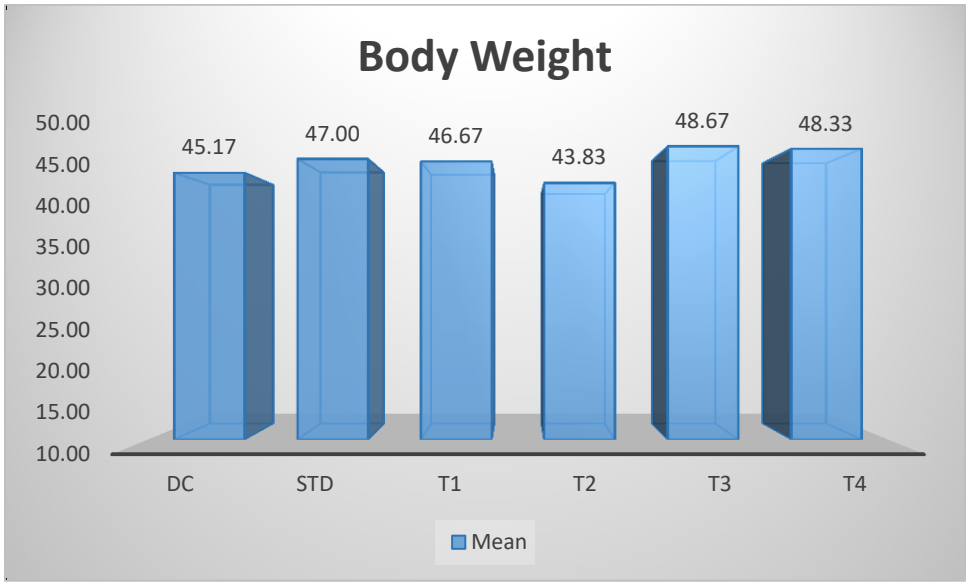


6.23.2.Effect of test drug on body weight (g) (Hot Plate) as per results 5.40.2:

Body Weight	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	45.17	8.08	3.30	0.529	0.753
Pentazocine- 1.4 mg/kg	6	47.00	7.29	2.98		
T 1 –*Hinguleshvara Rasa* (8.13 mg/kg)	6	46.67	6.41	2.62		
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	6	43.83	5.34	2.18		
T3- *Pippali (Piper*	6	48.67	4.72	1.93		

***longum* Linn) (97.50 mg/kg)**						
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	6	48.33	4.80	1.96		
Total	36	46.61	6.03	1.00		

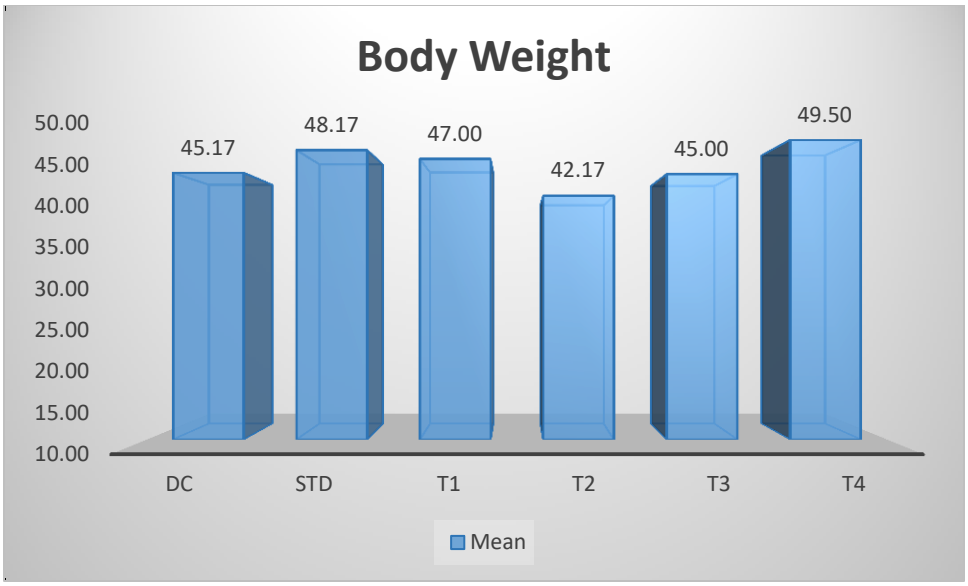
One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among six groups.



6.23.3.Effect of test drug on body weight (g) (Formalin test) as per results 5.40.3:

Body Weight	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	45.17	6.74	2.75	1.250	0.311
Diclofenac (10mg/Kg)	6	48.17	6.85	2.80		
T 1 – *Hinguleshvara Rasa* (8.13 mg/kg)	6	47.00	4.73	1.93		
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	6	42.17	4.54	1.85		
T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	6	45.00	5.22	2.13		
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	6	49.50	5.86	2.39		
Total	36	46.17	5.83	0.97		

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among six groups.



6.23.4.Effect of test drug on Acetic Acid induced Writhing as per results 5.40.4:

Decrease in frequency of writhing indicates analgesic effect. In following test, Test 2 (*Shuddha Hingula* Red Cinnabar:HgS) is showing decreased frequency i.e. 19 and test 1 *(Hinguleshvara Rasa)* - 29.67 as compared to disease control. However, Std group has frequency of 16. It means the test drug although shows analgesic activity, it is not as good as the std. drug. At the same time test 3 *(Pippali Piper longum* Linn)and T4 (*Shuddha Vatsanabh ,Aconitum balfourii* Stapf) show increase in writhing frequency indicating negative effect.

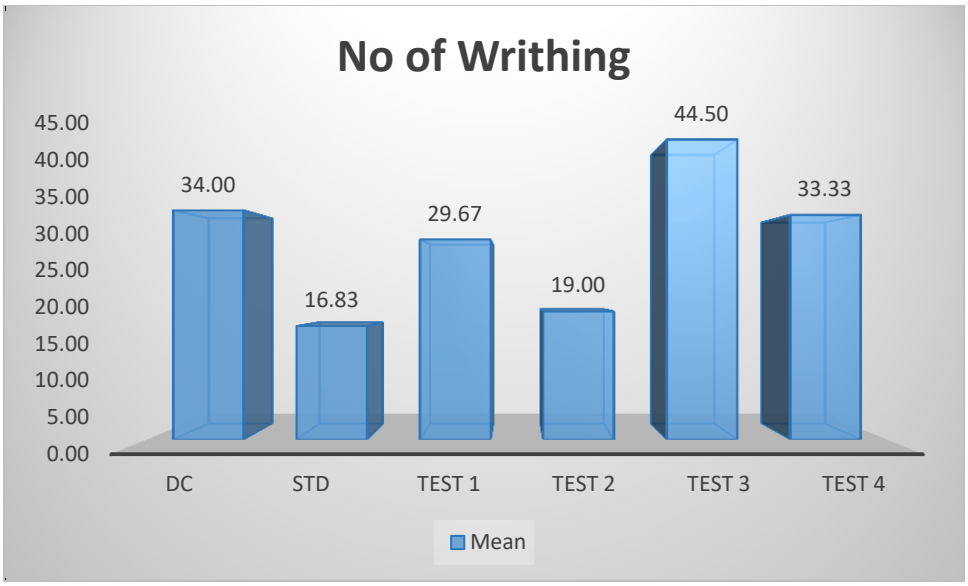
No. of writhing	N	Mean	SD	SE	F-Value	P-Value
Disease Control	6	34.00	12.60	5.14	4.968	0.0019718*
Diclofenac (10mg/Kg)	6	16.83	7.41	3.03		
T 1 – *Hinguleshvara Rasa* (8.13 mg/kg)	6	29.67	9.07	3.70		
T2- *Shuddha*	6	19.00	3.35	1.37		

Hingula **(Red Cinnabar :HgS) (16.25 mg/kg)**						
T3- ***Pippali (Piper longum*** **Linn) (97.50 mg/kg)**	6	44.50	17.42	7.11		
T4- ***Shuddha Vatsanabh (Aconitum balfourii*** **Stapf) (2.03 mg/kg)**	6	33.33	12.58	5.14		
Total	36	29.56	14.17	2.36		

***P<0.05**

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among six groups.

Further we can observe that, mean value for **T3-** ***Pippali (Piper longum*** **Linn)** group is maximum while mean value for Diclofenac (standard control group) is minimum among six groups.



Writhing test is a chemical method used **to induce pain of peripheral origin by injection of irritant principles like phenylquinone, acetic acid in mice**. Analgesic activity of the test compound is inferred from decrease in the frequency of writhings. In the above test number of writhings in standard drug Diclofenac (16.83+/-7.41) are observed to be minimum among all groups. Whereas, number of writhings are highest in test drug 3 *Pippali* group (44.50 +/- 17.42). The difference is statistically significant (P<0.05). It shows that analgesic activity induced by the standard drug is much more as compared to all the test drugs. Within the test drugs *Shodhita Hingula* is showing better analgesic activity (19+/-3.35). However, the study drug *Hinguleshvara Rasa* did not exhibit any significant analgesic activity (29.27+/-9.07) in comparison to other groups. Although it was better than *Pippali* (44.50 +/- 17.42).

6.23.5.Effect of test drug on hot plate analgesia as per results 5.40.5

Hot plate test is based on the principle that when rodents are placed onto a hot surface they will initially demonstrate the aversive effects of the thermal stimulus by licking their paws and, ultimately, by overt attempts to escape the environment (jumping). Substances that alter nociceptive threshold either increase the latency to licking/jumping (analgesic effect) or decrease it (hyperalgesic effect).

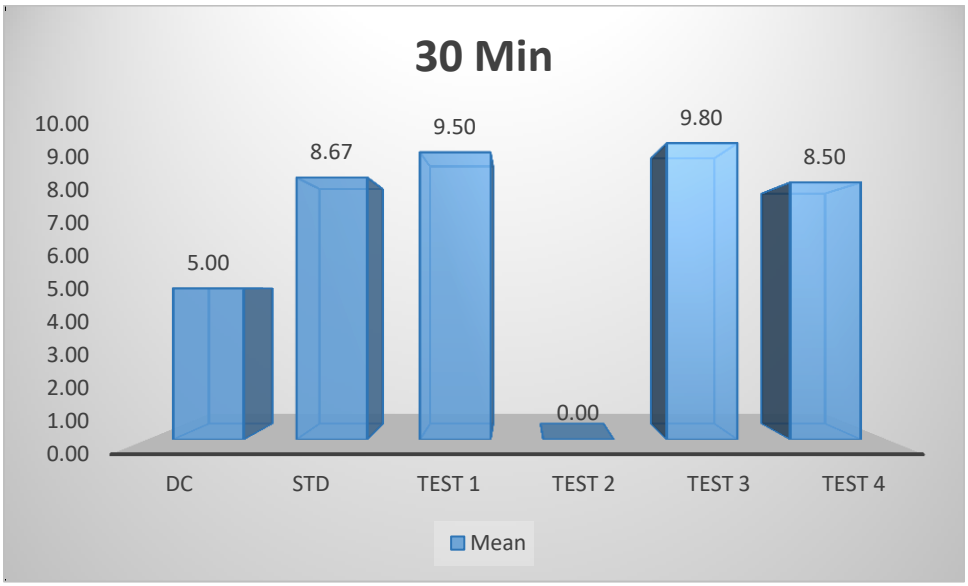
6.23.5.a.Paw licking time at the end 30 minutes

	N	Mean	SD	SE	F-Value	P-Value
		Seconds				
Disease Control	6	5.00	0.89	0.37	12.481	0.0000491*
Pentazocine- 1.4 mg/kg	6	8.67	0.82	0.33		
T 1 – *Hinguleshvara Rasa* (8.13 mg/kg)	4	9.50	1.91	0.96		
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	0	0.00	0.00	0.00		
T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	5	9.80	1.64	0.73		
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	2	8.50	0.71	0.50		
Total	23	8.09	2.25	0.47		

* <0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among six groups.

Further we can observe that, mean value for Pippali T3 group is maximum while mean value for disease control group is minimum among six groups.



6.23.5.b.Paw licking time at the end 60 minutes

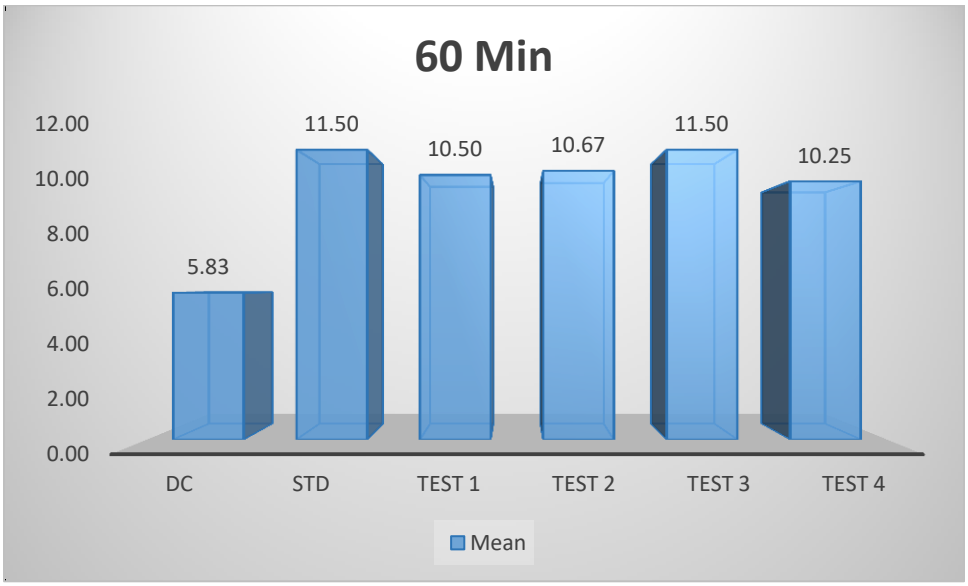
	N	Mean	SD	SE	F-Value	P-Value
		Seconds				
Disease Control	6	5.83	0.75	0.31	9.037	0.0006902*
Pentazocine- 1.4 mg/kg	2	11.50	2.12	1.50		
T 1 – *Hinguleshvara Rasa* (8.13 mg/kg)	2	10.50	2.12	1.50		
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	3	10.67	2.08	1.20		

T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	2	11.50	2.12	1.50		
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	4	10.25	1.26	0.63		
Total	19	9.21	2.72	0.62		

* <0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among six groups.

Further we can observe that, mean value for Pippali T3 group is maximum while mean value for disease control group is minimum among six groups.



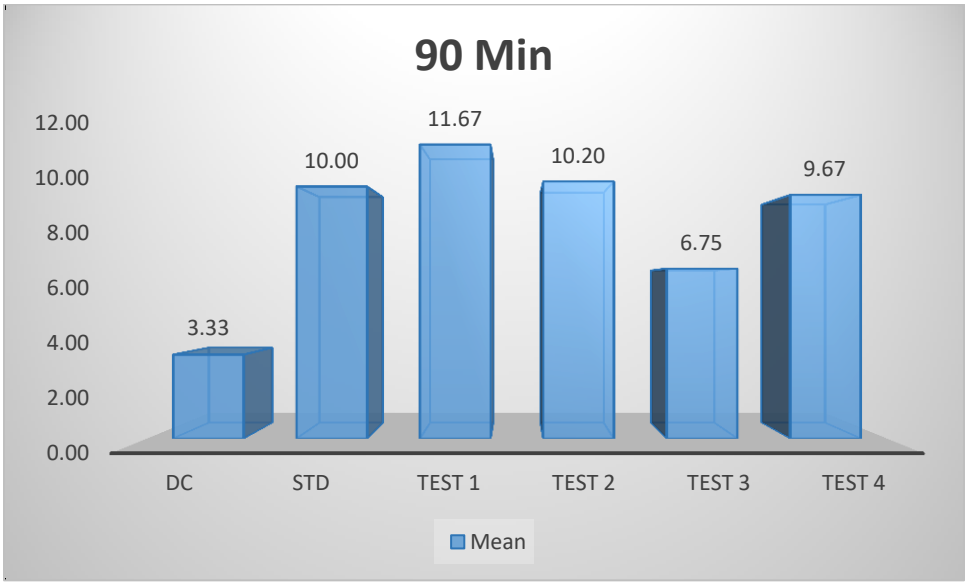
6.23.5.c.Paw licking time at the end 90 minutes

	N	Mean	SD	SE	F-Value	P-Value
		Seconds				
Disease Control	6	3.33	0.82	0.33	20.731	0.0000003*
Pentazocine- 1.4 mg/kg	5	10.00	0.71	0.32		
T 1 – *Hinguleshvara Rasa* (8.13 mg/kg)	3	11.67	1.53	0.88		
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	5	10.20	1.30	0.58		
T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	4	6.75	2.75	1.38		
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	3	9.67	1.53	0.88		
Total	26	8.15	3.31	0.65		

* <0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among six groups.

Further we can observe that, mean value for *Hinguleshvara Rasa* T1 group is maximum while mean value for disease control group is minimum among six groups.



6.23.5.d.Paw licking time at the end 120 minutes

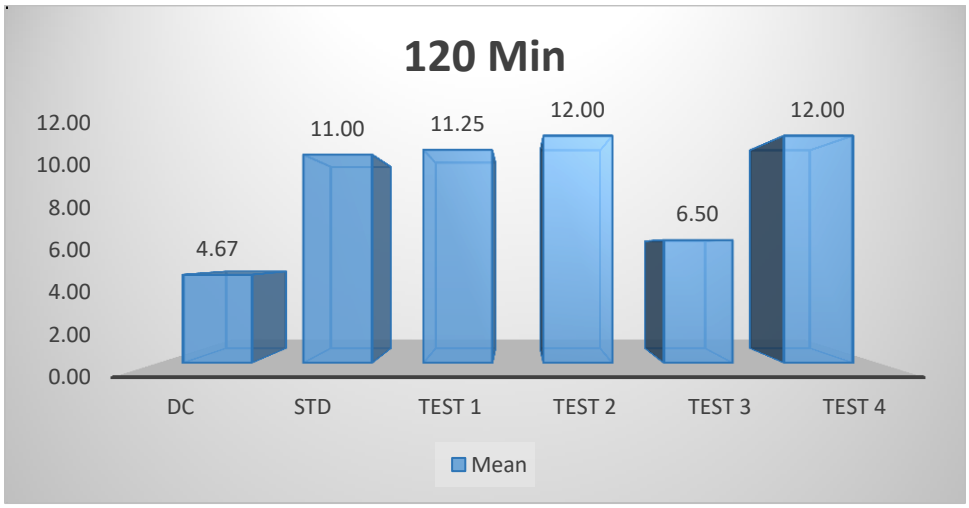
	N	Mean	SD	SE	F-Value	P-Value
		Seconds				
Disease Control	6	4.67	0.82	0.33	23.582	0.0000080*
Pentazocine- 1.4 mg/kg	2	11.00	0.00	0.00		
T 1 – *Hinguleshvara Rasa* (8.13 mg/kg)	4	11.25	0.96	0.48		
T2- *Shuddha*	1	12.00	0.00	0.00		

***Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)**						
T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	4	6.50	1.91	0.96		
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	1	12.00	0.00	0.00		
Total	18	8.06	3.30	0.78		

***P<0.05**

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is less than 0.05. Hence, we can conclude that. There is significant difference among six groups.

Further we can observe that, mean value for *Shodhita Hingula T2* and *Shodhita Vatsanabh T4* group is maximum while mean value for disease control group is minimum among six groups.



6.23.5.e.Paw licking time at the end of 30, 60, 90 and 120 minutes

	30	60	90	120		
	Minutes Mean	Minutes Mean	Minutes Mean	Minutes Mean	**F-Value**	**P-Value**
Disease Control	5.00	5.83	3.33	4.67+/- 0.082	10.718159	0.000286
Standard (**Pentazocine (1.4 mg/kg)**)	8.67	11.50	10.00	11.00+/- 0.00	9.261816	0.000635
T 1 – *Hinguleshvara Rasa* (8.13 mg/kg)	9.50	10.50	11.67	11.25+/- 0.96	9.057138	0.000715
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	0.00	10.67	10.20	12.00+/- 0.00	10.419772	0.000335
T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	9.80	11.50	6.75	6.50+/- 1.91	9.592594	0.000527
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	8.50	10.25	9.67	12.00+/- 0.00	9.418999	0.000581

***P<0.05**

It is observed that test *drug Hinguleshvara Rasa* shows maximum analgesic effect (11.67 seconds) at the end of 90 minutes following drug administration. Whereas, at the end of first thirty minutes following drug administration *Pippali (Piper longum* Linn)shows analgesic effect for a maximum duration (9.80 seconds) among all. This duration is more than even the standard drug Pentazocin (8.67 seconds.). At the end of first 30 minutes *Hinguleshvara Rasa* (9.50 seconds) and *Pippali (Piper longum* Linn (9.80 seconds) both showed more analgesia as compared to standard drug Pentazocin (8.67 seconds). Which indicates that *Hinguleshvara Rasa* and *Pippali Piper longum* Linn are more effective as analgesics than Pentazocin in first thirty minutes. Similarly, at the end of 120 minutes also the study drug *Hinguleshvara Rasa* (11.25+/- 0.96 seconds) is observed to show more effective analgesic than Pentazocin (11 seconds). Interestingly, initiation of paw licking in *Shuddha Hingula* (12 seconds) and *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (12 seconds) groups was more than Pentazocin (11 seconds) at the end of 120 mins. Thus the analgesic effect of the study drug *Hinguleshvara Rasa*, test drugs *Shuddha Hingula* and test drug *Shuddha Vatsanabh (Aconitum balfourii* Stapf) is more marked than standard drug Pentazocine at the end of two hours.

Hot plate test is used to measure threshold of thermal pain. Increase in time to lick the paw indicates analgesic effect. Less the time to withstand the heat and lick the paw indicate diminished analgesic effect. In the present study Test 1 *Hinguleshvara Rasa*,3*(Pippali Piper longum* Linn and T 4 *Shuddha Vatsanabh, Aconitum balfourii* Stapf) show increase pain threshold T 4 *Shuddha Vatsanabh ,Aconitum balfourii* Stapf) is similar to std group. Thus the analgesic effect of test 1 *Hinguleshvara Rasa,* and 3 *(Pippali Piper longum* Linn is better than std Pentazocine.

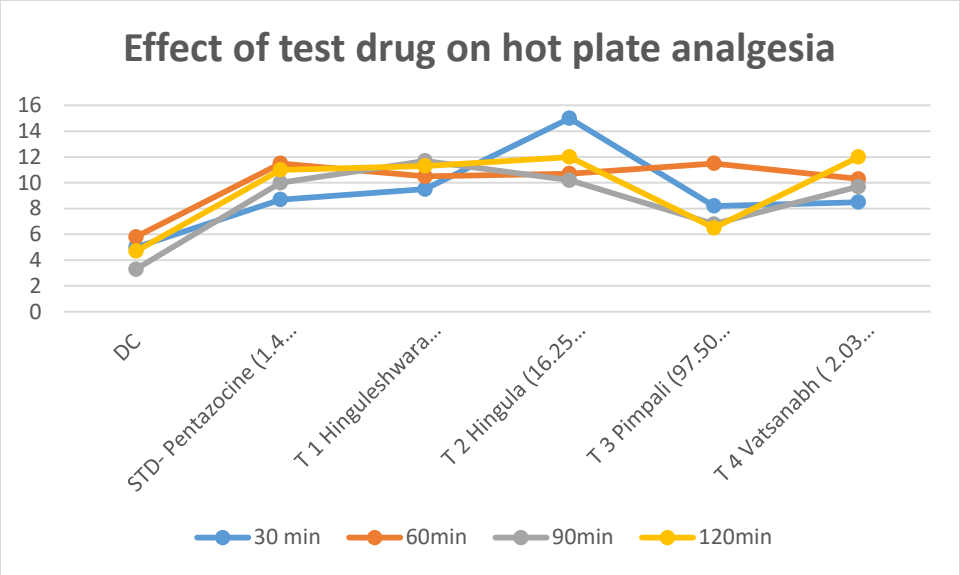
The hot plate analgesia, the reaction time for paw licking was increased in all the test groups and Standard group as compared to diseased control at 30, 60, 90 and 120 min.

Effect of test drug on hot plate analgesia

Group	Reaction in Sec					
	30 min	**60min**	**90min**	**120min**	**F-Value**	**P-Value**
DC	5	5.8	3.3	4.7	10.718159	0.000286
STD- Pentazocine (1.4 mg/kg)	8.7	11.5	10	11	9.261816	0.000635
T 1 –*Hinguleshvara Rasa* (8.13 mg/kg)	9.5	10.5	11.7	11.3	9.057138	0.000715
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	15	10.7	10.2	12	10.419772	0.000335
T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	9.8	11.5	6.8	6.5	9.592594	0.000527
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	8.5	10.3	9.7	12	9.418999	0.000581

***P<0.05**

Repeated Measures ANOVA Test is carried out to test significance in all groups at different time points. Above table shows that, P-Value for all six groups are less than 0.05. Hence, we can conclude that, there is significant change observed in mean at different time points.



6.24Effect of test drug on formalin induced licking

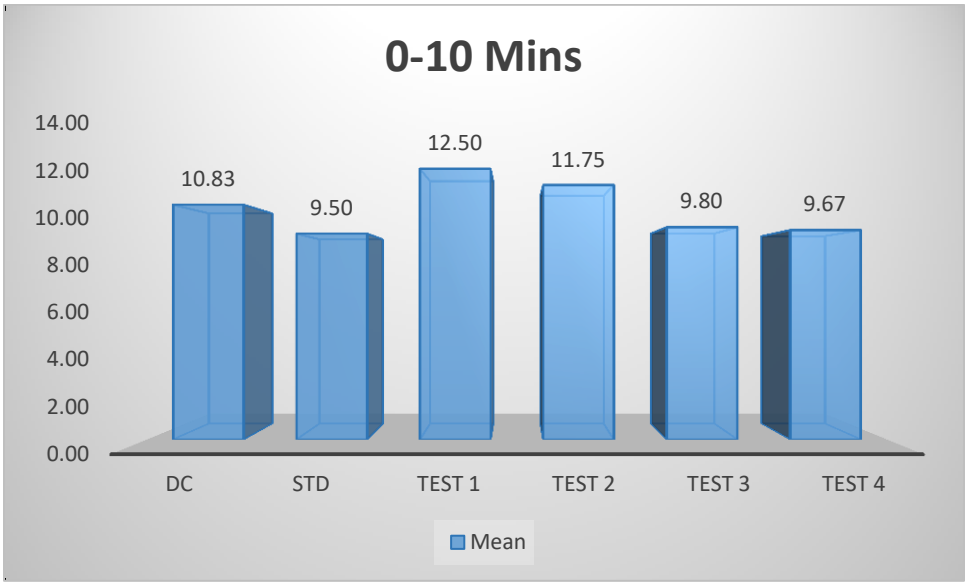
6.24.1(number of times of licking within 0-10 minutes)

	N	Mean	SD	SE	F-Value	P-Value
		Seconds				
Disease Control	6	10.83	2.32	0.95	0.178	0.9676985
Diclofenac (10mg/Kg)	4	9.50	7.23	3.62		
T 1 – *Hinguleshvara Rasa* (8.13 mg/kg)	4	12.50	4.65	2.33		
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	4	11.75	7.59	3.79		
T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	5	9.80	7.12	3.18		

T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	3	9.67	5.51	3.18		
Total	26	10.69	5.35	1.05		

***P<0.05**

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among six groups.



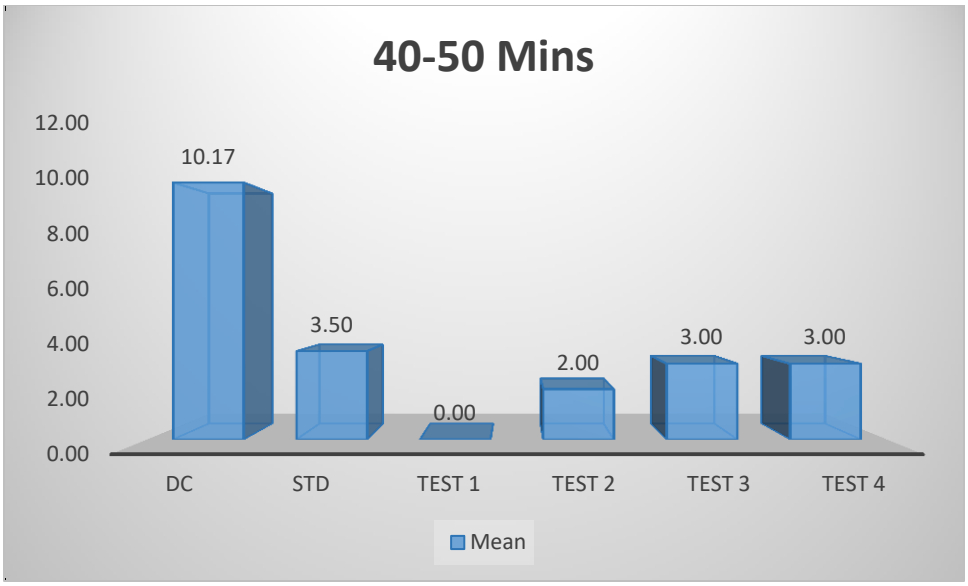
6.24.2.(number of times of licking within 40-50 minutes)

	N	Mean	SD	SE	F-Value	P-Value
		Seconds				
Disease Control	6	10.17	6.05	2.47	1.775	0.2178217
Diclofenac (10mg/Kg)	4	3.50	3.32	1.66		

T 1 – *Hinguleshvara Rasa* (8.13 mg/kg)	0	0.00	0.00	0.00		
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	1	2.00	0.00	0.00		
T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	1	3.00	0.00	0.00		
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	2	3.00	1.41	1.00		
Total	14	6.14	5.48	1.46		

*p<0.05

One Way Analysis of Variance Test (ANOVA) test is carried out for comparison among six groups. From above table, we can observe that, P-Value is greater than 0.05. Hence, we can conclude that. There is no significant difference among six groups.



6.24.3a.Mean of effect of test drug on formalin induced licking

Group	No. of times of licking	
	0-10 min	40-50mins
DC	10.8	10.2
STD- Diclofenac (10mg/Kg)	9.5	3.5
T 1 –*Hinguleshvara Rasa* (8.13 mg/kg)	12.5	0.0
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	11.8	2.0
T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	9.8	3.0
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	9.7	3.0

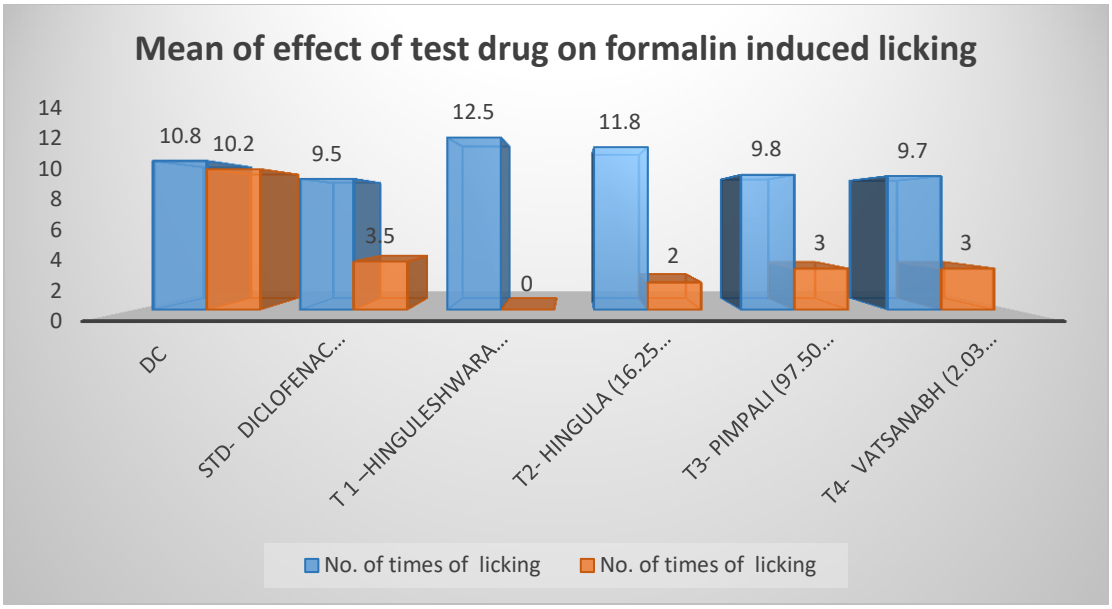
No significant difference was observed in the formalin induced licking in 0-10 min but a significant decrease in the licking at the site of formalin injection was seen after 40-50 minutes which shows the analgesic effect of the test drugs.

6.24.3b(number of times of licking within 0-10 and 40-50 minutes)

Group	No. of times of licking			
	0-10 min	**40-50mins**	**t-Value**	**P-Value**
DC	10.8	10.2	4.670	0.0008809*****
STD- Diclofenac (10mg/Kg)	9.5	3.5	5.377	0.0003113*****
T 1 –*Hinguleshvara Rasa* (8.13 mg/kg)	12.5	0	8.877	0.0000047*****
T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg)	11.8	2	5.300	0.0003474*****
T3- *Pippali (Piper longum* Linn) (97.50 mg/kg)	9.8	3	4.836	0.0006851*****
T4- *Shuddha Vatsanabh (Aconitum balfourii* Stapf) (2.03 mg/kg)	9.7	3	5.413	0.0002961*****

***P<0.05**

Unpaired t- Test is carried out to test significance in all groups at different time points. Above table shows that, P-Value for all six groups are less than 0.05. Hence, we can conclude that, there is significant change observed in mean at different time points.



In acetic acid induced writhing, the no. of writhing observed were less in STD (Diclofenac) and T2- *Shuddha Hingula* (Red Cinnabar :HgS) (16.25 mg/kg) treated group as compared to diseased control, which indicate the analgesic activity.

The hot plate analgesia, the reaction time for paw licking was increased in all the test groups and Standard group as compared to diseased control at 30, 60, 90 and 120 min.

No significant difference was observed in the formalin induced licking in 0-10 min but a significant decrease in the licking at the site of formalin injection was seen after 40-50 minutes which shows the analgesic effect of the test drugs.

6.25 **Probable mode of action of *Hinguleshvara Rasa***

In the present study ***Hinguleshvara Rasa*** **was** found to produce antipyretic and analgesic activity in experimental animals. These activities were comparable with the standard drug (Paracetamol for antipyretic),(Diclofenac Sodium&Pentzocin for Analgesic)used in this study.

Vatsanabh the principal ingredient fot his formulation plays a significant role in producing the antipyretic and analgesic activity. Hingula on account of its being Yogavahi character and

Deepan action and Pippali due to its Deepan and Pachana action help in enhancing the activity of Vatsanabh.

Antipyretic and analgesic activity of Vatsanabha, the principal ingredient of this formulation is known to be due to its Amapachaka and Svedajanaka property.

Aconitum plants have been widely used for analgesia since ancient time. A large number of earlier studies have confirmed that *Aconitum* alkaloids exert remarkable protective effects on the nervous system.

Aconitum has also been widely used as analgesic and antispasmodic drugs since ancient times, and *Aconitum*

alkaloids exert prominent central analgesic effects without addiction, which are considered as the potential active ingredients of new analgesics. In addition, *Aconitum* alkaloids, as a type of naturally active ingredients with significant anticholinesterase activity and neuroprotective effect, have shown potential therapeutic effects on a variety of nervous system diseases, and their clinical therapeutic effects remains to be further studied.

Isotalatizidine exerted analgesic effects by activating the ERK1/2-CREB pathway and mediating the expression of dynorphin A in microglia cells Bulleyaconitine A also displayed long-acting local anesthetic properties both *in vitro* and *in vivo*, and often used in the treatment of chronic pain[269].

CONCLUSION

This study was aimed to assess antipyretic and analgesic activity *of Hinguleshvara Rasa*. Primary objective of this study included preparation of *Hinguleshvara Rasa*, physicochemical analysis and characterization of *Hinguleshvara Rasa*. Physicochemical parameters like total ash, moisture content, thin layer chromatography etc of *Hinguleshvara Rasa* were studied. Secondary objectives included the study of all three ingredients of *Hinguleshvara Rasa* and *Hinguleshvara Rasa* itself for an assessment of their antipyretic as well as analgesic activity.

The study was conducted in three steps 1. Preparation of *Hinguleshvara Rasa*, 2. Physicochemical analysis and 3. Assessment of Antipyretic and Analgesic activity in experimental animals.

Following conclusions can be drawn from the first step of study i.e. Preparation of Hinguleshvara Rasa:

1. *Hinguleshvara Rasa* was prepared with *Shuddha Hingula (*Red Cinnabar: HgS), *Shuddha Vatsanabh* (*Aconitum balfourii* Linn) *and Pippali (Piper longum* Linn) in equal proportion (250 g each) using method described in the classical text *Bhaishajya Ratnavali Jvara chikitsa (*5/72). The weight of final product was observed reduced by 14 g. (1.86%) may be due to loss incurred during manual handling in processing.
2. It was also observed that the formulation ingredients 20.72% of Hingula gets lost during its wet grinding in ginger juice during its shodhana process.
3. Approx 34.12% of Vatsanabh is lost during its *shodhana* in Gomutra(Cow urine) .

Following conclusions can be drawn from the physicochemical testing

1. Reduction of number of spots during comparison of Thin layer chromatographs of *Ashuddha and Shuddha Vatsanabha* indicating that some of the phytochemical ingredients are lost during its *shodhana.*
2. Aconitine content of Vatsanabha was observed markedly reduced following its *shodhana.*
3. while total alkaloid content was observed increased due to its processing with cow urine.

4. Increase in the Particle size of formulation ingredients was observed after comparison of the ingredient before and after the wet grinding. This was due the the coating of the *Bhavana Dravya* on the particles of the ingredients as well as due to agglomeration of the particles.
5. The product *Hinguleshvara Rasa* was found to contain alkaloids Piperine (0.059%) and total alkaloids (o.48%). The Aconitine content in *Hinguleshvara Rasa* was observed to be 0.0022%. The observation concludes that even after *shodhan of Vatasnabha*, some amount of Aconitine remains in *Vatsanabh*, which is transferred to the final product *Hinguleshvara Rasa*. Which underlines the importance of caution to be observed during its therapeutic use.

Following conclusions can be drawn from the experimental study of *Hinguleshvara Rasa*

1. Acute oral toxicity study of *Shuddha Hingula, Shuddha Vatsanabh and Hinguleshvara Rasa* was performed as per OECD-423 guidelines. No toxic effects in the form of mortality or pathological changes in organs were observed following oral administration of ***Hinguleshvara Rasa*** in therapeutic dose in the experimental animals.
2. It can be concluded on the basis of antipyretic study that although in initial period the antipyretic activity of the standard drug Paracetamol is more pronounced than that of the test drug ***Hinguleshvara Rasa***. However, the activity is almost comparable in later stages (24 hrs) with the standard drug Paracetamol. Still, it can be said that the test drug ***Hinguleshvara Rasa*** can't substitute the standard drug Paracetamol as far as its antipyretic activity is considered.
3. Writhing test, Hot plate analgesia and Formalin induced licking tests were utilized for testing analgesic activity of the test drug. On the basis of these tests, it can be concluded that the analgesic effect of ***Hinguleshvara Rasa*** is as good as the standard drug Diclofenac for Writhing test and Formalin test, Pentazocin for Hot plate Test. On the basis of this study, it is concluded that ***Hinguleshvara Rasa*** is having significant antipyretic and analgesic activity. However, although its antipyretic activity is not comparable with the standard drug Paraetamol, ***Hinguleshvara Rasa*** is as effective as standard drugs Diclofenac and Pentazocin in it's analgesic effect.
4. It is also concluded that the ingredients ***of Hinguleshvara Rasa*** are found potent analgesic but weak in antipyretic effect.

8. BIBLIOGRAPHY

1) Yadav Trikamji Aacharya, Charaka Samhita of Agnivesh Munshiram Mohanlal Publication Pvt Ltd Bombay 4th Edition 1992 Ch. Ni. 1/35pg 203
2) Yadav Trikamji Aacharya, Charaka Samhita of Agnivesh Munshiram Mohanlal publication pvt ltd Bombay 4th Edition 1992 Ch.chi. 3/31 pg 400
3) Junaid Niazietal Antiinflammatory and antipyretic activity of aleuritismoluccanaleaves, Asian journal of Pharmaceutical and clinical research volm 3 issue 1 Jan –Mar 2010,pg 35-37.
4) H.G.Vogel, Drug discovery and evolution 2nd revised edn, Antipyretc action in rats,H.4.0.2,pg777
5) Ref: Hunskaar, Steinar, Odd-Geir Berge, and Kjell Hole. "Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic acid and indomethacin in the formalin test." Pain 25.1 (1986): 125-132
6) H.G. Vogel, Drug discovery and evolution 2nd revised edn formalin test in rats H.1.2.10,pg 702
7) Ref : S R Jaiswal, S D Sontakke, Experimental evaluation of analgesic and anti-inflammatory activity of simvastatin and atorvastatin Indian Journal of pharmacology2012-ncbi.nlm.nih.gov
8) H.G.Vogel, Drug discovery and evolution 2nd revised edn Writhing Test,H.2.0.2,pg 716
9) Tripathi k d essentials of medical pharmacology 7th edn Jaypee brothers medical Publishers Pvt Ltd New Delhi 2013
10) Ghosh m n fundamentals of experimental pharmacology3rd edn Kolkata Hilton and company 2005
11) Menéndez, Luis, Ana Lastra, Agustín Hidalgo, and Ana Baamonde. "Unilateral hot plate test: a simple and sensitive method for detecting central and peripheral hyperalgesia in mice." Journal of neuroscience methods 113, no. 1 (2002): 91-97.
12) H.G.Vogel, Drug discovery and evolution 2nd revised edn .Hot plate Method.H.1.2.4,pg 696
13) Koteshvara (2005) preparation, Physico chemical analysis and its clinical efficacy on Aamvata w.s.r.to Rheumatoid arthritis.P.G Dessertation, Rajiv Gandhi University.

14) R S Sarashetty and Bagewadi Physicochemical analysis and antipyretic activity of Hinguleshvararasa in Albino Rat's PG Dessertation RGUHS Karnataka AVS's Ayurved Mahavidyalaya PGCRC, Bijapur(India)2007.

15) Thakur S K Hinguleshvara Rasaka Pratishyay Jvaramein Amayik Prayog PG Dr B R Ambedkar University, Patna, Bihar(India)2000

16) Sadanand Sharma, Rasatarangini, Motilal Banarasidas, Delhi(India),2000, Tarang 24/26-31

17) Shri Gulraj Sharma Mishra, Ayurved Prakash, Chaukhamba Bharti Academy, Reprint 1999,2/72,pg 274

18. Sadanand Sharma, Rasatarangini, Motilal Banarasidas, Delhi(India),2000,P-654

19 Shri Nagindas Chhaganlal Shah, Bharat Bhaishajya Ratnakar, volm 5,B Jain Publishers Pvt. Ltd. New Delhi,110055,2nd Edn,1928,reprint 1999 ,pg 491-492

20 Govind Dasa, Bhaishajya Ratnavali, Hindi Commentry by Ambikadatta Shastri,14thEdn, Chaukhamba Sanskrit Sansthana,Varanasi(India)1999,pg 82

21 Pdt Dattaram Chaube, Bruhad Rasaraj Sundaram, Chaukhambaorientalia Varanasi,2ndEdn,Pg 253

22 Shri.Baidyanathtanay, Shalinathkrut,'Rasamanjiri' Chaukhaba Sanskrut Pratishthana Delhi,110007,1st Edn 1995,pg 84

23 Chaitra.L.V & Jayalaxmi, Critical review of Hinguleshvara Rasa-A Herbomineral Formulation. Int J Ayu Pharm Chem2020 Vol 13 Issue 1,pg 156-161

24. Shri Nagindas Chhaganlal Shah, Bharat Bhaishajya Ratnakar, volm 5,B Jain Publishers Pvt.Ltd. New Delhi,110055,2nd Edn,1928,reprint 1999 ,pg 491-492

25. Vagbhata,'Rasaratnasamucchaya'2ndHindi commentary by Pdt Dharmanand Sharma, Motilal Banarasidas,1962,P-62

26. Shri Nagindas Chhaganlal Shah, Bharat Bhaishajya Ratnakar, volm 5,B Jain Publishers Pvt.Ltd.New Delhi,110055,2nd Edn,1928,reprint 1999 ,pg 491-492

27. Koteshvara(2005) preparation, Physico chemical analysis and its clinical efficacy on Aamvata w.s.r.to Rheumatoid arthritis.P.G Dessertation, Rajiv Gandhi University.

28. R S Sarashetty and Bagewadi Physicochemical analysis and antipyretic activity of Hinguleshvararasa in Albino Rat's PG Dessertation RGUHS Karnataka AVS's Ayurved Mahavidyalaya P GCRC, Bijapur (India) 2007.

29. Thakur S K Hinguleshvara Rasa ka Pratishyay Jvaramein Amayik Prayog PG Dr B R Ambedkar University, Patna, Bihar(India) 2000
30. (Sujatha K and Rashma Saokar Role of Bhavana process in the antipyretic effect of Hinguleshvara Rasa, Dept of Rasashastra & BK SDM Institute of Ayurveda & Hospital Bengluru Europian Journal of Biomedical and Pharmaceutical Sciences2019,vilm 6,Issue 8,451-455)
31. Abhishek Chatterjee &etal, In vitro anti-inflammatory and antioxidant activities of Hinguleshvara Rasa Based Herbomineral formulation

Asian Journal of Pharmaceutical and clinical research vol 11, special issue2,2018,24-27

32. (Dr Dinesh Gupta, Dr P Koteshvara Dr Jagdish Mitti & Dr Mallikarjun Patil Pharmaceutical–Analytical study of Hinguleshvara Rasa Rasamruta world's first e journal of Ayurvedarasamruta,4:5 June 2012)
33. (Kashinath & Shivleelaetal Evalution of Analgesic Activity of Bruhat Hinguleshvara Rasa, world Journal of Pharmaceutical Research,Volm 6,Issue 8,2017,1540-1544)
34. (Kashinath & Shivleelaetal Evalution of Antipyretic Activity of Bruhat Hinguleshvara Rasa Journal of Biological and Scientific opinion vol 5 (4)2017,53-54)
35. Sadanand Sharma, Rasatarangini,Motilal Banarasidas, Delhi(India),2000, Tarang 24/26-31
36. Shri Gulraj Sharma Mishra, Ayurved Prakash, Chaukhamba Bharti Academy, Reprint 1999,2/72,pg 274
37. Vagbhatacharya, Rasaratnasamucchaya, commentary by DA Kulkarni, meherchand Lachmandas publication, New Delhi, reprint 1998,3/120,pg64
38. Dr Indradev Tripathi .Rasarnava, Chaukhamba Sanskrit series of Varanasi,2nd edn 1978, Seventh patal, pg 93-94
39. Vagbhatacharya, Rasaratnasamucchaya, commentary by DA Kulkarni, meherchand Lachmandas publication, New Delhi, reprint 1998,3/120, pg 64
40. Gopal Krishna bhatta Rasendra Sara Sangraha, Chaukhamba Sanskrita Sansthana Varanasi, Delhi,st edn 2007, chapter 1/114-115 pg 43 For synonym 1/226 pg72
41. Shri Madhav Ayurved Prakash by Gularaj Sharma Mishra, Chukhamba Bharti Academy, Varanasi, Reprint 1999,2/1,pg 252
    For synonym2/69pg 272

42. Bhudeb Mookerjei, Rasajalanidhior ocean of Indian chemistry and alchemy,Parimal publication,Delhi,Vol[m] 2,Edn 1990,chapter 2,pg 224.
43. Bhavmishra pranit Bhavaprakash, Krushnachandra Chunekar Chaukhamba Bharti Academy, Varanasi, Reprint 1998,Dhatvadivarga,101/pg 614
    For Synonym types & properties 102-103/pg 615
44. S.V Radhakrishna Shastri, Anandkandam published by TMSSM Library Tanjore1952, Kriyakaranvishranti Ullasa 1,pg 542-543
45. Vd Datto Ballal Borkar Sarth Rasachandanshu, Gajanan book Depot,Pune,3rd edn,273pg56
    Vd Datto Ballal Borkar Sarth Rasachandanshu, Gajanan book Depot Pune,3rd edn,293-300pg63,64
46. Aacharya Somdeva, Rasendra Chudmani treanslated by Siddhinandan Mishra Chaukhamba orientalia Varanasi 2nd edn 1999,pg 195-196
47. Shri Madhav Ayurved Prakash by Gularaj Sharma Mishra, Chukhamba Bharti Academy, Varanasi, Reprint 1999,2/69pg 272
48. Aacharya PV Sharma, Dhanvantari Nighantu, Chaukhambaorientalia Varanasi,2ndedn 1998,Suvarnadi varga6/37-39,pg185
49. Dr Indradev Tripathi. Rasarnava, Chaukhamba Sanskrit series of Varanasi,2nd edn 1978, Seventh patal,pg 93-94
50. Pt. Aryadas Kumara Singh , Mahaushadhinighantu, The Vidyabhavan Ayurved Granthmala, Chaukhambavidyabhavan Varanasi 1stedn 1971,pg 173-174
51. Shrikaidevpandit virachit Kaidevnighantu (Pathyapathyavibodhak) Aacharya P.V Sharma, Chaukhamba Orientalia, Varanasi, 1st edn,1976,Dhatuvarga2/60-63,pg 283are new synonyms mentioned.
52. Shrimadvagbhatacharya virachit Ashtang Nighantu,Viprakirna prakarana27/292
53. Gopal Krishna Bhatta Rasendrasarasangraha, Chaukhamba Sanskrutasansthana Varanasi, Delhi, stedn 2007,chapter 1/226 pg72
54. Sadanand Sharmana Rasatarangini, Motilal Banarasidas,Delhi,Reprint 2004,tarang 9/1-2, pg198
55. Bhavmishra pranit Bhavprakash, krushnachandra Chunekar Chaukhamba Bharti Academy, Varanasi, Reprint 1998, Dhatvadivarga, 102-103/pg 615

56&57 Shri Madhav Ayurved Prakash by Gularaj Sharma Mishra, Chaukhamba Bharti Academy, Varanasi, Reprint 1999,2/70-71pg 273

58. Vagbhatacharya, Rasaratnasamucchaya, commentary by DA Kulkarni, meherchand Lachmandas publication, New Delhi, reprint 1998,3/139,pg68

59. Sadanand Sharmana Rasatarangini, Motilal Banarasidas, Delhi,Reprint 2004,tarang 9/3,pg199

60. Shri Madhav Ayurved Prakash by Gularaj Sharma Mishra, Chaukhamba Bharti Academy, Varanasi, Reprint 1999,2/70-71pg 273

61. Vagbhatacharya, Rasaratnasamucchaya, commentary by DA Kulkarni, meherchand Lachmandas publication, NewDelhi, reprint 1998,3/120,pg64

62. Pt. Aryadas Kumara Singh , Mahaushadhinighantu, Thevidyabhavanayurvedgranthmala, Chaukhambavidyabhavan Varanasi 1st edn 1971,pg 173-174

63. Dr Indradev Tripathi .Rasarnava, Chaukhamba Sanskrit series of Varanasi,2nd edn 1978, Seventh patal,pg 93-94

64. Aacharya P V Sharma, Dhanvantari Nighantu, Chaukhamba orientalia Varanasi,2nd Edn , 1998,Suvarnadivarga ,pg 185&275

65. Aacharya Krushnchandra Chunekar, Bhavprakash Nighantu Chaukhamba Bharti academy , Varanasi,reprint 1998, Dhatvadi varga pg 615 .

66. Sri gopal Krishna, Rasendrasarsangraha, Jayakrishnadas Haridas Gupta, Chaukhamba Sanskrit Series Banarasa 4th edn 1953,adhyay 1, pg 63-64

67. Vagbhatacharya, Rasaratnasamucchaya, commentary by DA Kulkarni, meherchand Lachmandas publication, NewDelhi,reprint 1998,3/120,pg64

68. Sadanand Sharmana Rasatarangini, Motilal Banarasidas, Delhi, Reprint 2004,tarang 9/3,pg199

69. Shri Madhav Ayurved Prakash by Gularaj Sharma Mishra, Chaukhamba Bharti Academy, Varanasi, Reprint 1999,2/70-71pg 273

70&71Vagbhatacharya, Rasaratnasamucchaya, commentary by DA Kulkarni, meherchand Lachmandaspublication, NewDelhi,reprint 1998,3/120,pg64

72 Dr Indradev Tripathi .Rasarnava, Chaukhamba Sanskrit series of Varanasi,2nd edn 1978, Seventh patal 7/52,pg 93-94

73&74 Gopal Krishnabhatta Rasendrasarasangraha, Chaukhamba Sanskrutasansthana Varanasi, Delhi, Stedn 2007,chapter 1/226 pg72

75 Dr Indradev Tripathi .Rasarnava, Chaukhamba Sanskrit series of Varanasi,2nd edn 1978, Seventh patal, pg 93-94

76 Shri Madhav Ayurved Prakash by Gularaj Sharma Mishra, Chaukhamba Bharti Academy, Varanasi, Reprint 1999,2/83-86 pg 273

77 Sadanand Sharmana Rasatarangini, Motilal Banarasidas, Delhi, Reprint 2004,tarang 9/3,pg199

78 Dr. Nalin D Shah&etal, Comparative XRF study of Hingula purified in two different media,www.ayurpub.com,Mar-Apr2018Vol 3,issue2

79 Dr. R. Surjith*1 etalImpact of shodhana w.s.r to heavy metal on Hingula with Lakoochaphalaswarasa (Artocarpuslakoocha), wjpmr, 2018,4(7), 196-199

80 A Comparative experimental evaluation of toxicological effects of Shodhita and Ashodhita Hingula in Albino Rats by Ambujakshi V M, Dissertation submitted to Rajiv Gandhi University of Health Sciences Karnataka Bengaluru.

81 Dr Indradev Tripathi, Rajanighantu of Pandit Narahari, Chaukhamba Krishnadas Academy, Varanasi,3rd edition 2003, Pippalydi varga pg 136-137

82 The Ayurvedic Pharmacopoeia of India ,part 1,volme 4,1st edition,Govt of India,ministry of health and family welfare,department of yoga,naturopathy,,unani Siddha and homeopathy(AYUSH)New Delhi ,pg 91-92

Documentation and publication division Central council for research in Ayurveda and siddha, Janakpuri New Delhi110058, Edn.2001, pg 472

83. Vd.VM Gogte, Ayurvedic pharmacology, and therapeutic uses of medicinal plants, dravyagunavignyan, Chaukhamba Publication, New Delhi, Reprint2009, part 2, pg 424-427

84. Dr.Gyanendra Pandey, Dravyaguna Vijnana, volume 3, Chaumba, Krishnadas, academy, Varanasi, Reprint 2014, pg 117

85. Quality standards of Indian Medicinal plants, Indian Council of Medical Research, New Delhi 2003, volume 1, pg 168-173

86. P, C Sharma, M.B .Yelne, T J Dennis Database on medicinal plants used in Ayurveda Vol.3,

87. E-Nighantu copyrightz@CCRAS New Delhi-All rights reserved e Nighantu designed and developed by National Institute of Indian Medical heritage Hyderabad,

88. AshalathaM, Rekha B Sannapanawar, A, A review article on Pippali (Piper longum Linn), IAMJ vol 3, Issue 9, Sept 2015

89-90) Dr Krushnachandra Chunekar, Bhavmishrapranit Bhavaprakash nighantu Chaukhamba Bharti Academy Varanasi, reprint 1998,pg 15-16,19

91) Sharagdharamishra Sharangdhar Samhita, Chaukhamba orientalia,4thEdn.2000,pratham khand adhyay 1/44,pg 11

92) Sharagdharamishra Sharangdhar Samhita, Chaukhamba orientalia,4thEdn.2000,pratham khand adhyay 1/46,pg 11

93-94) Dr Krushnachandra Chunekar, Bhavmishrapranit Bhavaprakash nighantu Chaukhamba Bharti Academy Varanasi, reprint 1998,pg 15-16,19

95. Meghan Pathak et al /A toxicity study of Pippali (Piper longum) fruit. International Journal of Pharmaceutical & biological Archives2013; 4(1):89-93

96. Analgesic, Antipyretic and Ulcerogenic effect of piperine: an active ingredient of Pepper, Evan prince Sabina, Ayesha Nasreen,Mahima Vedi,Mahabookhan Rasool,Evan Prince et al/J.Pharm.Sci.& Res.Vol5(10),2013,203-206

97. Vagbhata, Pandit Dharmanand Sharmana Rasaratnasamucchaya II Motilal Banarasidas P.B.75, Nepalikhapra Varansi(1962), adhyay 28/2

98. Aacharya shri Gulrajasharmamishra, Rasakamdhenu, Chaukhambaorientaliya Varanasi,1st part,2ndEdn 1999,Pratham upakaranpad, chaturthodhhyay, pg 114-115

99. Shri Gularajsharma Mishra, Ayurveda Prakash, Chaukhamba Bharti Academy, Varanasi, reprint1999,adhyay6/33,pg488

100. Aacharya shri Gulrajasharmamishra, Rasakamdhenu, Chaukhambaorientaliya Varanasi,1st part,2ndEdn 1999,Pratham upakaranpad, chaturthodhhyay,pg 109

101. Sadanand Sharmana, Rasatarangini, Motilal banarasidas,11t Edn, Reprint 2000, tarang 24/8

102. Aacharya shri Gulrajasharmamishra, Rasakamdhenu, Chaukhambaorientaliya Varanasi,1st part,2ndEdn 1999, Pratham upakaranpad, chaturthodhhyay,pg 114-115

103. Vd Laxmipati Shastri, Yogratnakar ChaukhambaPrakashan,Varanasi Edn 2012 pg 166

104. (Vaidya.Vishnu Madhav Gogte, Ayurved Pharmacology & Therapeutic Uses of medicinal plants, Dravyaguna Vidnyan, Chaukhamba Publication ,New Delhi, Edn, reprint 2009,part 2,pg 481-483)
105. Sadanand Sharmana, Rasatarangini,Motilal banarasidas,11t Edn,Reprint 2000, tarang 24/12-14
106. Aacharya shri Gulrajasharmamishra, Rasakamdhenu, Chaukhambaorientaliya Varanasi,1st part,2ndEdn 1999, Pratham upakaranpad, chaturthodhhyay, shloka26-28,pg 109
107. Sadanand Sharmana, Rasatarangini, Motilal banarasidas,11thEdn,Reprint 2000, tarang 24/12-14
108. (Vaidya. Vishnu Madhav Gogte, Ayurved Pharmacology & Therapeutic Uses of medicinal plants, Dravyaguna Vidnyan, Chaukhamba Publication ,New Delhi, Edn,reprint 2009,part 2,pg 481-483)

109-110 2250The Ayurvedic Pharmacopoea of India part 1 vol 4,1stEdn,Govt of India Ministry of health and family welfare, Dept of Indian system of Medicine & Homoeopathy ,NewDelhi,pg 171-172

111. Shri Gularajsharma Mishra, Ayurveda Prakash, Chaukhamba Bharti Academy, Varanasi, reprint1999,adhyay6/40pg489
112. Sadanand Sharmana, Rasatarangini, Motilal banarasidas,11th Edn, Reprint 2000, tarang 24/11

113-115 (Vaidya.Vishnu Madhav Gogte, Ayurved Pharmacology & Therapeutic Uses of medicinal plants, Dravyaguna Vidnyan, Chaukhamba Publication ,New Delhi, Edn, reprint 2009,part 2,pg 481-483)

116. Aacharyashri Gulrajasharmamishra, Rasakamdhenu, Chaukhambaorientaliya Varanasi,1st part, 2ndEdn 1999,Pratham upakaranpad, chaturthodhhyay,pg 109
117 Sadanand Sharmana, Rasatarangini, Motilal banarasidas,11t Edn, Reprint 2000, tarang 24/15
118. Shri Gularajsharma Mishra,Ayurveda Prakash,Chaukhamba Bharti Academy, Varanasi,r eprint1999,adhyay6/41-42,pg490
119. Vd Yadavtrikamji Aacharya, Sushruta Samhita, Chaukhamba orientalia,Varanasi,6th edn 1997, Kalpsthana, Adhyay2/5&12,pg 564

120. L.B.Singh, Poisonous (Visa)plants in Ayurveda, Chaukhamba Sanskrita Bhavan,Varanasi,1st Edn,1997,pg 37
121. Datta S. C. & Mukerji B. (1950). Pharmacognosy of Indian root and rhizome drugs. Pharmacognosy Lab. Bull, 1, pp. 14-23
122. Pandey and Ansari medicinal plant(Aconite), Nat. Integrated Med. Assoc, Vol XXNo10,1978
123. The Ayurvedic Pharmacopoea of india part 1 vol 4,1stEdn,Govt of India Ministry of health and family welfare, Dept of Indian system of Medicine & Homoeopathy, NewDelhi,pg 171-172
124. IUCN red listed medicinal plants of Siddha
125. Vd Adavtrikamji Aacharya, Charaksamhita, Chaukhambasurbharti, Varanasi, reprint 2011,chi adhyay 23/11,pg571
126. Vd Adavtrikamji Aacharya, Charaksamhita, Chaukhambasurbharti, Varanasi, reprint 2011,chi adhyay1-3/29 pg 385
127. Vd Adavtrikamji Aacharya, Charaksamhita, Chaukhambasurbharti, Varanasi,reprint 2011,chi adhyay3/309pg426
128. Acharya Vagbhat, Ashtang Hruday,edited by Harishastri paradkar, Krishnadas adcademy Varanasi. Reprint 2000.
129. Dr.J L N Shasry & Dr Tanuja Nesri, A textbook of dravyagna Vijnana, Chaukhambaorientalia, Varanasi, volm 2,1st Edn2018,pg 302-318

130-131 Sadanand Sharmana, Rasatarangini, Motilal banarasidas,11t Edn,Reprint 2000, tarang 24/17&18

132. PK Sarkar etal Evalution of Effect of Shodhana treatment on pharmacological activities of Aconite, Ind J Pharm Edu Res Jul-Sept,2012/Vol46/Issue3
133. Sadanand Sharmana, Rasatarangini, Motilal banarasidas,11th Edn, Reprint 2000, tarang 24/17&18
134. Shri Gularajsharma Mishra, Ayurveda Prakash, Chaukhamba Bharti Academy, Varanasi, reprint1999,adhyay6/54-59,pg492-493

135-136 Dr.J L N Shasry & Dr Tanuja Nesri, A textbook of dravyagna Vijnana, Chaukhambaorientalia, Varanasi,volm 2,1st Edn2018,pg 302-318

137. Bhavmishra, Bhavprakash commentary by Krushnachandra Chunekar, Chaukhamba Bharti Academy, Varanasi, Reprint 1998, Vishavarga204,pg 634
138. Sadanand Sharmana, Rasatarangini, Motilal banarasidas,11t Edn,Reprint 2000, tarang 24/26-31,pg 653
139. Vagbhata, Pandit Dharmanand Sharmana Rasaratnasamucchaya II Motilal Banarasidas P.B.75, Nepalikhapra Varansi(1962),adhyay 28/2
140. Aacharyashri Gulrajasharmamishra, Rasakamdhenu, Chaukhambaorientaliya Varanasi,1st part,2ndEdn 1999,Pratham upakaranpad, chaturthodhhyay, 62-63,pg 114-115
141. Vd Laxmipati Shastri, Yogratnakar Chaukhamba Prakashan, Varanasi Edn 2012 pg 166
142. Vagbhata, Pandit Dharmanand Sharmana Rasaratnasamucchaya II Motilal Banarasidas P.B.75, Nepalikhapra Varansi (1962),adhyay 28/2
143. Aacharyashri Gulrajasharmamishra, Rasakamdhenu, Chaukhambaorientaliya Varanasi,1st part,2ndEdn 1999, Pratham upakaranpad, chaturthodhhyay, 51,pg 113
144. Shri Gularajsharma Mishra, Ayurveda Prakash, Chaukhamba Bharti Academy, Varanasi, reprint1999,adhyay6/71,pg495
145. Sadanand Sharmana, Rasatarangini, Motilal banarasidas,11t Edn, Reprint 2000, tarang 24/66,pg 660
146. Dr K.S. Narayan Reddy, The essentials of Forensic medicine and Toxicology,K.Suguna Devi publication, Hydrabad .32nd Edn2013,pg 587)
147. The Ayurvedic Pharmacopoea of India part 1 vol 4,1stEdn,Govt of India Ministry of health and family welfare, Dept of Indian system of Medicine & Homoeopathy, New Delhi,pg 171-172
148. Neha Arya and Hemantkumar Comparative Physico-chemical profile of Vatsanabh (Aconitum Ferox, Ranunculaceae) Mula processed through cow's urine and cow's milk.Int J Res Ayurveda Pharma.8(5), 2017
149. PK Sarkar etal Evalution of Effect of Shodhana treatment on pharmacological activities of Aconite, Ind J Pharm Edu Res Jul-Sept, 2012/Vol46/Issue3
150. The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda, Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi. Determination of Moisture Content (Loss on Drying)2.2.9,pg 160

151. The Ayurvedic Pharmacopoeia of India part I,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi. Determination of Total Ash content,2.2.9 ,pg 159

152. The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi.iddha & Homoeopathy (AYUSH)New Delhi. Determination of Acid insoluble Ash ,2.2.4,pg 159

153. Determination of Loss on ignition Hinguleshvara Rasa, Inhouse method given by bhide research foundation

154 Gravimetric method given by Vasu research laboratory.

155 Inhouse method given by Vasu research laboratory

156 www.youtube.com

157. The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda, Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi.iddha & Homoeopathy (AYUSH)New Delhi., Determination of Moisture Content (Loss on Drying)2.2.9,pg 160

158. The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda, Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi. Determination of Total Ash content,2.2.9 ,pg 159

159. Determination of Acid insoluble Ash,2.2.4,pg 159,The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda, Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi.iddha & Homoeopathy (AYUSH)New Delhi.

160 Comparative study of physicochemical properties of Ashuddha and Shuddha parade with special reference to Rasonshodhita Parada,by Vd Sharadini Ketkar, dissertation submitted to Swami Ramanand Teerth Marathwada University,Nanded.2003-2005.

161 TLC: of Ardrak juice (Juiceof Zingiber officinale Rosc) 2.2.12,164
The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi.iddha & Homoeopathy (AYUSH)New Delhi.

162. Detection of Heavy metals like Cadmium, Arsenic, Mercury &Lead from Ardrak juice (Juice of Zingiberofficinale Rosc The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda, Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi.iddha & Homoeopathy (AYUSH)New Delhi.

163 Johansen DA Plant micro technique ,(McGraw-Hill Book Company Inc ,Newyork and Landon)1940,182

164. Indian Medicinal Plants. Vol. II. Dehradun: International Book Distributers. 2006. pp. 1459-60.

165. Pandey G. Medicinal Plants of Himalaya.Vol II. 1st ed. Delhi: Sri Satguru Publications. 2000; pp. 252

166. Wallis TE.Text book of Pharmacognosy. 5th ed. New Delhi: CBS Publishers and Distributors; 2002. pp. 578-581.

167. Ibidem (7). Khandelwal KR, Sethi V. pp. 20.1-20.5.

168. Ibidem (7).Khandelwal KR, Sethi V. pp. 2.1-2.6.

169 Lohar DR. Protocol for Testing, Ayurvedic, Siddha, Unani medicines, government of India.Appendix-2. Ghaziabad: Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory for Indian Medicines; 2007. pp. 41-44.

170 Wallis TE.Text book of Pharmacognosy. 5th ed. New Delhi: CBS Publishers and Distributors; 2002. pp. 578-581.

171. Ibidem (7). Khandelwal KR, Sethi V. pp. 8.1-8.3.

172. Metcalfe CR, Chalk L. Anatomy of the Dicotyledons.Vol. 1. Great Britain; Oxford University Press: 1950, pp. 1328-1348.

173 Ibidem (7).Khandelwal KR, Sethi V. pp. 5.2-5.5.

174 Wallis TK analytical Microscopy.3rdedn (J&AChurchilLtd,Landon)1965,111

175. TLC Method of Vatsanabh (Aconite Root) 2.2.12,164

The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda, Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi.iddha & Homoeopathy (AYUSH)New Delhi.

176., The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi.iddha & Homoeopathy (AYUSH)New Delhi. Determination of Foreign Matter 2.2.2,pg 159

177. The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi. Determination of Moisture Content (Loss on Drying)2.2.9,pg 160

178. The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi.Determination of Total Ash content,2.2.9 ,pg 159

179. Determination of Acid Insoluble Ash2.2.4,pg 159, The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi.

180. Determination of Alcohol Soluble Extractive,2.2.6,pg 160, The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi.

181. Determination of Water Soluble Extractive)2.2.7,pg 160, The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi.

182. Johansen DA Plant micro technique, (McGraw-Hill Book Company Inc, Newyork and Landon)1940,182

183 Indian Medicinal Plants. Vol. II. Dehradun: International Book Distributers. 2006. pp. 1459-60.

184. Pandey G. Medicinal Plants of Himalaya.Vol II. 1st ed. Delhi: Sri Satguru Publications. 2000; pp. 252

185. Wallis TE.Text book of Pharmacognosy. 5th ed. New Delhi: CBS Publishers and Distributors; 2002. pp. 578-581.

186. Ibidem (7).Khandelwal KR, Sethi V. pp. 20.1-20.5.

187. Ibidem (7).Khandelwal KR, Sethi V. pp. 2.1-2.6.

188. Lohar DR. Protocol for Testing, Ayurvedic, Siddha, Unani medicines, government of India.Appendix-2. Ghaziabad: Department of AYUSH, Ministry of Health & Family Welfare, Pharmacopoeial Laboratory for Indian Medicines; 2007. pp. 41-44.

189. Wallis TE.Text book of Pharmacognosy. 5th ed. New Delhi: CBS Publishers and Distributors; 2002. pp. 578-581.

190. Ibidem (7).Khandelwal KR, Sethi V. pp. 8.1-8.3.

191. Metcalfe CR, Chalk L. Anatomy of the Dicotyledons.Vol. 1. Great Britain; Oxford University Press: 1950, pp. 1328-1348.

192. TLC Method 2.2.12,pg 164, The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn , Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi.

193 Determination of Foreign Matter 2.2.2,pg 159, The Ayurvedic Pharmacopoeia of India part I, volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda, Yoga, -Naturopathy, Unani,Siddha & Homoeopathy (AYUSH)New Delhi.

194 The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi. Determination of Moisture Content (Loss on Drying)2.2.9,pg 160

195. The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi.Determination of Total Ash content,2.2.9 ,pg 159

196. The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi. Determination of Acid Insoluble Ash2.2.4,pg 159

197.Determination of Alcohol Soluble Extractive,2.2.6,pg 160The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi.

198. Determination of Water Soluble Extractive,2.2.7,pg 160,The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi.

199. (The Ayurvedic Pharmacopoeia of India part 2, volume 3 first edn 2010reprint January 2011, 2.4.1pg 174-175&180

200. Counting of cells and spores in microorganisms, Experiment in microbiology, plant pathology, & biotechnology,New age international pvt Ltd publisher New Delhi,th revised edn 2003,reprint2014,pg 69-71

201. Dr Pulok Mukherjee,Quality control of Herbal drugs, Business Horizon Publication Delhi,1st edn 2002,Estimation of Total solid 9.2pg 192

202. Estimation of total alkaloid inhouse method given by Vasu research lab

203. Aacharya Vagbhata Rasaratnasamuchhaya, Dr Ashok Satpute, Chaukhamba Sanskrit Pratishthan Delhi Shodhana of Hingula(Red cinnabar: HgS) adhyay 3/136 ,pg82.

204 Shri Madhava,Ayurved Prakash,commentary by Gulrajsharma Mishra,Chaukhamba Bharti Academy Varanasi,reprint 1999, Shodhana of Vatsanabh (Aconitum balfourii Stapf) adhyay 6/49-50pg 491

205 (Ambikadattashastri, Bhaishajya Ratnavali,Chaukhamba Sanskrit Sansthana 9th Edn 1991,5/483,pg 82)

206 Paget G E and Barnes, J.M.'Toxicity tests' In:"Evaluation of drug activities pharmacometrics" (Laurene,D.R.and Bacharach,A.L eds)Vol 1,Academic press London 1964 pp -135-165

207. (sharangdharacharya,SharangdharaSamhita,GudharthdipikaChaukhambaorientalia Varanasi 4thedn 2000,Madhyam khandaadhyay 6/8 pg 178

208 (sharangdharacharya,SharangdharaSamhita,GudharthdipikaChaukhamba orientalia Varanasi 4thedn 2000,pratham khandaadhyay 1/20&32 pg 7-9)

209. (Sadanand Sharmana, Rasatarangini Motilal Banarasidas 11thedn, Reprint 2000,24/66 pg 660.)

210.(Shri Madhav Ayurved Prakash commentary by Gulraj Sharma Mishra Chaukhamba Bharati Academy reprint 1999,2/76 pg 275)

211. (Ambikadattashastri, Bhaishajya Ratnavali,Chaukhamba Sanskrit Sansthana 9th Edn 1991,5/483,pg 82)

212. Junaid Niazi etal Antiinflammatory and antipyretic activity of aleuritis moluccana leaves ,Asian journal of pharmaceutical and clinical research volm 3 isue 1 Jan –March 2010,pg 35-37

213 Nirmal kumar etal Analgesic and antipyretic ativities of methanol extract and its fraction from the root of Schoenoplectus grossus Hindawi publishing corporation evidence based complementary and Alternative medicine volm 2016, 8 pages,article Id 3820704

214) H.G.Vogel,Drug discovery and evolution 2nd revised edn,Antipyretc action in rats,H.4.0.2,pg777

215. Ref: Hunskaar, Steinar, Odd-Geir Berge, and Kjell Hole. "Dissociation between antinociceptive and anti-inflammatory effects of acetylsalicylic acid and indomethacin in the formalin test." Pain 25.1 (1986): 125-132

216. H.G.Vogel,Drug discovery and evolution 2nd revised edn formalin test in rats H.1.2.10,pg 702

217 Ref : S R Jaiswal,S D Sontakke, Experimental evaluation of analgesic and anti-inflammatory activity of simvastatin and atorvastatin Indian Journal of pharmacology2012-ncbi.nlm.nih.gov

218 H.G.Vogel,Drug discovery and evolution 2nd revised edn Writhing Test,H.2.0.2,pg 716

219 Menéndez, Luis, Ana Lastra, Agustín Hidalgo, and Ana Baamonde. "Unilateral hot plate test: a simple and sensitive method for detecting central and peripheral hyperalgesia in mice." Journal of neuroscience methods 113, no. 1 (2002): 91-97.

220. Tripathi k d essentials of medical pharmacology7th ednJaypee brothers medical publishers pvt ltd new delhi 2013

221. Ghosh m n fundamentals of experimental pharmacology3rd edn Kolkata Hilton and company 2005

222. H.G.Vogel,Drug discovery and evolution 2nd revised edn .Hot plate Method.H.1.2.4,pg 696

223 Ambikadattashastri, Bhaishajya Ratnavali, Chaukhamba Sanskrit Sansthana 9th Edn 1991,5/483,pg 82)

224 Sadanand Sharma, Rasatarangini, Motilal Banarasidas, Delhi(India),2000, Tarang 24/26-31

225 Shri Gulraj Sharma Mishra, Ayurved Prakash, Chaukhamba Bharti Academy, Reprint 1999,2/72,pg 274

226 Aacharya Vagbhata Rasaratnasamuchhaya, Dr Ashok Satpute, Chaukhamba Sanskrit Pratishthan Delhi Shodhana of Hingula(Red cinnabar: HgS) adhyay 3/136 ,pg82.

227 Dr Pulok Mukherjee,Quality control of herbal drugs, published by business horizon New

Delhi,1st edn 2002,9.6 determination of moisture content ,pg 193

228 Sharangdharacharya, Sharangdhara Samhita, chaukhamba orientalia,Varanasi 4th Edn 2000,pra.kha. 1/44-46 pg11

229 (The Ayurvedic Pharmacopoeia of India part 2, volume 3 first edn 2010reprint January 2011, 2.4.1pg 174-175&180

230 Counting of cells and spores in microorganisms, Experiment in microbiology, plant pathology,&biotechnology,New age international pvt Ltd publisher New Delhi,th revised edn 2003,reprint2014,pg 69-71

231 The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi,Determination of Foreign Matter ,pg 91-92

232 Sharma P and etal,Pharmacognostical, physicochemical and phytochemical status of Piper longum Linn fruits, IJPER,2019,volm 1,issue1,19-24

233 Amrita Pincha, Dhrubjyoti Sarma, Bishnu Prasad Sarma, detailed Pharmacognostical, physicochemical and phytochemical study of Pippali fruits (Piper longum Linn) available in Assam. Int J Ayu Pharm chem. 2017 volm 6 issue 3221-227.

234 The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi., Determination of Moisture Content (Loss on Drying)pg 91-92

235 Sharma P and etal,Pharmacognostical, physicochemical and phytochemical status of Piper longum Linn fruits,IJPER,2019,volm 1,issue1,19-24

236 Amrita Pincha, Dhrubjyoti Sarma, Bishnu Prasad Sarma, detailed, Pharmacognostical, physicochemical and phytochemical study of Pippali fruit s(Piper longum Linn) available in Assam.Int J Ayu Pharm chem. 2017 volm 6 issue 3221-227.

237 The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi. Determination of Total Ash content,pg 91-92

238 Sharma P and etal , Pharmacognostical, physicochemical and phytochemical status of Piper longum Linn fruits,IJPER,2019,volm 1,issue1,19-24

239 Amrita Pincha, Dhrubjyoti Sarma, Bishnu Prasad Sarma, detailed, Pharmacognostical, physicochemical and phytochemical study of Pippali fruit (Piper longum Linn) available in Assam.Int J Ayu Pharm chem. 2017 volm 6 issue 3221-227.

240 The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi, Determination of Acid Insoluble Ash,pg91- 92,

241. Sharma P and etal ,Pharmacognostical, physicochemical and phytochemical status of Piper longum Linn fruits,IJPER,2019,volm 1,issue1,19-24

242. Amrita Pincha, Dhrubjyoti Sarma, Bishnu Prasad Sarma,detailed, Pharmacognostical, physicochemical and phytochemical study of Pippali fruit (Piper longum Linn) available in Assam.Int J Ayu Pharm chem. 2017 volm 6 issue 3221-227.

243. The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi ,Determination of Alcohol Soluble Extractive,pg91-92

244. Amrita Pincha, Dhrubjyoti Sarma, Bishnu Prasad Sarma, detailed, Pharmacognostical, physicochemical and phytochemical study of Pippali fruit (Piper longum Linn) available in Assam.Int J Ayu Pharm chem. 2017 volm 6 issue 3221-227.

245. The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India,Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi, Determination of Water Soluble Extractive)pg 91-92

246. Amrita Pincha, Dhrubjyoti Sarma, Bishnu Prasad Sarma, detailed, Pharmacognostical, physicochemical and phytochemical study of Pippali fruit (Piper longum Linn) available in Assam.Int J Ayu Pharm chem. 2017 volm 6 issue 3221-227.

247. TLC Method, The The Ayurvedic Pharmacopoeia of India part I ,volm IV,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi, pg 91-92248. Estimation of total alkaloid inhouse method given by Vasu research lab.

249. Estimation of piperine content from Pippali Piper longum Linn inhouse method given by Vasu research lab

250 Shri Gulraj Sharma Mishra, Ayurved Prakash, Chaukhamba Bharti Academy, Reprint 1999,2/72,pg 274

251 (The Ayurvedic Pharmacopoeia of India part 2, volume 3 first edn 2010reprint January 2011, 2.4.1pg 174-175&180

252 Counting of cells and spores in microorganisms, Experiment in microbiology, plant pathology, & biotechnology, New age international pvt Ltd publisher New Delhi,th revised edn 2003,reprint2014,pg 69-71

253.The Ayurvedic Pharmacopoeia of India part I, volm II,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy,Unani,Siddha & Homoeopathy (AYUSH)New Delhi., Determination of Moisture Content (Loss on Drying),pg 171-172

254. Neha Arya & Hemant kumar/Int.J.Res. Ayurveda Pharm.8(5), 2017, Comparative physicochemical profile of Vatsanabh (Aconitum ferox ,Ranunculaceae)mula processed through cow's urine and cow's milk.

255. The Ayurvedic Pharmacopoeia of India part I ,volm II,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda, Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH) New Delhi. Determination of Total Ash content,pg 172-174

256. Neha Arya & Hemant kumar/Int.J.Res.Ayurveda Pharm.8(5),2017,Comparative physicochemical profile of Vatsanabh (Aconitum ferox ,Ranunculaceae)mula processed through cow's urine and cow's milk.

257.Determination of Acid Insoluble Ash, The Ayurvedic Pharmacopoeia of India part I ,volm II,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda,Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi,Pg.172-174

258 Neha Arya & Hemant kumar/Int.J.Res. Ayurveda Pharm.8(5), 2017, Comparative physicochemical profile of Vatsanabh (Aconitum ferox, Ranunculaceae) mula processed through cow's urine and cow's milk.

259 Determination of Alcohol Soluble Extractive ,pg 172-174, The Ayurvedic Pharmacopoeia of India part I ,volm II,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda, Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi.

260. Neha Arya & Hemant kumar/Int.J.Res. Ayurveda Pharm.8(5),2017, Comparative physicochemical profile of Vatsanabh (Aconitum ferox ,Ranunculaceae) mula processed through cow's urine and cow's milk.

261. The Ayurvedic Pharmacopoeia of India part I ,volm II,1st Edn ,Govt of India, Ministry of Health and Family welfare ,Dept of Ayurveda, Yoga, -Naturopathy, Unani, Siddha & Homoeopathy (AYUSH)New Delhi, Determination of Water Soluble Extractive ,pg 172-174,

262. Neha Arya & Hemant kumar/Int.J.Res. Ayurveda Pharm.8(5),2017, Comparative physicochemical profile of Vatsanabh (Aconitum ferox, Ranunculaceae) mula processed through cow's urine and cow's milk.

263.(The Ayurvedic Pharmacopoeia of India part 2, volume 3 first edn 2010reprint January 2011, 2.4.1pg 174-175&180

264.A Comparative experimental evaluation of toxicological effects of Shodhita and Ashodhita Hingula in Albino Rats by Ambujakshi V M, Dissertation submitted to Rajiv Gandhi University of Health Sciences Karnataka Bengaluru.

265. Meghana Pathak et al /A toxicity study of Pippali (Piper Longum) fruit. International Journal of Pharmaceutical & biological Archives2013; 4(1):89-93

266. S.L.Deore etal/journal of young Pharmacists5(2013)3-6

267. PK Sarkar etal Evalution of Effect of Shodhana treatment on pharmacological activities of Aconite, Ind J Pharm Edu Res Jul-Sept,2012/Vol46/Issue3

268. (Sujatha K and Rashma Saokar Role of Bhavana process in the antipyretic effect of Hinguleshvara Rasa, Europian Journal of Biomedical and Pharmaceutical Sciences 2019, vilm 6, Issue 8,451-455)

269 MI Li[1],LI Yu-Chen *etal*

" A systematic review of pharmacological activities, toxicological mechanisms and pharmacokinetic studies on *Aconitum* alkaloids".*Chin J Nat Med*, 2021, **19**(7): 505-520. doi: 10.1016/S1875-5364(21)60050-X

270 Karambelkar Sharadini A, Savrikar Shriram S,Vatsanabha (Aconite)-A review, world journal of Pharmaceutical Research Vol.2,issue5,2022.

271 Karambelkar Sharadini A, Savrikar Shriram S, Hinguleshvara Rasa-A review IAMJ April 2022.

ASSESSMENT OF ANTIPYRETIC AND ANALGESIC ACTIVITY OF HINGULESHVARA RASA IN EXPERIMENTAL ANIMALS

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80 RECOMMENDATION

This study was aimed to assess antipyretic and analgesic activity *of Hinguleshvara Rasa*. Primary objective of this study included preparation of *Hinguleshvara Rasa*, physicochemical analysis and characterization of *Hinguleshvara Rasa*. Physicochemical parameters like total ash, moisture content, thin layer chromatography etc of *Hinguleshvara Rasa* were studied. Secondary objectives included the study of all three ingredients of *Hinguleshvara Rasa* and *Hinguleshvara Rasa* itself for an assessment of their antipyretic as well as analgesic activity.

The study was conducted in three steps 1. Preparation of *Hinguleshvara Rasa*, 2. Physicochemical analysis and 3. Assessment of Antipyretic and Analgesic activity in experimental animals.

Following conclusions can be drawn from the first step of study i.e. Preparation of Hinguleshvara Rasa:

1. *Hinguleshvara Rasa* was prepared with *Shuddha Hingula (*Red Cinnabar: HgS), *Shuddha Vatsanabh* (*Aconitum balfourii* Linn) *and Pippali (Piper longum* Linn) in equal proportion (250 g each) using method described in the classical text *Bhaishajya Ratnavali Jvara chikitsa (*5/72). The weight of final product was observed reduced by 14 g. (1.86%) may be due to loss incurred during manual handling in processing.
2. It was also observed that the formulation ingredients 20.72% of Hingula gets lost during its wet grinding in ginger juice during its shodhana process.
3. Approx 34.12% of Vatsanabh is lost during its *shodhana* in Gomutra(Cow urine) .

Following conclusions can be drawn from the physicochemical testing

1. Reduction of number of spots during comparison of Thin layer chromatographs of *Ashuddha and Shuddha Vatsanabha* indicating that some of the phytochemical ingredients are lost during its *shodhana.*
2. Aconitine content of Vatsanabha was observed markedly reduced following its *shodhana.*
3. While total alkaloid content was observed increased due to its processing with cow urine.

4. Increase in the Particle size of formulation ingredients was observed after comparison of the ingredient before and after the wet grinding. This was due the the coating of the *Bhavana Dravya* on the particles of the ingredients as well as due to agglomeration of the particles.
5. The product *Hinguleshvara Rasa* was found to contain alkaloids Piperine (0.059%) and total alkaloids (o.48%). The Aconitine content in *Hinguleshvara Rasa* was observed to be 0.0022%. The observation concludes that even after *shodhan of Vatasnabha*, some amount of Aconitine remains in *Vatsanabh*, which is transferred to the final product *Hinguleshvara Rasa*. Which underlines the importance of caution to be observed during its therapeutic use.

Following conclusions can be drawn from the experimental study of *Hinguleshvara Rasa*

1. Acute oral toxicity study of *Shuddha Hingula, Shuddha Vatsanabh and Hinguleshvara Rasa* was performed as per OECD-423 guidelines. No toxic effects in the form of mortality or pathological changes in organs were observed following oral administration of ***Hinguleshvara Rasa*** in therapeutic dose in the experimental animals.
2. It can be concluded on the basis of antipyretic study that although in initial period the antipyretic activity of the standard drug Paracetamol is more pronounced than that of the test drug ***Hinguleshvara Rasa***. However, the activity is almost comparable in later stages (24 hrs) with the standard drug Paracetamol. Still, it can be said that the test drug ***Hinguleshvara Rasa*** can't substitute the standard drug Paracetamol as far as its antipyretic activity is considered.
3. Writhing test, Hot plate analgesia and Formalin induced licking tests were utilized for testing analgesic activity of the test drug. On the basis of these tests, it can be concluded that the analgesic effect of ***Hinguleshvara Rasa*** is as good as the standard drug Diclofenac for Writhing test and Formalin test, Pentazocin for Hot plate Test. On the basis of this study, it is concluded that ***Hinguleshvara Rasa*** is having significant antipyretic and analgesic activity. However, although its antipyretic activity is not comparable with the standard drug Paraetamol, ***Hinguleshvara Rasa*** is as effective as standard drugs Diclofenac and Pentazocin in it's analgesic effect.
4. It is also concluded that the ingredients ***of Hinguleshvara Rasa*** are found potent analgesic but weak in antipyretic effect.