"Comparative Physicochemical Characterization and *in vivo* anti-ulcer activity of Aamalaki Mashi"

A thesis submitted to

TILAK MAHARASHTRA VIDYAPEETH, PUNE

For the Degree of

DOCTOR OF PHILOSOPHY (Ph.D.)

Subject	**:**	**Rasa Shastra & Bhaishajya Kalpana**
Under Faculty of	**:**	**Ayurved**
Name of Candidate	**:**	**Vaidya Miss. Nilambari Mohan Patil**
Under the Guidance of Name of the Guide	**:**	**Prof. Dr. Vilas A. Dole**
Name of the Department	**:**	**Ayurved**
Month & Year	**:**	**December 2016**

DECLARATION

I hereby declare that the thesis entitled '**Comparative physicochemical characterization and *in vivo* anti-ulcer activity of Aamalaki Mashi'** completed and written by me has not previously formed the basis for the award of any degree or other similar title or any other university or examining body.

Place - Pune

Date – 15/12/2016

Vd. Miss. Nilambari Mohan Patil

CERTIFICATE

This is to certify that the thesis entitled, '**Comparative physicochemical characterization and *in vivo* anti-ulcer activity of Aamalaki Mashi'** which is being submitted herewith for the award of the Degree of Vidyavachaspati (Ph.D.) in Ayurveda of Tilak Maharashtra Vidyapeeth, Pune is the result of original research work completed by **Vd. Miss Nilambari Mohan Patil** under my supervision and guidance. To the best of my knowledge and belief the work incorporated in this thesis has not formed the basis for the award of any degree or similar title of this or any other University or examining body.

Place - Pune
Date – 15/12/2016

Prof. Dr.Vilas A.Dole
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Guru Sakshat Parah Bramhah Tasmai Shri. Gurave Namah!!"

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Research Scholar

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INTRODUCTION

Rasashastra and Bhaishajya Kalpana are the integral parts of Ayurveda which mainly deals with Ayurvedic pharmaceutics. In Ayurvedic pharmaceutics, use of herbal products, animal products, marine products and mineral products has been in used since time immemorial to prepare potent medicines. For the development of an excellent medicine, there must be alterations in the properties of the raw drug. These alterations are done for improvement and enhancement of therapeutic properties and minimizing the harmful effects. All these alterations can be achieved with the help of Sanskara like Toya sannikarsha, Agni, Saucha, Mantana, Desha, Kala, Vasana, Bhavana, Kalaprakarsha and Bhajana[1].

संस्कारो हि गुणान्तराधानमुच्यते । ते गुणास्तोयसन्निकर्षशौचमंथनदेशकालवासनभावनादिभिः

कालप्रकर्षभाजनादिभिश्चाधीयन्ते ।। च.वि.१/१२

Using all the above mentioned Sanskara, Panchavidha Kashaya Kalpana[2] viz. Swarasa, Kalka, Kwatha, Hima and Phanta are prepared. Though these five basic preparations are effective, there are some limitations like palatability, odor, color, shelf life, mode of action, etc. so as to overcome this problem, Acharyas using Yukti Pramana designed some derived formulations. These are Churna, Vati-Guti, Varti, Avaleha, Asava, Arishta, Kshara Kalpana, Siddha ghruta, Siddha tailam, Lepa, Malhara, etc.

Mashi Kalpana is one of the derived formulation mentioned in our ancient Ayurvedic texts which is obtained by incinerating the raw medicinal drug. Even though Acharya Charaka has mentioned about this Kalpana and its utility, nowadays there is very restricted use of this dosage form. Also, it is less known to common people and very

1. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Vimansthan 1/12, pp 233.

few Ayurvedic practitioners use it regularly in their practice. Presently, mainly Mashi formulations like Triphala Mashi, Aamalaki Mashi, Ela Mashi, Mayurpiccha Mashi are in use.

In today's date, it is really a virtuous approach in the field of Ayurvedic research that researchers should concentrate towards this overlooked dosage form.

Aamalaki Mashi in which the main ingredient is Aamalaki, inspite of no textual reference is in use since a long time in ethnomedical system in treating gastric disorders like Amlapitta (hyperacidity), Adhmana and Aanaha (abdominal distension). It was first prepared by Late Vaidya B.V. Gokhale. At present, one of the proprietary medicine of Rasashala 'Charcosal (Vatanashak)' contains Aamalaki Mashi as the main ingredient and is mainly used in treating flatulence, dyspepsia, indigestion, nausea and vomiting. This particular symptom complex can be seen in various gastro-intestinal disturbances and one such condition is peptic ulceration. Presently Acid peptic diseases are very commonly found of which gastric ulcers are predominantly seen. Gastric ulcers are a type of peptic ulcer disease which can be a serious gastrointestinal disorder requiring a well targeted therapeutic strategy. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence[3]. Currently there is no cost-effective treatment that meets all these goals. There are number of drugs in Modern medicine available for the treatment of these ulcers, but clinical evaluation of these drugs show higher incidence of relapse, side-effects and drug interactions. This has been the rationale for the development of new anti-ulcer drugs in which the herbal drugs can offer a better protection and decreased relapse.

Thus it is a logical approach to drug discovery by screening traditional herbal products instead of synthesized chemical molecules. So Aamalaki Mashi was undertaken as an archetype which may prove to be useful in treating acid peptic disorders. Also the pharmaceutical, analytical, toxicity and efficacy data of Aamalaki Mashi is not available till today. Taking into consideration this rationale, this hypothesis is put forth with an intention to standardize this formulation, obtain relevant toxicological data and prove its anti ulcer activity with animal experimentation. Scrutinizing the Ayurvedic drugs through established and

universally accepted quality control and toxicological protocols will help us to achieve better results and strengthen the faith of people in this precious and timeless medicinal science globally[4]. This can be useful in generating a systemic and scientific data of Aamalaki Mashi which can be further used as a reference for efficacy and clinical studies to describe newer therapeutic uses.

4. The Modern Ayurveda: Milestones Beyond the Classical Age edited by C. P. Khare, Chandra Kant Katiyar, Evaluation of efficacy and safety of herbal/Ayurvedic medicines by Arun Gupta and Chandrakant Katiyar, CRC press, version date: 20120119, pp325.

AIM AND OBJECTIVES

Aim:

To compare physicochemical characterization and *in vivo* anti ulcer activity of Aamalaki Mashi.

Objectives:

1. To standardize and validate the process of preparation of Mashi with Bahirdhuma and Antardhuma methods.
2. To analyze different samples of Mashi using standard physicochemical and analytical methods.
3. To evaluate safety profile of Mashi samples using standard *in vivo* toxicity tests.
4. To compare Mashi samples prepared by Bahirdhuma and Antardhuma methods using analytical and *in vivo* assays.

LITERATURE REVIEW

1] Mashi Kalpana

Mashi Kalpana is one of the formulations described in Ayurveda.

For the ease, literature study is divided into the following heads –

1) Samhita period
2) Sangraha period
3) Other medical sciences
4) Other relevant information

1) Samhita period [5,6,7,8] –

In Charak Samhita, Mashi is obtained by burning animal product like hair, hoof, horn, skin, etc. and is used in disorders like Shwasa and Kasa.

In Sushrut Samhita, Mashi is described as a formulation prepared by incinerating the skin, hair, hoof, horn, and bones of quadruped animals. Mashi is prepared by both the methods i.e. Bahirdhuma i.e. heating in open air and Antardhuma i.e. heating in very limited air supply.

In both these Samhita, Mashi is used for internal use as well as external applications. Kashyap Samhita has also mentioned Mashi formulation using drug material of animal origin.

5. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Chikitsasthan 10/40, pp 476.
6. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati PrakashanVaranasi. Chikitsasthan 17/119, pp 538.
7. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. Sushrutasamhita Chikitsasthna9/17, pp 444.
8. Kashyapa Samhita or Vruddhajivak Tantra by Vruddhajivak revised by Vatysa with Sansrit introduction by Pandit Hemraj Sharma with Vidyotini Hindi Commentary by Shri Satyapal Bhishagacharya, Chaukhamba Sanskrit Sansthana, Varanasi, Chikitsasthana Dwivraniya, pp 126.

2) Sangraha period [9,10]**–**

In Astanga Sangraha and Astanga Hridaya many similar references of Mashi have been noted. A wide range of drug material of plant, bird and animal origin like Triphala, Ashwaganda, elephant's tusk, peacock's feather, hoof, skin, and hair of quadruped animals are used for Mashi preparation by Bahirdhum and Antardhuma methods.

चतुष्पाच्चर्मरोमास्थिखुरश्रृंगोद्भवां मषीम् ।

तथैव वाजिगन्धाया लिह्यात् श्वासी कफोल्बणाः ॥ अ.स.चि.६/२०

These are used for external application as well as internally with proper Anupana. Kapala, an earthen utensil is specifically described to prepare Triphala Mashi.

Chakradutta[11] has described some Mashi formulations like Mrigashringa Bhasma in Hridroga, Hastidanta Mashi in Indralupta and Khalli, Pasharajju Mashi in Apasmara, etc. These preparations are used for both external and internal purpose.

Along with the use of herbal and animal origin drug material for Mashi preparation, Sharangdhara[12] has mentioned use of foodgrain like Yava i.e. Barley for Mashi preparation. In the preparation of Triphala Mashi, instead of Kapala, Kataha (utensil made from iron) is advised by Sharangdhara.

9. Shrimad Vruddhavagbhata Virachita Ashtang Sangraha, Indu Teeka, by Vd.Anant Damodar Aathavale, Chaukhamba Pratishtan, Varanasi, Chikitsa Sthana 6/41-42, pp 44.
10. Shrimad Vruddhavagbhata Virachita Ashtang Sangraha, Indu Teeka, by Vd.Anant Damodar Aathavale, Chaukhamba Pratishtan, Varanasi, Uttara Sthana 39/9, pp 215.
11. Cakradatta compiled by Mahamahopadhyaya Caraka Caturanan Srimaccakrapanidatta with Tattvacandrika explanation and Annotations of Sri Siwdas Sen, Chaukhamba Orientalia, Apasmara Chikitsa, Udbandhana Rajju Bhasma Prayoga,12,pp 260.
12. Sharangdhar Samhita by Pandit Sharagdharacharya son of Pandit Damodar with the Commentary Adhamalla Dipika and Kashirams Gudartha Dipika edited by PanditParshuram Shastri Vidyasagar, Chaukhamba Orientalia Varanasi, Uttar Khanda 11/109-110, pp 367-368

Yogaratnakar[13] and Bhaishajya Ratnavali[14] have described similar Mashi formulations. Different Mashi formulations are also found in Siddha Yoga Sangraha, Lanka Bhaishajya Manimala, Rasatantra Saar and Siddha Bhaishajya Manimala. Also, in Chikitsa Prabhakar many Mashi formulations used by ancient vaidyas have been noted.

3) Other Medical Sciences –

- **Pharmacological aspect-**

Medicinal charcoal is prepared by carbonizing wood, cellulose residues or coconut shells out of contact of air. Carbonizing dense woods produces the most effective gas absorbing charcoals in particular log wood, lignum vitae and coconut shells. Charcoal made by burning wood, coconut shell or even in an atmosphere containing minimum oxygen, acquires good adsorbent properties.

Adsorbents[15] are used in medicine to remove gases, toxins and poisons. Adsorption is a physical phenomenon exhibited by colloidal particles whereby substances are bound or made to adhere to the absorbent. The large surface area of the colloidal particle is responsible to thus attract and hold the unwanted substances like toxins, bacteria, noxious fumes and gases.

- **Yunani medicine**[16] **–**

Mashi Kalpana has been described in Yunani medicine. The word “Ehraka” or “Sokhta” are the synonyms used for Mashi. Both the methods of preparation i.e. Bahirdhuma and Antardhuma are mentioned. The source of raw material for preparation of Mashi is of plant, animal and mineral origin. Few of the examples are quoted below,

13. Yogaratnakara with Vidyotini Hindi Commentary by Vaidya Lakshmipati Shashtri edited by Bhishagratna Bramha Shankar Shashtri, Chaukhamba Prakashana, Varanasi, Kshudra Roga, Indralupta Chikitsa 2,pp 279
14. Bhaishajya Ratnavali of Shri Govind Das Vidyotini Hindi commentary by Shri Ambikadatta Shashtri, edited by Shri Rajeshwardatta Shashtri, Chaukhamba Prakashan, Varanasi, ,Kshudra Roga Chikitsa 60/40,pp 950.
15. Adsorption effect of activated charcoal on enterohemorrhagic Escherichia coli. Naka K1, Watarai S, Tana, Inoue K, Kodama Y, Oguma K, Yasuda T, Kodama H. 2001 Mar;63(3):281-5, PMID: 11307928
16. Yunani Dravyaguna Darshan, part 1- Hakim Daljit Singh, Ayurvedic and Tibbi Academy, U.U Lucknow, 1972.

Aababil Muharakh – Mashikrita Aababil

Kahruva Sokhta – Mashikrita Trunakanta Mani

Marjan Sokhta – Mashikrita Praval Shankha

Sangabasari – Mashikrita Karpara

Mashikrita Katran

Mashikrita Hastidant

Sarpa Mashikrita

Kachuva Mashikrita

4. Other relevant Information –

In ancient times, Mashi was used to prepare a particular kind of black ink. This ink was prepared by mixing with water and gum in a specific proportion. Many of the manuscripts of the ancient times were written with this ink. The famous poet Sant Kabir Das had quoted about Mashi[17] in one of his poem.

सात समुन्दर की मषी कसें लेखनी सब बनराई ।

धरनी सब कागज करो हरिगुन लिखें न जाई ।।

17. Santkabirdas.blogspot.com

II] Conceptual study of Aamalaki

Origin and importance[18]:

Skanda purana states that once the whole earth was submerged into the water and Lord Brahma was immersed in meditation of Parabrahma (Almighty Vishnu). He became so full of emotions that tears started coming down from his eyes and fell down on the earth. The Aamalaki tree germinated from those tears. The Aamalaki tree had many branches and was full of Aamalaki fruits. Since Aamalaki tree was the tree to manifest itself on the earth, it is known as 'Adiroha' (pre-eminent tree).

Photo-1: Aamalaki Fruit



Botanical name: *Phyllanthus emblica* Linn

Syn. *Emblica officinalis* Gaertn

Natural order: Euphorbiaceae

Classical names: Vayasya, Vrishya, Tishyaphala.

Vernacular names:

English – Indian gooseberry

Hindi – Amla, Aonla

Marathi – Avala

Kannada – Nelli, Nelika

Bengali – Amla, Amulati

Botanical description[19]:

A small or medium sized deciduous tree.

Leaves – subsessile, closely set along the branchlets, distichous, narrowly linear, obtuse, having appearance of primate leaves.

18. www.indianscriptures.com/vedic-lifestyle/food-and-health/amla
19. Database on medicinal plants used in Ayurveda, Vol 3 – P.C Sharma, M.B Yelne and T.J Dennis, CCRAS, 2001,pp11-16

Flowers – greenish-yellow, in axillary fascicles on the leaf bearing branchlets, often on the naked portion below the leaves.

Fruits – fleshy, globose with obscure vertical furrow, pale yellow.
Seeds – trigonous and 6 in number

Distribution:

Throughout tropical and subtropical India, chiefly in dry deciduous forests, ascending to 1400 meters on the Himalaya, Chota Nagpur, Bihar, Orissa, West Bengal, North Circars, Deccan, Karnataka and in Western Ghats.

Parts used:

Root bark, stem bark, leaf, fruit and seed.

Action and Uses:

The root bark is astringent and is useful in ulcerative stomatitis and gastric ulcer. The bark is astringent and useful in gonorrhoea, jaundice, diarrhoea and myalgia. The flowers are cooling and aperient. The leaves are useful in conjunctivitis, inflammation, dyspepsia, diarrhoea and dysentery. The fruits are astringent, cooling, anodyne, carminative, digestive, laxative, alterant, alexeteric, aphrodisiac, diuretic, antipyretic, tonic and trichogenous. They are useful in diabetes, cough, asthma, bronchitis, headache, ophthalmic disorders, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, haematemesis, inflammations, anaemia, emaciation, hepatic disorders, jaundice, strangury, diarrhoea, dysentery, intrinsic haemorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and greyness of hair. Seeds are reported to be useful in asthma, bronchitis and biliousness.

Ayurvedic properties [20]**:**

वयस्यामलकी वृष्या जातीफलरसं शिवम् ।

धात्रीफलं श्रीफलं च तथामृतफलं स्मृतम् ॥

त्रिष्वामलकमाख्यातं धात्री तिष्यफलाऽमृता ॥३८॥

हरीतकीसमं धात्रीफलं किन्तु विशेषतः ।

रक्तपित्तप्रमेहघ्नं परं वृष्यं रसायनम् ॥३९॥

हन्ति वातं तदम्लत्वात्पितं माधुर्यशैत्यतः ।

कफं रुक्षकषायत्वात्फलं धात्र्यास्त्रिदोषजित् ॥४०॥

यस्य यस्य फलस्येह वीर्य भवति यादृश्यम् ।

तस्य तस्यैव वीर्य्येण मज्जानमपि निर्दिशेत् ॥ ४१॥

हरितक्यादि वर्ग भावप्रकाशनिघण्टु.

Rasa - Amla, Madhura, Kashaya, Tikta, Katu

Guna - Guru, Ruksha, Sheeta

Veerya - Sheeta

Vipaka - Madhura

Doshaghnata - Tridoshashamaka, especially Pittashamaka

Action on Mala – Anulomana

Action on Mutra - Mutrajanana

Rogaghnata - Paittikavikara, Daha, Paittika shirashoola, Mootravarodha, Netraroga, Khailtya, Palitya, Mastishka daurbalya, Drishtimandya, Indriya daurbalya, Aruchi, Trishna, Agnimandya, Vibandha, Yakridvikara, Amlapitta, Parinamashoola, Udarvarta, Udararoga, Arsha, Hridroga, Raktapitta, Raktavikara, Kasa, Shwasa, Yakshma, Shukrameha, Pradara, Garbhashaya daurbalya, Mootrakrichchhra, Paittika Prameha, Kushtha, Visarpa, Charmaroga, Jeerna Jwara, Kshaya, Daurbalya, Daha, Shotha

20. Bhavaprakashnighantu of Shri Bhava Mishra, commentary by Dr.K.C.Chunekar, edited by Dr.G.S.Pandey, Chaukhambha Bharati Academy, Haritakyadivarga, Amalaki 38-41,pp10.

Karma - Dahaprashamana, Chakshushya, Keshya, Medhya, Nadibalya, Balya, Rochana, Deepana, Anulomana, Amlatanashaka, Yakriduttejaka, Stambhana, Sransan, Hridya, Shonitasthapana, Kaphaghna, Vrishya, Garbhasthapana, Mootrala, Pramehaghna, Kushthaghna, Jwaraghna, Rasayana

Doses – Fruit powder – 3-6 gm.; Fresh juice – 10-20 ml.

Pharmacognosy:

Root Bark - It occurs in pieces, 4-8 cm long, 2-4 cm broad, curved. Outer surface rugged, often scaly without any lenticels, colour greenish-sepia to mouse-grey with cracks and fissures, with recurved edges and irregular wrinkles. Fracture fibrous to granular. Transverse section of root bark shows rectangular cork cells, outer cortex one layered, consisting of irregular parenchymatous cells with abundant starch grains, a few crystals and laticifers. Starch grains uniform in distribution, simple, oval, hilum and lamellae indistinct. Crystals are of two types – rhomboidal and rod shaped with arrow heads on both sides mostly distributed in secondary phloem and cortical tissue. Latex tube non articulated, frequently present in secondary phloem and cortex including ray cells. Phloem fibres present, having narrow apex. Phloem rays multiseriate.

Powder microscopy shows presence of rod shaped elongated crystal with arrow heads on both sides; fragments of fibres, cork cells and sclerotic nature of ray cells, starch grains.

Fresh fruit – Fruit Globose, 2.5-3.5 cm in diameter, fleshy, smooth with six prominent lines, greenish when tender, changing to light yellowish or pinkish when mature, with a few dark specks. Taste sour and astringent followed by delicately sweet taste. Transverse section of mature fruit shows an epicarp consisting of single layer of epidermis and 2-4 layers of hypodermis. Epidermal cells tubular in shape, covered externally with a thick cuticle and appear in surface view as polygonal. Hypodermal cells tangentially elongated, thick walled, smaller in dimension than epidermal cells. Mesocarp forms bulk of fruit consisting of thin walled parenchymatous cells with intercellular spaces, peripheral 6-9 layers smaller,ovoid or tangentially elongated while rest of the cells are larger in size, isodiametric and

radially elongated. Several collateral fibrovascular bundles are scattered throughout mesocarp consisting of xylem and phloem. Xylem fibres are elongated with narrow lumen and pointed end. Mesocarp contains large aggregates of numerous irregular silica crystals.

Dried mature fruit – Drug consist of curled pieces of pericarp of dried fruit. Bulk colour grey to black; pieces showing a broad, highly shrivelled and wrinkled external convex surface to somewhat concave, transversely wrinkled lateral surface, external surface shows few whitish specks, occasionally some pieces show a portion of stony testa. Texture rough, cartilaginous, tough. Taste sour and astringent. Transverse section of fruit shows epicarp consisting of single layered epicermis, cell appearing tabular and polygonal in surface view cuticle present. Mesocarp cells are tangentially elongated, parenchymatous and crushed, differentiated roughly into a peripheral 8 or 9 layers of tangentially elongated smaller cells, rest consisting of mostly isodiametric larger cells with walls showing irregular thickenings. Ramified vascular elements occasionally present. Stone cells present either isolate or in small groups towards endocarp. Vascular fibres pitted, walls appearing serrated due to the pit canals leading into lumen.

Fine powder shows epidermis with uniformly thickened straight walls, isodiametric parenchyma cells with irregular thickened walls, occasionally short fibres and tracheids.

Physical constituents –

a. Root bark – Total ash – 13.6 %; Acid insoluble ash -0.61 %; Alcohol soluble extractive – 7.1 %; Water soluble extractive – 72 %.
b. Fresh fruit – Foreign matter – Not more than 2 % ; Total ash – Not more than 7 %; Acid insoluble ash – Not more than 2 %; Alcohol soluble extractive – Not more than 40 %; Water soluble extractive – Not less than 50 %; Moisture content – Not less than 80 %.
c. Dried mature fruit – Foreign matter (seed and seed coat) – Not more than 3 %; Total ash – Not more than 7 %; acid insoluble ash – Not more than 2 %; Alcohol soluble extractive – Not less than 40 %; Water soluble extractive – Not less than 50 %.

Chemical Constituents -

A good source of vitamin C; carotene, nicotinic acid, riboflavin, D-glucose, D-fructose, myoinositol and a pectin with D- galacturonic acid, D- arabinosyl, D-xylosyl, L-rhamnosyl, D-glucosyl, D-mannosyl and D-galactosyl residues, embicol, mucic, Indole acetic acid and four other auxins – a1, a3, a4 and a5, two growth inhibitors- R1 & R2, phyllembic acid and phyllembin (fruits) and fatty acids (seed oil); leucodelphinidin, procyanidin, 3-0-gallated prodelphinidin and tannin (bark); ellagic acid, lupeol, oleanolic aldehyde and 0-acetyl oleanolic acid (root); tannins, polyphenolic compounds; 1, 2, 3, 6-trigalloyglucosem terchebin, corialgin, ellagic acid, alkaloids, phyllantidine and phyllantine (leaves & fruits).

Pharmacological Activities:

Spasmolytic, mild CNS depressant, hypolipidaemic, antiatherosclerotic, antimutagenic, antimicrobial, antioxidant, immunomodulatory, antifungal, antitumour, hypoglycaemic, anti-inflammatory, antibacterial, antiulcer, adrenergic potentiating, HIV -1 reverse transcriptase inhibitory action.

Toxicology:

Active crude alcoholic extract of plant was assayed for cellular toxicity to fresh sheep erythrocytes and found to have no cellular toxicity.

Therapeutic Evaluation:

During a clinical trial, an Ayurvedic preparation, Aamalaki Rasayana (chiefly consisting of *P. emblica* fruits), on 39 cases of duodenal ulcer and 21 cases of non-ulcer dyspepsia, was found to be very effective in 72 % patients with no recurrence of signs and symptoms for a period of 6 months to 3 years of follow up observations.

A clinical study was conducted on 25 patients of type II diabetes with a herbomineral proprietary preparation, of which fruit of *Phyllanthus emblica* was one of the constituents.The patients were administered 2 tablets 3 times a day in addition to regular sulphonyl ureas over a period of 6 weeks. It showed improvement in glycaemic parameters viz. fasting blood glucose, post lunch blood glucose and fructosamine levels. This indicates that the compound preparation can be useful

adjuvant in poorly controlled type II diabetes. The drug contained 19 ingredients, viz., 12 minerals and 6 herbals.

Clinical studies were conducted to investigate the effect of Aamalaki (*Phyllanthus emblic*) fruits on 20 patients of Amlapitta (acid gastritis). The drug was administered in doses of 3 gm 3 times in a day for 7 days. It was found effective in 85 % cases. It did not show any beneficial effect in cases of hypochlorhydria.

In a clinical trial a compound herbal preparation (S.G.I. Shwitradi lepa), of which *Phyllanthus emblica* was one of the ingredients, proved useful as an external application in vitiligo.

In a preliminary clinical trial on patients of bleeding piles, oral administration of multiherbal composite drug containing *Berberis aristata, Holarrhena antidysenterica, Picrorrrhiza kurroa, Mesua ferra, Terminalia chebula, Terminalia belerica and Emblica officinalis* to 22 patients showed significant decrease in bleeding and constipation by fourth week of therapy.

Formulation and Preparations:

Brahmarasayana, Chyavanaprasha, Dhatrilauha, Dhatrirasayana, Triphala churna, Amalakyavaleha, Amalakyadi Kwatha, Phalarishta, Dhatryarishta.

Propagation and Cultivation:

Phyllanthus emblica grows best on well drained fertile loamy soil. It thrives well on light as well as heavy soils. It is found even on moderately alkaline and acidic soils. Moderate warm climate favours for initiation of floral buds. It is usually propagated by seeds; it may also be propagated vegetatively by budding, cutting and inarching. Seedlings can be best raised in nursery by sowing mature seeds directly in polythene bags. Two seeds are dibbled in each bag. The seeds are collected in the month of April or May. Freshly collected fruits stored in tins give 70 to 80 % germination till three months. Adequate watering is necessary to keep the soil moist until the seedlings have put forth four or five leaves. The highest percentage of germination (92.5 %) has been attained by presowing treatment of seeds with 500 ppm GA3 for 24 hours. Planting is done without disturbance to root system in month of June or July.

The soil is mixed with 5-10 kg farm yard manure. In order to protect the attack of termite 5% BHC powder is mixed in the FYM. Pruning of the bearing plants should be done after the end of fruit bearing season. The young plants require watering at fortnightly interval, particularly till they are fully established. Watering of mature, bearing plants is also necessary from April to June at bi-weekly interval to secure higher fruit set and reduced fruit drop. Irrigation during October to December at 20 days intervals helps in better development of fruits. The tree generally starts bearing fruits after 6 to 8 years. The plant is reported to be affected by blue mold (caused by Penicillium islandicum) and fruit and leaf rust (caused by Revenellia emblicae).

III. AYURVEDIC PERSPECTIVE OF ACID PEPTIC DISEASES

Amashaya [21,22]-

The word Amashaya is composed of two words-

Ama - undigested food,

Ashaya - cavity, Akasha Mahabhuta pradhana.

Definition

१. नाभिस्तनान्तरं जन्तोरामाशय इति स्मृतः । च. वि.२/१७

२. यत्राशितपीतादिकं पच्यते स आमाशयः । सु.सू.२१/६ डल्हण

The organ located between Nabhi and Stana is called Amashaya. According to Dalhana the organ where the Ashita (eatables), Peeta (in liquid form) etc. are digested is known as Amashaya.

Matruja Avayava[23]

मातृज अवयव–.....आमाशयाश्च... । च. शा. ३/६

Amashaya is one of the Matruja Avayava.

Koshthanga [24]**-**

पञ्चदश कोष्ठाङ्गानि; तद्यथा–.....आमाशयश्च.... । च. शा. ७/१०.

Charakacharya has described Amashaya as one of the Koshthanga among the fifteen Koshtangas.

21. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Vimansthan 2 / 17, pp 240.
22. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. Sutrasthan. 21 / 6 Dalhan. pp 100.
23. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Sharirsthan 3 / 6, pp 310.
24. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Sharirsthan 7 / 10, pp 338.

Sthana [25,26,27,28,29,30,31]**-**

१. स्वेदो रसो लसीका रुधिरमामाशयश्च पित्तस्थानानि, तत्राप्यामाशयो विशेषेण पित्तस्थानम्... । च. सू.२०/८

पित्तस्थानेषु आमाशय इति आमाशयाधोभागः, श्लेष्मस्थानेष्वामाशय आमाशयोर्ध्वभागः । चक्रपाणि.

२. पक्वामाशयमध्यं पित्तस्य; आमाशयः श्लेष्मणः । सु.सू.२१/६

३. नाभिरामाशयः स्वेदो..... । अ.ह्र.सू.१२/२

आमाशयो–नाभेरुर्ध्वमपक्वान्नस्थानम् । हेमाद्रि

४. उरःकण्ठशिरःक्लोमपर्वाण्यामाशयो रसः । अ.ह्र.सू.१२/३

Amashaya is the site of Pitta and Kapha Dosha. Chakrapani has divided it into two parts i.e. Urdhwa Amashaya which is the region of Kapha and Adho Amashaya which is the region of Pitta.

पाचक पित्त–

...तत्र पक्वामाशयमध्यगम् । अ.ह्र.सू.१२/१०

Pitta which is situated between Pakvashaya and Amashaya is known as Pachak Pitta.

रंजक पित्त–

आमाशयाश्रयं पित्तं रञ्जकं रसरञ्जनात् । अ.ह्र.सू.१२/१२

25. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Sutrasthan 20 / 8, pp 113.
26. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. Sutrasthan 21 / 6, pp 100.
27. Ashtanga Hrudaya of Vaghbhata with commentaries 'Sarvangasundara' of Arundatta & 'Ayurvedrasayana' of Hemadri, annotated by Dr. Anna Moreshwar Kunte and Krshna Ramchandra Shastri Navre, edited by Pt Hari Sadashiva Shastri Paradkara Bhishgacharya, Chaukhamba Surbharati Prakashana, Varanasi. Sutra sthan 12 / 2, pp 192.
28. Ashtanga Hrudaya of Vaghbhata with commentaries 'Sarvangasundara' of Arundatta& 'Ayurvedrasayana' of Hemadri, annotated by Dr. Anna Moreshwar Kunte and Krshna Ramchandra Shastri Navre, edited by Pt Hari Sadashiva Shastri Paradkara Bhishgacharya, Chaukhamba Surbharati Prakashana, Varanasi. Sutrasthan 12/3, pp 192.
29. Ashtanga Hrudaya of Vaghbhata with commentaries 'Sarvangasundara' of Arundatta & 'Ayurvedrasayana' of Hemadri, annotated by Dr. Anna Moreshwar Kunte and Krshna Ramchandra Shastri Navre, edited by Pt Hari Sadashiva Shastri Paradkara Bhishgacharya, Chaukhamba Surbharati Prakashana, Varanasi. Sutrasthan 12/10, pp193
30. Ashtanga Hrudaya of Vaghbhata with commentaries 'Sarvangasundara' of Arundatta& 'Ayurvedrasayana' of Hemadri, annotated by Dr. Anna Moreshwar Kunte and Krshna Ramchandra Shastri Navre, edited by Pt Hari Sadashiva Shastri Paradkara Bhishgacharya, Chaukhamba Surbharati Prakashana, Varanasi. Sutrasthan 12/ 13, pp194.
31. Ashtanga Hrudaya of Vaghbhata with commentaries 'Sarvangasundara' of Arundatta & 'Ayurvedrasayana' of Hemadri, annotated by Dr. Anna Moreshwar Kunte and Krshna Ramchandra Shastri Navre, edited by Pt Hari Sadashiva Shastri Paradkara Bhishgacharya, Chaukhamba Surbharati Prakashana, Varanasi. Sutrasthan 12/16, pp 194.

Pitta located in Amashaya imparts red colour to Rasa and hence is known as Ranjaka Pitta.

क्लेदक कफ–

…यस्त्वामाशयसंस्थितः ।।

क्लेदकः सोऽन्नसङ्घातक्लेदनात्… । अ.ह्र.सू.१२/१६

Amashaya is the region of Kledaka Kapha which moistens the ingested food, disintegrates, breaks and liquefies it.

Srotasa [32,33]**-**

१.अन्नवहानां स्रोतसामामाशयो मूलं वामं च पार्श्वं,……। च.वि.५/८

२. अन्नवहे द्वे, तयोर्मूलमामाशयोऽन्नवाहिन्यश्च धमन्यः…… । सु.शा.९/१२

Amashaya is the moolsthana of Annavahasrotasa.

Kala [34]**-**

षष्ठी पित्तधरा; या चतुर्विधमन्नपानमामाशयात् प्रच्युतं पक्वाशयोपस्थितं धारयति । सु.शा.४/१८

षष्ठी पित्तधरा नाम पक्वामाशयमध्यस्था, सा ह्यन्तरग्न्यधिष्ठानतयाऽऽमपक्वाशययोर्मध्ये चतुर्विधमन्नं बलेन विधार्य पित्ततेजसा शोषयन्ती पचति ।। अ.सं. शा.५/

Ashaya[35]**-**

आशयास्तु…आमाशयः,… । सु.शा.५/८

Sushrutacharya has mentioned Amashaya as one of the Ashaya.

Snayu[36]**-**

आमपक्वाशयान्तेषु बस्तौ च सुषिराः खलु । सु.शा.५/३२

Amashaya is comprised of Sushir Snayu.

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32. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Vimansthana 5/8, pp 250.
33. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. Sharir 9/12, pp 386.
34. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. Sharir4/18, pp 356.
35. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. Sharir 5 / 8, pp 364
36. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. Sharir 5 / 32, pp 361.

Peshi[37]**-**

द्वे हृदयामाशययोः.... । सु.शा.५/३७

Peshis are circular and longitudinal in Urdhwa and Adho Amashaya.

Dhamani[38]**-**

धमनी– (अधोगामी)

आमपक्वाशयान्तरे च त्रिधा जायन्ते...। सु.शा.९/७

Disease[39]**-**

द्वे रोगानीके आशयभेदेन–आमाशयसमुत्थं, पक्वाशयसमुत्थं चेति । च.वि.६/३

आमाशयसमुत्थेन आमाशयाश्रयाः कफपित्तजाः सर्वे गदा गृह्यन्ते...। चक्रपाणि

There are two groups of diseases according to site of origin- diseases originated from Amashaya and that from Pakvashaya.

Digestion[40,41,42]**-**

तत्र, आमाशयः पित्ताशयस्योपरिष्टात् तत्प्रत्यनीकत्वादूर्ध्वगतित्वात्तेजसः, चन्द्र इव आदित्यस्य, चतुर्विधस्याहारस्याधारः;स च तत्रौदकैर्गुणैराहारः प्रक्लिन्नो भिन्नसंघातः सुखजरो भवति ॥

माधुर्यात् पिच्छिलत्वाच्च प्रक्लेदित्वात्तथैव च ॥

आमाशये संभवति श्लेष्मा मधुरशीतलः ॥ सु.सू.२१/१२–१३

Amashaya is situated above the Pittashaya where it receives the four types of food. In Amashayathe food moistened by the aqueous qualities, gets disintegrated and thus is digested easily.

37. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. Sharir 5/37, pp 368.
38. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. Sharir 9/7 384.
39. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Vimansthana 6/3, pp252.
40. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. Sutrasthan 21/13, pp102.
41. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Chikitsa 15/10, pp512.
42. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Vimansthana 2 / 17 – 18, pp 240.

Due to Madhurya (sweetness), Picchilatva (sliminess) and Prakledatva (moistening) Madhur (Sweet) and Sheeta (cold) gunatmak Shleshmana, is produced in Amashaya.

अम्ल अवस्थापाक–

परं तु पच्यमानस्य विदग्धस्याम्लभावतः ।

आशयाच्च्यवमानस्य पित्तमच्छमुदीर्यते ।। च.चि.१५/१०

आशयात् आमाशयात् । चक्रपाणि

Amla Avasthapaka occurs in Amashaya. In this stage there is a secretion of Accha Pitta (clear Pitta).

अशितं खादितं पीतं लीढं चात्र विपच्यते ।

आमाशयगतः पाकमाहारः प्राप्य केवलम् ।

पक्वः सर्वाशयं पश्चाद्धमनीभिः प्रपद्यते ।। च.वि.२/१७–१८

Ashita (eatable), Khadita (chewable), Peeta (in form of liquids), and Leedha (lickables) food are digested in Amashaya. Thereafter it reaches to all the organs through Dhamani (blood vessels).

Samprapti [43,44,45,46]

The symptom complex mentioned in acid peptic diseases[47] which are mostly seen in peptic ulcers like abdominal pain, nausea, water brash, vomiting, loss of appetite and weight loss. Complications include bleeding, perforation and obstruction in the digestive tract can be correlated with many such similar symptoms which are akin to those mentioned in ancient Ayurvedic texts like Pittaja Gulma, Parinam Shula, Urvadja Amlapitta, Chardi, Annadrava Shula, etc.

43. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Sutrasthana 18/29, pp 107.
44. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Chikitsa 5/6-7, pp435.
45. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Nidan 3/8-9, pp209.
46. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Chikitsa 5/12-13, pp436.

Here for illustration purpose, Samprapti of Pittaja gulma has been explained.
In Charak Sutrasthana, Trishothiya Adhyaya, the definition of Gulma is mentioned.

यस्य वायु: प्रकुपितो गुल्मस्थानेऽवतिष्ठते ।
शोफं सशूलं जनयन् गुल्मस्तस्योपजायते ।। च.सू.१८/२९

When the vitiated Vata is located in the regions of Gulma, it causes swelling and pain which is known as Gulma.

While describing Gulma, Charak has mainly described Vataj Gulma. In context to Pittaj and Kaphaj Gulma, Charak has emphasized "तैरेव तु कर्शनै: कर्शितस्य" (Cha. Ni. 3/8-11). This signifies if a person is already emaciated (Karshit) due different factors of vitiation of Vata and still is exposed to etiological factors which aggravates Pitta and Kapha Doshas, can finally land up into Pittaja and Kaphaj Gulma respectively.
On this basis, Samanya Samprapti of Gulma is described as follows

कफं च पित्तं च स दुष्टवायुरुद्धूय मार्गान् विनिबद्धय ताभ्याम् ।
ह्रन्नाभिपार्श्वोदरबस्तिशूलं करोत्यथो याति न बद्धमार्ग: ।।
पक्वाशये पित्तकफाशये वा स्थित: स्वतन्त्र: परसंश्रयो वा ।
स्पर्शोपलभ्य: परिपिण्डितत्वाद्गुल्मो यथादोषमुपैति नाम ।। च.चि.५/६–७

Vitiated Vata is the important factor in all types of Gulma. Mahasrotas is already occupied with Rukshadi Gunas of Vata and this leads to Kha Vaigunya which is mainly responsible for manifestation of disease. In this Mahasrotas, Kupit / Akupit circulating (Paribhraman) Kapha and Pitta get sited (Sthana Sanshraya) and are responsible for formation of Shotha which is known as Gulma.

Pittaja Gulma Samprapti –

तैरेव तु कर्शनैः कर्शितस्याम्ललवणकटुकक्षारोष्णतीक्ष्णशुक्तव्यापन्नमद्यहरितकफलाम्लानां विदाहिनां च शाकधान्यमांसादीनामुपयोगादजीर्णाध्यशनाद्रौक्ष्यानुगते चामाशये वमनमतिवेलं संधारणं वातातपौ चातिसेवमानस्य पित्तं सह मारुतेन प्रकोपमापद्यते ।।

तत् प्रकुपितं मारुतं आमाशयैकदेशे संवर्त्य तानेव वेदनाप्रकारानुपजनयति, य उक्ता वातगुल्मे; पित्तं त्वेनं विदहति कुक्षौ हृद्युरसि कण्ठे च; स विदह्यमानः
सधूममिवोद्गारमुद्गिरत्यम्लान्वितं;गुल्मावकाशश्चास्य दह्यते दूयते धूप्यते ऊष्मायते स्विद्यति क्लिद्यति शिथिल इव स्पर्शासहोऽल्परोमाञ्चश्च भवति;
ज्वरभ्रमदवथुपिपासागलतालुमुखशोषप्रमोहविड्भेदाश्चैनमुपद्रवन्ति;हरितहारिद्रत्वङ्नखनयनवदनमूत्रपुरीषश्च भवति; निदानोक्तानि चास्य नोपशेरते, विपरीतान्युपशेरत इति पित्तगुल्मः ।। च.नि.३/८–९

कटवम्लतीक्ष्णोष्णविदाहिरुक्षक्रोधातिमद्यार्कहुताशसेवा ।
आमाभिघातो रुधिरं च दुष्टं पैत्तस्य गुल्मस्य निमित्तमुक्तम् ।।
ज्वरः पिपासा वदनाङ्गरागः शूलं महज्जीर्यति भोजने च ।
स्वेदो विदाहो व्रणवच्च गुल्मः स्पर्शासहः पैत्तिकगुल्मरुपम् ।। च.चि.५/१२–१३

If a previously emaciated person continuous etiological factors of Pitta like – consumption of Amla, Lavan, Katu, Kshara, Ushna, Tikshna, Shukta, Vyapanna Maddya, Haritaka, Amla Phala; Vidahi Shaka, Dhanya, Mansa, etc.; consumption of food during indigestion, excessively induced emesis in Ruksha Aamashaya, suppression of natural urges for a long time, exposure to extreme sun and wind, in this condition Pitta gets vitiated along with Vata.

Mahasrotas is already occupied with Rukshadi Guna of vata and this leads to kha vaigunya. In this mahasrotas, kupit circulating (paribhraman) pitta get sited (sthana sanshraya) and is responsible for formation of Shotha which is known as Pittaja Gulma. If the person continues with these etiological factors, it leads to formation of Vrana in Amashaya.

Charaka has mentioned the "Vata-pittaja mahasrotascha Vrana" in the Gulma chapter. According to him, any Shotha occuring in the Mahasrotasa due to vitiation of pitta by itself (Swatantra) or by any other cause(Paratantra) can be Gulma. He has excluded the Kapha caused Gulma here. It is mentioned in the Charak Samhita that Various Pittakara Ahara and Vihara are the Hetu of Pittaja Gulma. It has been mentioned in the lakshana that Shula occurs during the digestion process of food. This Shotha is with vrana and with tenderness at site.

Aamalaki Mashi has been hypothesized to treat the acid peptic condition therefore taking into consideration the various experimental *in vivo* models for gastro-intestinal disorders and their availability, ethanol induced gastric ulcers model in animals was used so as to know the efficacy of Aamalaki Mashi and accordingly plan it's study in humans. If Aamalaki Mashi shows remarkable results in the gastric ulcers in animals, then it can be an encouragement to think of its use at an appropriate stage in humans.

There is a difference between treating induced gastric ulcer in animals and gastric ulcer in humans due to improper dietary habits. The induced ulcer in animals is a one-time procedure whereas the improper food consumption in humans is an on-going process. The continuous irritation to Gastric tissue due to those food substances causes gastric ulcer. This process cannot be stopped until and unless the causative factors are avoided completely.

Gastric ulcer gets resolved within a stipulated time in animals and recurrence is very rare as it is artificially induced. Whereas in humans it gives a temporary relief when the diet is corrected but it should be followed strictly so as to get a long term relief.
In humans, the Nidan Parivarjan concept along with psychosomatic condition should also be considered as it's a well-known fact that Hurry, Worry and Curry are responsible for peptic ulceration.

Ayurveda has various drugs which act on the Pitta causing the Gastric ulcer viz. Kamadudha, Yashtimadhu Ghruta, etc. Aamalaki Mashi is in use in ethno-medical system for treating flatulence, dyspepsia, vomiting, etc. Therefore, an attempt has been made to evaluate the efficacy of Aamalaki Mashi in gastric ulcer condition.

IV] MODERN PERSPECTIVE OF ACID PEPTIC DISEASE

"Acid peptic disease[47]" is a collective term used to include many conditions such as gastro-esophageal reflux disease (GERD), gastritis, gastric ulcer, duodenal ulcer, esophageal ulcer, Zollinger Ellison Syndrome (ZES) and Meckel's diverticular ulcer. The commonest ulcers are the gastric and the duodenal ulcer.

Symptoms of peptic ulcers include abdominal pain, nausea, water brash, vomiting, loss of appetite and weight loss. Complications include bleeding, perforation, obstruction in the digestive tract and sometimes cancer.

For present study, *in vivo* anti-ulcer study is considered therefore literature review regarding peptic ulcers has been done

Ulceration[48] - This refers to a site of inflammation where an epithelial surface (Skin, gastric epithelium, colonic mucosa, bladder epithelium) has become necrotic and eroded, often with associated subepithelial acute and chronic inflammation. This can occur as a consequence of toxic or traumatic injury to the epithelial surface (eg- peptic ulcers). The peptic ulcer of the stomach or duodenum illustrates the typical features. There is usually an early intense neutrophilic infiltrate with associated vascular dilatation. In chronic lesions where there has been repeated insult, the area surrounding the ulcer develops fibroblastic proliferation, scarring and accumulation of chronic inflammatory cells.

Stomach - The main layers of the stomach from outside to inside are as follows -

a) Mucus membrane.

b) Mucosa.

c) Muscularis mucosae.

d) Submucosa.

47. www.medindia.net/diseaseinfo

48. S. Das, *A Concise textbook of surgery*, 3/e, 2004, Ch.44

Various types of Gastric disorders are the cause of different diseases viz. chronic gastritis, peptic ulceration, etc. Bacteria like Helicobacter pylori are usually a cause of gastrointestinal infection. At times, congenital anomalies are also seen.

Commonly seen symptoms - initially heartburn and vague epigastric pain. In case of the breach of gastric mucosa and bleeding, hematemesis or melena may be seen. The stomach lumen generally has acidic environment and hence the blood if present quickly congeals and turns brown.

The damaging causes for gastric mucosa are mainly Helicobacter pylori, urease, toxins, gastric acidity, peptic enzymes and drugs.

PEPTIC ULCER[49]

Overview-

Occurrence of any ulcer in the digestive system is known as peptic ulcer. Pepsin is normally present with hydrochloric acid in stomach lining.

There are 2 main types according to site of peptic ulcer -

a) Gastric

b) Duodenal

Rare peptic ulcers may be seen in the following -

i) Cardiac end of Oesophagus.

ii) Meckel's diverticulum (due to presence of ectopic gastric mucosa).

iii) In any segment of bowel (anastomotic ulcer) which has been surgically anastomized to gastric fundus.

Another classification is -

a) Acute ulcers - They are usually shallow and multiple. The most common causative factor is consumption of NSAIDs and sometimes stress and hence are also called as 'Stress ulcers'. Sepsis and undrained pus are also seen as aetiological factors. Patients on steroids may also develop acute ulcers called 'Steroid ulcer'.

49. Kumar et al, *RobbinsBasic Pathology,* 7/e (Elsevier, Pennsylvania, 2005 Ch.2

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b) Chronic ulcers -They are usually single, deep and schirrous. They occur in any part of the gastrointestinal tract exposed to aggressive action of acid/pepsin juices.

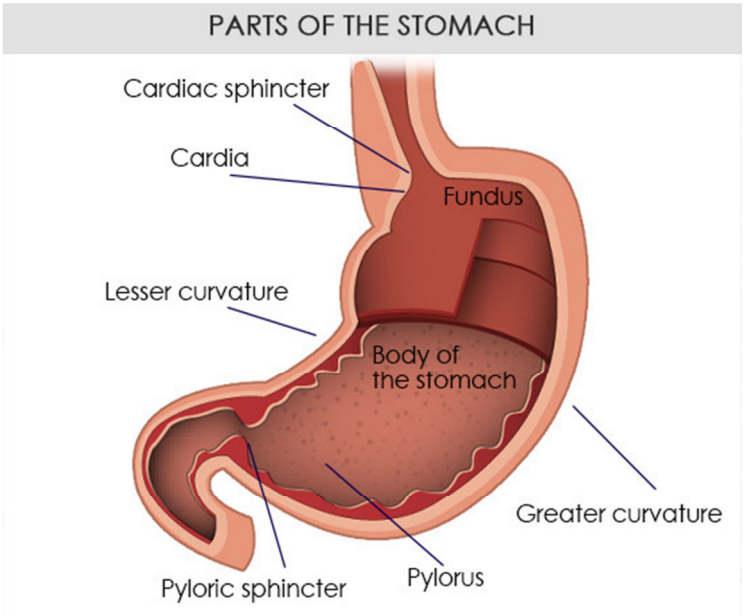
GASTRIC ULCER - These ulcers occur in the stomach. The patients in this case secrete normal or below normal amount of acid. Very few patients may demonstrate acid hypersecretion.

Pathophysiology of Gastric ulcer -

Stomach - It is a very important and active part of the gastrointestinal tract and metabolism of the human body.

Anatomically the stomach is divided into five main regions viz. Cardia, Fundus, Body or Corpus, Antrum and Pyloric sphincter.

Fig. 1: Parts of the Stomach



The wall of stomach (gastric wall) mucosa, submucosa, muscularis propria, serosa. The two compartments of normal gastric mucosa are -

a) Foleolar – It is leaflike and hence the name and is the superficial compartment.

b) Deep Glandular compartment.

The foleolar compartment is relatively uniform throughout the stomach whereas the deep glandular compartment exhibits major differences in thickness in different regions of stomach. This compartment consists of gastric glands which vary according to the different regions of stomach. These are-

i. The Cardia glands - consists of mucous secreting cells.

ii. Oxyntic glands - The term Oxyntic means acid forming. Also called as gastric or fundic glands are found in fundus or body and contain parietal cells, chief cells and scattered endocrine cells.

iii. Antral or Pyloric glands - They contain the mucous secreting cells and endocrine cells. The Mucous cells secrete mucuos and pepsinogen I and II.

The parietal cells have the vesicles on apical membrane which contain the proton pump. A unique mechanism of hydrogen -potassium -ATPase (H+, K+, ATPase) pumps hydrogen across membranes in exchange for Potassium ions. The parietal cells also do the function of secreting the intrinsic factor.

iv. Chief cells - They secrete the proteolytic proenzymes I and II. Pepsinogens are activated to pepsin at low luminal pH and it is inactivated above pH value 6.0 at the duodenum.

v. Endocrine or enteroendocrine cells- These produce histamine which binds to Histamine- 2 (H2) receptor on the parietal cells resulting in an increase in acid production. They are also referred as 'enterochromaffin like cells' (ECL cells). D cells (producing somastosatin) and X cells (producing endothelin) from the gastric mucosa are also included in ECL cells. They play an important role in modulating acid production.

Gastric mucosal physiology:

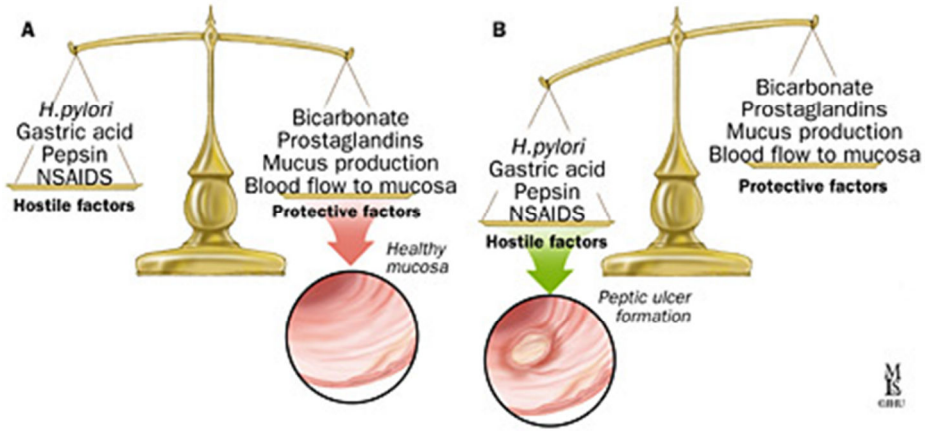
There are two main factors in Ulcer formation. They are as follows -

1) Aggressive (Hostile).

2) Defensive (Protective).

The integrity of the upper gastrointestinal tract depends upon hostile factors such as gastric acid, H. pylori, NSAIDs and Pepsin and protective factors such as prostaglandins, mucuos, bicarbonates and blood flow to mucosa affecting the mucosa in the gastrointestinal tract.

Fig. 2: The Balance of Aggressive and Defensive factors in peptic ulcer formation.



There is a well-known dictum 'no acid, no ulcer'. Indeed, inhibition of acid secretion by pharmacological blockade of histamine H2 receptor using drugs viz. cimetidine or ranitidine relieves a majority of peptic ulcers. Removal of acid facilitates healing of ulcer. However Histamine -H2 antagonists relieve the ulcer but are not a solution to the underlying cause. Recurrence of ulceration after the treatment cessation is common.

Acid secretion - The Gastric physiology mainly depends on the secretion of Hydrochloric acid which is divided in their phases-

1) Cephalic phase- It is mediated by vagal activity.
2) Gastric phase- It is mediated by vagal impulses and also involves gastrin release from endocrine cells, G cells in the antral glands.
3) Intestinal phase - It involves a number of polypeptides besides gastrin. The signals converge on gastric parietal cell and activation of proton pump takes place.

Acetylcholine released from cephalo-vagal and gastro-vagal afferents stimulate the parietal cell and cause subsequent activation of proton pump.

Oxyntic gland and ECL cell plays a central role. Gastrin and vagal afferents induce release of histamine from ECL cell and thus stimulating the H-2 receptor on parietal cell.

Mucosal protection - The mucosal barrier protects the gastric mucosa from autodigestion. The gel like gastrin mucuos barrier is composed of different biochemical constituents. Mucus mainly contains water, bicarbonate, proteins and lipids. The lipids bound to gastric mucosal surface from excess luminal H+ exposure and oxygen radical attack.

Bicarbonate secretion - It is secreted in the stomach at a basal rate amounting to 5% to 10% of maximal acid output. Prostaglandins are potent stimulants of gastric and duodenal bicarbonate secretion and are known for cytoprotective properties of these groups of compounds.

Mucosal blood flow - If the vascular perfusion is sufficient, the epithelial damage does not progress to necrosis of deep layers of mucosa. The rapid repair mechanism is termed 'restitution' and involves migration of healthy epithelial cells from gastric pits to denuded basement membrane.

A protective layer of mucous over the denuded region is called as 'Mucoid cap'. The basement membrane is highly sensitive to damage by acid, this protection is crucial for restitution to take place. Prostaglandins play a significant role in maintenance of mucosal blood flow during period of epithelial repair.

Prostaglandin synthesis- Prostaglandins favour production of mucuos and bicarbonates and inhibit acid secretion by parietal cells. They also improve the mucosal blood flow by their vasodilatory action of prostaglandin E and I.

Their stimulatory actions for secretions of mucuos and bicarbonates strengthen the mucuos-bicarbonate barrier.

Nitric oxide (NO) in GI tract - Nitric oxide plays a vital role in various physiological functions of GI tract including mucosal blood flow, maintenance of mucosal integrity and vascular tone.

Main causes of Gastric ulcer -

1) Diminished mucosal resistance - This is due to lowering of ability to resist the effect of acid pepsin digestion.
2) Pyloro-duodenal reflux - Regurgitated bile and other duodenal juices are the main cause of pre-ulcerative superficial gastritis. Some gastric ulcers occur in the fundus or at the cardia.
3) Deficient mucous barrier - Normally a surface layer of mucous protects the digestive effect of the hydrochloric acid and pepsin. If this mucous barrier becomes deficient, gastric ulcer may develop.
4) Mucosal trauma - About a major number of gastric ulcers occur at the lesser curve of the stomach (Magenstrasse) as this part is more exposed to injurious effects of heat and trauma.
5) Local ischemia - Arteriovenous shunts which are present in the submucosa of the stomach are under control of sympathetic nervous system. Excessive stress may cause diminished blood supply to the mucous membrane of stomach leading to ulcer formation.
6) Non-Steroidal Anti-inflammatory Drugs (NSAIDs) - The drugs of this group are called 'Ulcerogenic drugs'. They seem to disrupt the prostaglandin driven support of mucosal barrier. Prostaglandins are related to production of mucosal gel layer which provides a protective barrier to gastric and duodenal lining.
7) Helicobacter pylori - It is a gram negative spirochete bacterium which exists in the deep mucosal layer of antrum. H. pylori secrete a number of enzymes and chemicals which reduce the viscosity of mucous. It was formerly known as Campylobacter pylori organism. It has a strong capability of urease production. It splits Urease into Urea and releases cytotoxic Ammonia (NH_3) can be a cause of increased acidity enabling it to survive.

Epidemiology-

In case of Gastric ulcer, it is found to have normal or below normal decreased acid secretion which is often associated with decreased mucosal defence. Gastric ulcers tend to be large, comparatively heal slowly and prone to recur.

Pharmacological treatment of Gastric Ulcer-

The recent medicinal treatment involves use of medicines with H-2 blockers and Proton pump inhibitors (PPI). Also, NSAIDs which are a known cause should be avoided.

The mechanism of drugs is-

A) To counteract aggressive factors like gastric acid, pepsin, bile acid, irritant food ingredients, exposure to NSAIDs.

B) To stimulate mucosal defences by mucous, bicarbonates, facilitate blood flow, prostaglandins (PG), Nitric oxide (NO),etc.

Reduction in Gastric acid secretion -

1) H2 antihistamines- Eg- Ranitidine, Famotidine, Roxatidine.

2) Proton pump inhibitors - Eg- Omeprazole, pantoprazole, Lansoprazole.

3) Anticholinegics - Eg- Propantheline, Pirenzepine, Doxepine.

4) Prostaglandin analogues - Eg- Mizoprostol, Enprostil.

Neutralization of Gastric acid -

1) Systemic - Eg- Sodium bicarbonate, Sodium citrate.

2) Non-systemic - Eg- Magnesium hydroxide, magnesium carbonate, magnesium trisilicate.

Ulcer protective -

Eg- Sucralfate, colloidal bismuth subcitrate.

Ulcer healing drugs -

Eg- Carbenoxolone sodium, deglycyrrhinised Loquorice.

Anti H. pylori drugs -

Eg- Amoxicillin, Clarithomycin, Metranidazole, Tinidazole, Tetrcycline.

Ayurved is an ancient Indian science and various herbs and minerals are described in it for various diseases. Some of the constituents of the specialized treatments procedures like rejunevation therapy (Rasayana) have also shown to possess anti-ulcer charateristics. They have also exhibited protective effect. Gastro-protective / anti-ulcer properties of most commonly used herbs include flavanoids (viz. quercetin, naringin, silymarin, anthcyanosides, sopharadin, derivatives), saponins, tannins, gums and mucilages.

V] TOXICOLOGICAL STUDIES[50]

Toxicological screening methods and toxicological research on individual substances developed in the mid-1900s, and environmental toxicological studies developed in the mid-20th century.

The use of animals in toxicity studies began in 1920, when J. W. Trevan proposed the use of the 50% lethal dose (LD50) test to determine the lethal dose of individual chemicals. After the introduction of LD50, a FDA scientist John Draize developed a method for testing eye and skin irritation using rabbits, and this method was widely accepted for testing the effects of chemicals and pharmaceuticals on the eye and skin. Later, the US National Cancer Institute (NCI) developed a test to identify carcinogenic chemicals through the daily dosing of rats and mice for 2 years. In the early 1960s, thousands of babies were born with debilitating birth defects caused by thalidomide. After this, all the regulatory agencies concentrated on determining the toxicity profiles of all pharmaceutical substances available for regular patient use and made mandatory the submission of toxicity profiles of investigational new drugs (IND). In the late 1980s, the Organisation for Economic Co-operation and Development (OECD) and the International Conference on Harmonization (ICH) brought out the guidelines for toxicity testing of pharmaceutical substances.

Before conducting any toxicological testing in animals or collecting tissue/cell lines from animals, the study should be approved by the Institute Animal Ethics Committee (IAEC) or the protocol should satisfy the guidelines of the local governing body. The guidelines for conducting experiments and regulatory requirements vary from region to region.

In India, the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines should be followed for the maintenance of experimental animals. In India along with OECD guidelines, Schedule Y toxicity

50. Toxicological screening S Parasuraman, J Pharmacol Pharmacother, April-June 2011, 2(2):74-79, PMCID:PMC3127354

guidelines are followed for toxicity studies. The pre-clinical toxicology unit of NIN in association with CCRAS has designed protocol for Bhasmas which includes acute and long term testing as per international guidelines (WHO/OECD).

There are different types of toxicity studies as laid down by OECD, USFDA, etc. These are acute oral toxicity testing, acute toxicity testing for inhalation, acute toxicity testing for topical preparations, skin sensitization tests, repeated dose toxicity testing, mutagenicity testing, subchronic oral toxicity testing, chronic oral toxicity testing, carcinogenicity testing, one-generation reproduction toxicity testing, two-generation reproduction toxicity studies, toxicokinetics, neurotoxicity studies in rodents, developmental toxicity/embryotoxicity studies and genetic toxicity testing.

VI] EXPERIMENTAL MODELS FOR ANTI ULCER ACTIVITY *IN VIVO*

There are several models that are used to evaluate antiulcer medicines[51,52]. However, the choice of a suitable model has proven to be difficult as each model has significant advantages as well as disadvantages. The choice of a particular model is sometimes influenced by local resources, the objectives of the study, the hypothesis being tested, or research questions being answered by the researcher. The choice of model may also depend on the relevance to the type of peptic ulcer disease under investigation. Generally, preclinical experiments should be carried out *in vivo* and supported, when possible, with *in vitro* studies to explore the mechanisms of action of drug candidates with antiulcer activity.

Some challenges associated with the various models for peptic ulcers are that, apart from information on their pathophysiological and biochemical bases being scanty, they are also scattered in the literature and not easy to find. These models can serve as tools that could help to better understand the pathophysiological mechanisms, such as antisecretory, gastro-cytoprotective, gastro healing and antioxidant mechanisms underlying medicines or agents that have antiulcer effects.

Peptic ulcers can be induced by physiological, pharmacological or surgical manipulations in several animal species. However, most experiments in peptic ulcer studies are carried out in rodents. Several models are used experimentally for testing or evaluating antipeptic ulcer activity of drugs/agents, and these include the following:

(i) water-immersion stress or cold-water-restraint or cold-restraint stress,
(ii) NSAIDs- (indomethacin, aspirin, and ibuprofen) induced gastric ulcers,
(iii) ethanol-induced gastric ulcers,
(iv) acetic acid-induced gastric ulcers,
(v) histamine-induced gastric ulcers,

51. H. Gerhard Vogel (Ed) Drug Discovery and Evaluation: Pharmacological Assay, 3rd edition, 1236 Chapter J – Activity on Gastrointestinal Tract.
52. Ulcers, Volume 2013 (2013), Article ID 796405, 12 pages, *In Vivo* Models Used for Evaluation of Potential Antigastroduodenal Ulcer Agents- Michael Buenor Adinortey,Charles Ansah, Isaac Galyuonand Alexander Nyarko, http://dx.doi.org/10.1155/2013/796405

(vi) reserpine-induced gastric ulcers,
(vii) serotonin-induced gastric ulcers,
(viii pylorus-ligated-induced peptic ulcers,
(ix) diethyldithiocarbamate- (DDC)-induced peptic ulcers,
(x) methylene blue-induced ulcers,
(xi) ischemia-reperfusion- (I-R-) induced gastric ulcers,
(xii) cysteamine-induced duodenal ulcers,
(xiii indomethacin-histamine-induced duodenal ulcers,
(xiv ferrous iron-ascorbic acid-induced gastric ulcers,
(xv) acetic acid-*H. pylori*-induced ulcers.

All *in vivo* animal models can be used to investigate the preventive or curative properties of medicines or drugs depending on the time of induction of the peptic ulcer. For preventive studies, it is advisable to pretreat animals for at least two weeks before the ulcerogen is administered to induce the peptic ulcer, after which measurement of the degree of ulcers is taken with the appropriate index to determine the extent of ulcer prevention achieved. In the case of healing or curative models, the ulcers are induced after which the animals are treated for at least two weeks followed by measurement of the extent of ulcers with the appropriate index to determine the degree of healing of the ulcers.

1. Pylorus Ligation in Rats (SHAY Rat)

A simple and reliable method for production of gastric ulceration in the rat based on ligature of the pylorus has been published by Shay et al. (1945). The ulceration is caused by accumulation of acidic gastric juice in the stomach. The model is useful for evaluating the effects of anti-secretory drugs that reduce secretion of gastric aggressive factors such as acid and pepsin. The model is also useful for assessing the cytoprotective effects of drugs that increase secretion of mucus.

[

2. Indomethacin Induced Ulcers in Rats

Nonsteroidal anti-inflammatory agents, like indomethacin and acetyl-salicylic acid, induce gastric lesions in man and in experimental animals by inhibition of gastric cyclo-oxygenase resulting in less formation of prostacyclin, the predominant prostanoid produced in the gastric mucosa. This phenomenon has been employed in

the development of NSAIDs-induced gastric ulcer models in rats. The model is important in investigating the potential usefulness of anti-secretory and cytoprotective agents since the underlying pathophysiology involves gastric acid secretion and mucosal prostaglandin synthesis.

3. Ethanol Induced Mucosal Damage in Rats (Cytoprotective Activity)

Intragastric application of absolute ethanol is a reproducible method to produce gastric lesions in experimental animals (Robert et al. 1979; Szabo et al. 1981). These lesions can be at least partially inhibited by various drugs, such as some prostaglandins. The protective effect against various irritants has been called cytoprotective activity (Robert 1979; Robert et al. 1979).

4. Water-Immersion-Stress-Induced Ulcer Model

Various physical and psychological stressors cause gastric ulceration in humans, and rat models have been developed to mimic the disease condition in humans. This model employs the restraint technique developed by Brodie and Hanson coupled with the cold-water or ordinary-water immersion method by Levine. The combination of these methods is reported to be synergistic in inducing acute stress lesion in rats, arising mainly from physiological discomfort. Gastric ulcers induced by cold-water-restraint stress (CWRS) or cold-restraint stress (CRS) or water-immersion stress (WIS) in rats or mice are known to resemble human peptic ulcers, both grossly and histopathologically. The model is widely used and is reported to be useful for assessing or studying the effects of agents/medicines on the healing of ulcers in rats.

5. Acetic Acid-Induced Gastric Ulcers

One of the least understood aspects of peptic ulcer is the chronicity of the disease that is characterized by repeated episodes of healing and re-exacerbation, a phenomenon which is a challenge to both patients and physicians. Most experimental ulcerative lesions heal quickly in a few days without scar formation and do not re-ulcerate spontaneously. Takagi et al.developed a model for inducing chronic gastric ulcer in rats by means of submucosal injection of acetic acid and reported on the healing process of lesions for extended intervals after the ulcer preparation. The experimental gastric ulcer was considered as chronic due to its persistence for a long time and resemblance to human chronic ulcer both grossly and histologically.

The acetic acid model is suitable for chronic peptic ulcers. It is used to evaluate the effect of potential drugs or to test materials on the healing process of chronic peptic ulcers and also to screen for their antisecretory and cytoprotective effects.

6. Histamine-Induced Gastric Ulcer

Gastric ulceration is mediated by several factors including the release of histamine, and this is the basis for the histamine model for producing ulcers. Histamine does not only enhance gastric acid secretion, but it also causes disturbances of the gastric mucosa, microcirculation, abnormal motility, and reduction in mucus production. The mechanism by which histamine induces gastric ulcers is through its potent acid stimulating and vasodilating capability, which leads to increased vascular permeability. These pharmacological effects of histamine underlie the histamine-induced ulcer model and hence its usefulness in evaluating antisecretory effects of potential drugs against ulcers and agents that function as H2-receptor antagonists.

7. Reserpine-Induced Peptic Ulcer

Scientists have also used reserpine to induce ulcers. Reserpine-induced gastric ulceration has been attributed to the degranulation of gastric mast cells consequent to liberation of histamine, believed to be mediated by the cholinergic system.

8. Serotonin-Induced Gastric Ulcer

Serotonin, which has also been used to induce ulcers, is known to cause vasoconstriction, thereby reducing gastric mucosal blood flow (GMBF) resulting in acute mucosal injury.

9. Diethyldithiocarbamate-Induced Gastric Ulcer

The diethyldithiocarbamate model is used to assess the antioxidant activities of drugs in the prevention of gastric damage. This model is also used to assess the cytoprotective actions of potential drugs. Diethyldithiocarbamate has been reported to induce antral lesions through the mobilization of superoxide and hydroxyl radicals. Superoxide radical and hydroxyl radicals play a pathogenic role in the induction of this ulcer.

10. Methylene Blue-Induced Ulcer

Methylene blue (MB), a synthetic drug is known to uncouple ATPases and generate superoxide radical ions. MB has been used as an ulcerogenic agent to study antiulcer agents and their mechanisms of action. It can be used as a pharmacological tool to screen various antiulcer agents, which modulate the H+/K+ ATPase system. When administered to animals, MB induces gastric and duodenal lesions. The compound also inhibits nitric oxide synthase activity and hence reduces the bioavailability of nitric oxide. Additionally, MB has affinity for acetylcholine or muscarinic receptors and has been reported to inhibit cholinesterase activity. This implies that the model could be used for assessing antiulcer agents with anticholinergic effects and proton-pump inhibitory activity.

11. Ischemia-Reperfusion (I-R) Gastric Ulcer Model

The gastrointestinal (GI) mucosa is very sensitive to ischemia. Reperfusion of the GI following ischemia causes erosion and ulceration in the gastric mucosa due to the formation of free radicals.

12. Cysteamine-Induced Duodenal Ulcers

A duodenal ulcer in rats induced by cysteamine HCl was first described by Selye and Szabo. Cysteamine induced duodenal ulcer in rat has been widely used as a model of peptic ulcer disease. This chemically induced ulcer resembles duodenal ulcer in man with respect to its location, histopathology and some aspects of pathophysiology. Although the mechanism involved in ulcer production has not been fully elucidated, generally it is reported that Cysteamine stimulates gastric acid secretion rate and inhibits the alkaline mucus secretion from Brunner's glands in the proximal duodenum resulting in the formation of duodenal ulcer.

13. Indomethacin, Plus Histamine-Induced Duodenal Ulcer

Another method for inducing duodenal ulcers described by Takeuchi et al. involves administering indomethacin and histamine to rats. The development of duodenal lesions induced by indomethacin and histamine in rats is due to both an increase in gastric acid secretion and an impairment of acid-induced duodenal HCO3- secretion. This model for duodenal ulcers is useful for studying the pathogenesis of duodenal ulcers and for screening antiduodenal ulcer drugs or agents.

14. Ferrous Iron-Plus Ascorbic Acid-Induced Gastric Ulcer Model

This type of gastric ulcer model is induced by the local injection of ferrous iron with ascorbic acid (Fe/AS A) solution into the gastric wall. The ulcers produced resemble human gastric ulcers that penetrate the muscularis mucosa. Lipid peroxidation mediated by oxygen radicals plays a crucial role in the pathogenesis of the gastric ulceration induced by the Fe/AS A solution.

PREVIOUS WORK DONE

1. Pune University – Dissertation for M.D (Rasashastra)
   "Study of Mashi Kalpana with reference to the pharmaceutical and analytical assessment of Aamalaki Mashi." – Vaidya Nilambari M Patil (Dec 2007)
2. Evidence Based Complementary and Alternative Medicine. 2008 March - Exploring of Antimicrobial Activity of Triphala Mashi – an Ayurvedic Formulation – Yogesh S Biradar, Sheetal Jagtap, K R Khandelwal and Smita Singhania (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2249739/#)
3. Indian Journal of Traditional Knowledge Vol 6(2)-April 2007 -pp 292-297 - Preparation, method of optimization and physicochemical evaluation of traditional formulation, *Triphala Mashi* - Yogesh S Biradar, Piush Sharma & K R Khandelwal
4. Journal of Pharmaceutical and Scientific Innovation 2015 - Masi Kalpana : A novel dosage form – Hussain Gazala
5. Journal of Herbal Pharmacotherapy, Vol 7, 2008, Issue 3-4, Evaluation of Anti-Diarrhoeal Property and Acute Toxicity of Triphala *Mashi*, an *Ayurvedic* Formulation – Y S Biradar, R. Singh, K. Sharma, K. Dhalwal, S. L. Bodhankar & K. R. Khandelwal
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8. Pharmacognostical and pharmacological study of Kukkutanda twak Bhasma and Unripe Coconut Husk Mashi with reference to Ayurvedic Formulation Processing Techniques- Baheti, Akshay M, Issue date 23/02/2011, Published by University of Pune (dl.unipune.ac.in:8080/jspui/handle/123456789/5218)

RATIONALE FOR SELECTION OF AAMALAKI MASHI IN TREATMENT OF GASTRIC ULCER

Ayurveda considers human being as a whole. It believes that imbalance in the Dosha, Dhatu and Mala are responsible for manifestation of disease and the restoration of their balance helps to eliminate the disease. The main aim of Ayurveda is to maintain the health of a healthy person and to cure the disease. A huge number of formulations are being used to achieve the objectives of Ayurveda[53].

प्रयोजनं चास्य स्वस्थस्य स्वास्थ्यरक्षणमातुरस्य विकारप्रशमनं च ।। च.सू.३/२६

Rasashastra and Bhaishajya Kalpana are the integral parts of Ayurveda which mainly deals with Ayurvedic pharmaceutics. Various dosage forms in form of Kwatha, Asava, Avaleha, Tailam, Ghruta, Vati, Bhasma, Parpati, Pottali, etc are discussed in these subjects. As per the necessity various dosage forms are prepared but factors like dushyam, desham, balam, kalam, etc. are need to be taken into consideration and then only a particular preparation can be designed. For the development of such an excellent drug, there must be alterations in the properties of the drug. These alterations are done for improvement and enhancement of therapeutic properties and minimizing the harmful effects. All these alterations can be achieved with the help of Samskara like Toya sannikarsha, Agni, Saucha, Mantana, Desha, Kala, Vasana, Bhavana, Kalaprakarsha and Bhajana[1].

संस्कारो हि गुणान्तराधानमुच्यते । ते गुणास्तोयसन्निकर्षशौचमंथनदेशकालवासनभावनादिभि: कालप्रकर्षभाजनादिभिश्चाधीयन्ते ।। च.वि.१/१२

Using all the above mentioned Sanskara, Panchavidha Kashaya Kalpana[2] viz. Swaras,

53. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Sutrasthan 3/26, pp 26.
1. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Vimansthan 1/12, pp 233.
2. Charak Samhita of Agnivesha, elaborated by Charaka and Drudhabala with the Ayurvedadipika commentary by Chakrapanidatta, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashan Varanasi. Sutrasthan 4/7, pp 31.

Kalka, Kwatha, Hima and Phanta are prepared. The ideology behind the origin of these five pharmaceutical preparations is –

1. To make various dosage forms from a single drug
2. To obtain active ingredients of the drug.
3. To make easy assimilation in the body.
4. To achieve desired action.
5. To make drugs of different potencies.

Though these five basic preparations are effective, there are some limitations like palatability, odor, color, shelf life, mode of action, etc. so as to overcome this problem, the Acharyas designed some derived formulations using Yukti Praman.

Mashi Kalpana is one of the derived formulation mentioned in our ancient Ayurvedic texts which is obtained by Agni Sanskara on the raw medicinal drug. This dosage form is less known to common people and sometimes not considered by our Ayurvedic practitioners as compared with other dosage forms.

In our classical Ayurvedic texts, there are number of Mashi formulations mentioned which are prepared using herbal and animal product. There are mainly two methods designed for their preparation – Bahirdhuma and Antardhuma. Mashi can be prepared using a single drug or by combination of many drugs. To name a few, Ashwagandha, Ela, Mayurpiccha, Vacha are examples of Mashi prepared from single drug whereas Triphala (combination of Haritaki, Bibhitaki and Aamalaki) Mashi is an example of polyherbal combination. Triphala Mashi is a very commonly used formulation in treatment of Dant roga and Upadansha by Ayurvedic physicians. In todays date, it is really a virtuous approach in the field of Ayurvedic research that researchers have started concentrating towards this overlooked dosage form. There are many research papers published on Triphala Mashi dealing with its standardization, pharmacognostical profile, acute toxicity, anti microbial activity and anti diarhhoeal property. Along with the therapeutic uses mentioned in our ancient texts, this kind of research have helped to establish new therapeutic utilities of Triphala Mashi. Of the three individual drugs of Triphala, Aamalaki is one of the important drug.

Aamalaki, Embelica officinalis Gaertn (Family: Euphorbiaceae) is one of the traditional medicine which is extensively used in Ayurveda for its Rasayana property

as well as for treating diabetes, asthma, cough, hyperacidity, peptic ulcers, dyspepsia, anemia, inflammations, diarrhea, jaundice, skin diseases, greyness of hair, menorrhagia and cardiac disorders. The pharmacological activities previously reported are spasmolytic, antioxidant. hypolipidaemic, antiatherosclerotic, antimicrobial, immunomodulatory, hypoglycemic, antitumor and anti ulcer[19].

In our ancient Ayurvedic texts, there are specific norms assigned for a proper Aushadha (one of the factor of Paad Chatustya)[54] which along with the remaining three factors is responsible for treating diseased condition.

भिषक् द्रव्याण्युपस्थाता रोगी पादचतुष्टयम् ।
चिकित्सितस्य निर्दिष्टं प्रत्येकं तच्चतुर्गुणम् ॥ अ.ह्र.सू.१/२७

बहुकल्पं बहुगुणं सम्पन्नं योग्यमौषधम् ॥ अ.ह्र.सू.१/२८

As per these criteria, Aamalaki is Uttam Bheshaja[55] (ideal drug) since,

1. It is available in abundance, though it is a seasonal plant.
2. It is easily available.
3. It is cost effective.
4. It is used to cure disease, maintain health and has rejuvenating property.

Various dosage form prepared from the same drug exhibit different pharmacological action. Chavanprashavaleha containing Aamalaki as its main ingredient has rejuvenation property whereas Aamalaki Churna is mostly used in treating diabetes. Likewise oil prepared from Aamalaki is useful to stop premature greying of hairs.

19. Database on medicinal plants used in Ayurveda, Vol 3 – P.C Sharma, M.B Yelne and T.J Dennis, CCRAS, 2001,pp11-16
54. Ashtanga Hrudaya of Vaghbhata with commentaries 'Sarvangasundara' of Arundatta & 'Ayurvedrasayana' of Hemadri, annotated by Dr. Anna Moreshwar Kunte and Krshna Ramchandra Shastri Navre, edited by Pt Hari Sadashiva Shastri Paradkara Bhishgacharya, Chaukhamba Surbharati Prakashana, Varanasi. Sutrasthan 1/27, pp 16.
55. Ashtanga Hrudaya of Vaghbhata with commentaries 'Sarvangasundara' of Arundatta & 'Ayurvedrasayana' of Hemadri, annotated by Dr. Anna Moreshwar Kunte and Krshna Ramchandra Shastri Navre, edited by Pt Hari Sadashiva Shastri Paradkara Bhishgacharya, Chaukhamba Surbharati Prakashana, Varanasi. Sutrasthan 1/28, pp17.

Aamalaki Mashi in which the main ingredient is Aamalaki inspite of no textual reference is in use since a long time in ethnomedical system in treating gastric disorders like Amlapitta (hyperacidity), Adhmana and Aanaha (abdominal distension). It was first prepared by Late Vaidya B.V.Gokhale. At present, one of the proprietary medicine of Rasashala 'Charcosal (Vatanashak)' contains Aamalaki mashi as the main ingredient and is mainly used in treating flatulence, dyspepsia, indigestion, nausea and vomiting. Presently Acid peptic diseases are very commonly found of which gastric ulcers are predominantly seen. Gastric ulcers are a type of peptic ulcer disease which can be a serious gastrointestinal disorder requiring a well targeted therapeutic strategy. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence[3]. Currently there is no cost-effective treatment that meets all these goals. There are number of drugs in Modern medicine available for the treatment of these ulcers, but clinical evaluation of these drugs show higher incidence of relapse, side-effects and drug interactions. This has been the rationale for the development of new anti-ulcer drugs in which the herbal drugs can offer a better protection and decreased relapse.

Thus it is a logical approach to drug discovery by screening traditional herbal products instead of synthesized chemical molecules. So we undertook Aamalaki Mashi as an archetype which may prove to be useful in treating acid peptic disorders. Also the pharmaceutical, analytical, toxicity and efficacy data of Aamalaki Mashi is not available till today. Taking into consideration this rationale, this hypothesis is put forth with an intention to standardize this formulation, obtain relevant toxicological data and prove its anti ulcer activity with animal experimentation. This can be useful in generating a systemic and scientific data of Aamalaki Mashi which can be further used as a reference for efficacy and clinical studies to describe newer therapeutic uses.

MATERIALS AND METHODS

Materials used for the dissertation and methodology followed during the project work was as follows:

MATERIALS –

1) For the literature study –

In this section a critical review of relevant literature regarding Mashi Kalpana, Aamalaki, Aamalaki Mashi, gastric ulcers, *in vivo* toxicity studies and *in vivo* anti ulcer activity as per Ayurvedic and modern literature was carried out which covered all related material from Ayurvedic Samhita, internet, research papers, research thesis and all authenticate available sources to generate a strong foundation for the study.

2) For pharmaceutical study –
   i) Procurement of Aamalaki and its authentication
   ii) Aamalaki Churna
   iii) Aamalaki Mashi
   iv) Equipments –

   All the necessary equipments needed for the preparation of Aamalaki Churna like Swedana Yantra, L.P.G, aluminium vessel, dryer, hammer mill, sieves, etc.

   The equipment used for the preparation of Mashi can be grouped according to the preparation method.

   a. For Bahirdhuma method –

      Earthen pan (round shaped)

      Size - Depth = 5 cm

      Diameter = 24 cm

      Height = 5 cms

      Stirrer

      L.P.G

      Retort stand

      Glass thermometer

   b. For Antardhuma method –

      Two Sharava (round shaped)

      Size - Depth = 4 cm

Diameter = 26 cm

Height = 4.5 cm

For preparation of Sharava samputa – cloth pieces, fullers mud and water

Puta –Laghuputa - 22X22X22 cm

Cow dungs – total 4.5 in number

Retort stand

K Thermocouple

Table No.1- Dimensions of cow dungs

	I cow dung	**II cow dung**	**III cow dung**	**IV cow dung**	**V cow dung**
Diameter (cm)	25X25 cm	23X24 cm	27X25 cm	25X25 cm	23X25 cm
Thickness (mm)	2.5 cm	2.3 cm	2.7 cm	2.6 cm	2.45 cm
Weight (gms)	310 gms	305 gms	320 gms	310 gms	308 gms

3) For physico-chemical characterization –

All the necessary equipment and instruments required for the physico-chemical characterization and analytical tests were of standard quality.

4) Toxicity studies-

Acute and sub acute toxicity studies were conducted as per 423 (Appendix -1) and 407 (Appendix – 2) guidelines of OECD respectively. Experimental animals – Swiss Albino mice were procured from authenticate source. All the necessary kits required for the biochemistry and pathological investigations were of standard quality.

5) *In vivo* anti ulcer models-

"Ethanol induced mucosal damage in Rats" animal model was used for present study.

METHODOLOGY

I. Procurement of Aamalaki –

a. Collection of plant material

The fruits of Aamalaki (*Embelica officinalis*) were procured from local areas of Pune (Maharashtra, India) in the month of December 2014.

b. Identification and Authentication

The fruits of *Embelica officinalis* were identified and authenticated at IDRL, Pune, India as per the norms described in Indian Pharmacopoeia[56].

II. Preparation of Aamalaki Churna:

The preparation process was as follows-

a. **Preliminary Process:**

This comprises of –

i. Swedana
ii. Separation of seeds
iii. Drying
iv. Preparation of Churna

i) Swedana process-

Material – Aamalaki fruits and water.

Equipments – Swedana Yantra or Steaming apparatus and L.P.G

Procedure-

1) An aluminium vessel having capacity of 10 litres was filled with water. A clean cloth was tied on the mouth of the vessel. Aamalaki fruits were placed on the cloth and the vessel was closed with another similar vessel upside down.
2) This whole set up is called as SwedanaYantra.
3) This apparatus was subjected for heating using L.P.G.
4) Heating was done slowly and steaming of Aamalaki fruits was achieved.
5) While heating precaution was taken that the flame doesn't reaches up to the cloth.

6) After one and half hour, the gas was lit off; the drug material was taken out.
7) The fruits were properly steamed and it was very easy to separate them from the seeds naturally.

ii) Separation of seeds –

After steaming process, the seeds were extracted manually and the remaining bulky portion was further subjected to drying.

iii) Drying –

1) The bulky fresh portion of the fruit was transferred to a tray.
2) This tray was kept in a dryer. The temperature was adjusted at 40°C.
3) This process was done for 8 hours per day for total 3 days.
4) After proper drying the fruit appeared brownish coloured and were taken out from the dryer.
5) This dried material was further used in the preparation of Churna.

iv) Preparation of Churna

1) The dried Aamalaki was grinded in a hammer mill using five different sieves.
2) After grinding, the Churna obtained was transferred to mechanical shifter.
3) In mechanical shifter it was sieved using 100 meshes.
4) Finally, fine powder of Aamalaki was obtained leaving behind the coarse powder.
5) Aamalaki powder was weighed and stored in clean container.
6) This powder was further used as the raw material for the pharmaceutical work.

III. Preparation of Aamalaki Mashi-

In Ayurvedic texts, the methods described to prepare Mashi are Bahirdhuma and Antardhumaa. The main principal behind the process is to convert the raw material into carbon or black ash by application of heat.

A. Bahirdhuma[57, 58] **–**

Bahirdhuma = bahi + dhuma

Where, bahi means out

Dhuma means smoke

Bahirdhuma means to let the smoke go out.

This is a type of open and direct heating in which the raw medicinal drug is heated or roasted in open environment.

कपालेत्रिफलादग्धासघृतारोपणंपरम् । अ.ह्र.उ.३४/६

कपाले–खर्परे । इण्दुटीका

दहेत्कटाहेत्रिफलांसामषीमधुसंयुता । शा.सं.उ.११/१०६

This method comprises following steps-

i. The raw material i.e. of herbal or animal origin is taken in an open pan and is kept on medium low flame.
ii. The mixture is continuously stirred so that each and each particle of the material receives equal quantum of heat.
iii. During this process of combustion, smoke starts appearing at the beginning and the material starts blackening.
iv. At this stage, the typical odour of combustion can be identified.
v. Ultimately, when the whole material turns black and appearance of fumes is completely stopped, the process is assumed to be completed.

57. Ashtanga Hrudaya of Vaghbhata with commentaries 'Sarvangasundara' of Arundatta & 'Ayurvedrasayana' of Hemadri, annotated by Dr. Anna Moreshwar Kunte and Krshna Ramchandra Shastri Navre, edited by Pt Hari Sadashiva Shastri Paradkara Bhishgacharya, Chaukhamba Surbharati Prakashana, Varanasi. Uttarstana34/06 pp 194.
58. Sharangdhar Samhita by Pandit Sharagdharacharya son of Pandit Damodar with the Commentary Adhamalla Dipika and Kashirams Gudartha Dipika edited by Pandit Parshuram Shastri Vidyasagar, Chaukhamba Orientalia Varanasi, Uttar Khanda 11/16.

B. Antardhumaa[59] **–**

Antardhumaa = antar + dhuma

Where, antar means inside

Dhuma means smoke

Antardhumaa means entrapping the smoke inside a closed system.

This type of closed and direct heating in which the drug material is heated in a closed system using Sharava samputa. The system is designed in such a manner that the smoke caused due to the combustion of the medicinal drug gets entrapped within the system.

अन्तर्धूमदग्धानीतिएतेषांरोमाणिघटमध्येनिक्षिप्यमुखंपिधायदग्धानीत्यर्थः ।।

सु.उ. ५०/१९–२० (डल्हणटीका)

The steps of Mashi preparation using this method are as follows.

i. The raw drug material is placed in one Sharava and another Sharava is placed on it upside down.
ii. With plaster of mud cloth the area of contact of the two Sharava i.e. Sandhibandha, is sealed.
iii. After the sealing is dried properly, the Sharava samputa is heated by the application of Puta.
iv. The type of Puta or amount of heat to be given depends upon the nature of raw material used.
v. After the Sharava samputa becomes Swangashita i.e. it cools by itself and returns to room temperature, the Saravasamputa is taken out from the Puta and individual Sharava are separated carefully by opening the seal.
vi. The final product obtained is black colored and with the help of mortar and pestle it is triturated till the complete mixture becomes homogeneously black and fine.

59. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. uttaratantra 50/19-20, pp 760.

End point of the preparation process of Mashi –

i. The final product obtained after heating must be completely black colour like Kajjala[60] i.e. collyrium.

मषीकज्जलंविशेषं । शा.सं.उ.११/२५–२६ (दीपिकाटीका)

ii. The fumes coming during the heating process are completely ceased[61].

दग्धमनिर्गतधूमंमृगश्रृगंगोघृतेनसहपीतम् । चक्रदत्त २६ शूलचिकित्सा

- In the present work, the preparation of Aamalaki Mashi was done in two parts:

a. Pilot Work

b. Actual Pharmaceutical Work

a. Pilot work

A pilot work was carried out to explore a standard 'Pharmaceutical Protocol' for the present study.

Purpose –

1. To make a supportive framework for doing pharmaceutical work.
2. For the assessment of,
   - ✓ Approximate time required for the procedure.
   - ✓ Temperature during the procedure.
   - ✓ Equipments used for the procedure.
   - ✓ Amount of raw material.
   - ✓ Changes occurring the procedure
   - ✓ To find out the yield of final drug
   - ✓ Organoleptic characteristics of the final product.

60. Sharangdhar Samhita by Pandit Sharagdharacharya son of Pandit Damodar with the Commentary Adhamalla Dipika and Kashirams Gudartha Dipika edited by Pandit Parshuram Shastri Vidyasagar, Chaukhamba Orientalia Varanasi, Uttar Khanda) 11/25-26.

61. Cakradatta compiled by Mahamahopadhyaya Caraka Caturanan Srimaccakrapanidatta with Tattvacandrika explanation and Annotations of Sri Siwdas Sen, Chaukhamba Orientalia, Kshudra Roga,Vyanga Chikitsa,46.pp 597.

Of these, following things were considered before the initiation of preparation process.

1. Optimization of Quantity-
   Mashi was prepared with 50 gms and 100 gms of Aamalaki Churna. In practice, it is essential to know the output of the final drug obtained which depends on the amount of raw material.
2. Optimization of Equipment-
   Literary review revealed that formulations containing Amla dravya should not be prepared in iron wares so earthen wares were used for Mashi preparation. Broad bottom Sharava rounded shaped were used for Mashi preparation. Use of stirrer was done in Bahirdhuma method for uniform distribution of heat to all the particles of the raw material.
3. Optimization of Temperature-
   Uniform and controlled heating is to be given during Mashi preparation due to which the changes occurring during the process can be studied in the detail and also to avoid Kshara formation.

On this basis, a pilot study was conducted with 50 gms and 100 gms of Aamalaki Churna using rounded shaped Sharava for Mashi preparation by Bahirdhuma and Antardhumaa method.

I. Preparation of Aamalaki Mashi by Bahirdhuma method

A. Expt no. A 1

Title – Preparation of Aamalaki Mashi by Bahirdhuma method.

Principle – Open heating

Date – 15/10/2014

Ingredients – Aamalaki Churna = 50 gms

Equipments – Rounded earthen pan, stirrer, glass thermometer, retort stand, L.P.G, weighing machine and watch.

Procedure –

1. An earthen pan containing Aamalaki Churna was kept on gas burner.
2. With the help of retort stand, glass thermometer was inserted in the Churna without touching the bottom of the pan.

3. The gas burner was ignited and the flame was adjusted to medium low.
4. Continuous stirring was done till the Churna became completely black colored and the fumes coming from the Churna were ceased.
5. At this stage, the pan was kept aside away from the flame and the final product was transferred to a clean plate.
6. During the process and at the end of the experiment, all the relevant observations were noted.

B. Expt no A 2

Title – Preparation of Aamalaki Mashi by Bahirdhuma method.

Principle – Open heating

Date – 20/10/2014

Ingredients – Aamalaki Churna = 50 gms

Equipments – Rounded earthen pan, stirrer, glass thermometer, retort stand, L.P.G, weighing machine and watch.

Procedure – Same as Expt no A1

C. Expt no. A 3

Title – Preparation of Aamalaki Mashi by Bahirdhuma method.

Principle – Open heating

Date – 24/10/2014

Ingredients – Aamalaki Churna = 50 gms

Equipments – Rounded earthen pan, stirrer, glass thermometer, retort stand, L.P.G, weighing machine and watch.

Procedure – Same as Expt no A 1

D. Expt no. A 4

Title – Preparation of Aamalaki Mashi by Bahirdhuma method.

Principle – Open heating

Date – 10/11/2014

Ingredients – Aamalaki Churna = 100 gms

Equipments – Rounded earthen pan, stirrer, glass thermometer, retort stand, L.P.G, weighing machine and watch.

Procedure – Same as Expt no A 1

E. Expt no. A 5

Title – Preparation of Aamalaki Mashi by Bahirdhuma method.

Principle – Open heating

Date – 26/11/2014

Ingredients – Aamalaki Churna = 100 gms

Equipments – Rounded earthen pan, stirrer, glass thermometer, retort stand, L.P.G, weighing machine and watch.

Procedure – Same as Expt no A 1

F. Expt no. A 6

Title – Preparation of Aamalaki Mashi by Bahirdhuma method.

Principle – Open heating

Date – 05/12/2015

Ingredients – Aamalaki Churna = 100 gms

Equipments – Rounded earthen pan, stirrer, glass thermometer, retort stand, L.P.G, weighing machine and watch.

Procedure – Same as Expt no A 1

G. Expt no. A 7

Title – Preparation of Aamalaki Mashi by Bahirdhum method.

Principle – Open heating

Date – 16/12/2014

Ingredients – Aamalaki Churna = 100 gms

Equipments – Rounded earthen pan, stirrer, glass thermometer, retort stand, L.P.G, weighing machine and watch.

Procedure – Same as Expt no A 1

II. Preparation of Aamalaki Mashi by Antardhumaa method

A. Expt no. B 1

Title – Preparation of Aamalaki Mashi by Antardhumaa method.

Principle – Closed heating

Date – 02/12/2014

Ingredients – Aamalaki Churna = 100 gms

Equipments –

a. Preparation of Sharava samputa – Two Sharava, fuller's earth (multani mati), water and pieces of cloth.
b. Mashi preparation – Sharava samputa containing Aamalaki Churna, 8 cow dungs, Laghu Puta, Til Tail, match box, weighing machine and watch.

Procedure –

1. A Sharava containing Aamalaki Churna was taken and another Sharava was placed on it upside down.
2. A mixture of fuller's earth and water was prepared and a piece of cloth was dipped in it so that the entire cloth was properly loaded with the mixture.
3. This mud cloth was wrapped around the area of contact of the Sharava.
4. After the mud cloth was completely dried, another mud cloth prepared in the similar manner was wrapped around it. Total seven mud cloths were used for wrapping and sealing.
5. Five cowdungs cut into small pieces were placed at the bottom of the Laghu Puta, the Sharava samputa was placed over it and the remaining 3 cowdungs pieces were placed covering the sides and the top of Sharava samputa.
6. Some drops of Til Tailam were sprinkled over the cowdungs and with the help of a match stick the cowdungs were subjected to fire.
7. Sharavasamputa was kept for Swangashita and the observations were noted.

B. Expt no. B 2

Title – Preparation of Aamalaki Mashi by Antardhumaa method.

Principle – Closed heating

Date – 26/12/2014

Ingredients – Aamalaki Churna = 100 gms

Equipments –

a. Preparation of Sharava samputa- Two Sharava, fuller's earth (multani mati), water and pieces of cloth.
b. Mashi preparation – Sharava samputa containing Aamalaki Churna, 6 cow dungs, Laghu Puta, Til Tailam, match box, weighing machine and watch.

Procedure –

1. Repeat step 1 to 4 as in Expt no B 1.

2. Four cowdungs cut into small pieces were placed at the bottom of the Laghu Puta, the Sharava samputa was placed over it and the remaining 2 cowdungs pieces were placed covering the sides and the top of Sharava samputa.
3. Some drops of Til Tailam were sprinkled over the cowdungs and with the help of a match stick the cowdungs were subjected to fire.
4. Sharava samputa was kept for Swangashita and the observations were noted.

C. Expt no. B 3

Title – Preparation of Aamalaki Mashi by Antardhumaa method.

Principle – Closed heating

Date – 03/01/2015

Ingredients – Aamalaki Churna = 100 gms

Equipments –

a. Preparation of Sharava samputa- Two Sharava, fuller's earth (multani mati), water and pieces of cloth.
b. Mashi preparation – Sharava samputa containing Aamalaki Churna, 4 cow dungs, Laghu Puta, Til tailam, match box, weighing machine and watch.

Procedure –

1. Repeat step 1 to 4 as in Expt no B 1
2. Two and a half cowdungs cut into small pieces were placed at the bottom of the Laghu Puta, the Sharava samputa was placed over it and the remaining one and a half cowdungs pieces were placed covering the sides and the top of Sharava samputa.
3. Some drops of Til Tailam were sprinkled over the cowdungs and with the help of a match stick the cowdungs were subjected to fire.
4. Sharava samputa was kept for Swangashita and the observations were noted.

D. Expt no. B 4

Title – Preparation of Aamalaki Mashi by Antardhumaa method.

Principle – Closed heating

Date – 10/01/2015

Ingredients – Aamalaki Churna = 100 gms

Equipments –

a. Preparation of Sharava samputa- Two Sharava, fuller's earth (multani mati), water and pieces of cloth.
b. Mashi preparation – Sharava samputa containing Aamalaki Churna, 4.5 cow dungs, Laghu Puta, Til Tailam, match box, weighing machine and watch.

Procedure –

1. Repeat step 1 to 4 as in Expt no B 1.
2. Three cowdungs cut into small pieces were placed at the bottom of the Laghu Puta, the Sharava samputa was placed over it and the remaining one and a half cowdungs pieces were placed covering the sides and the top of Sharava samputa.
3. Some drops of Til Tailam were sprinkled over the cowdungs and with the help of a match stick the cowdungs were subjected to fire.
4. Sharava samputa was kept for Swangashita and the observations were noted.

E. Expt no. B 5

Title – Preparation of Aamalaki Mashi by Antardhumaa method.

Principle – Closed heating

Date – 18/01/2015

Ingredients – Aamalaki Churna = 100 gms

Equipments –

a. Preparation of Sharava samputa- Two Sharava, fuller's earth (multani mati), water and pieces of cloth.
b. Mashi preparation – Sharavasamputa containing Aamalaki Churna, 5 cow dungs, Laghu Puta, Til Tailam, match box, weighing machine and watch.

Procedure –

1. Repeat step 1 to 4 as in Expt no B 1.
2. Three and a half cowdungs cut into small pieces were placed at the bottom of the Laghuputa, the Sharavasamputa was placed over it and the remaining one and a half cowdungs pieces were placed covering the sides and the top of Sharavasamputa.
3. Some drops of Til Tailam were sprinkled over the cowdungs and with the help of a match stick the cowdungs were subjected to fire.
4. Sharavasamputa was kept for Swangashita and the observations were noted.

Photo - 2 : PREPARATION OF AAMALAKI MASHI BY BAHIRDHUM METHOD



Equipment for Aamalaki Mashi preperation



In process + lump formation, colour changes



The mixture has turned black coloured



Final product - Aamalaki Mashi



Yield of Aamalaki Mashi

Photo - 3 : PREPARATION OF AAMALAKI MASHI BY ANTARDHUM METHOD



Laghuputa

Laghuputa filled with cowdungs & thermocouple

Placement of Sharava Samputa

Cowdungs covering the Sharava Samputa

Laghuputa subjected to heat treatment

Laghuputa subjected to heat treatment

Swangashita process

Sharava Samputa taken out from puta after swangashita

Final product - Aamalaki Mashi

b. Pharmaceutical Study –

A right product is the combination of only two components-

1. The right and genuine quality of raw material.
2. Accuracy in the process.

In case of pharmaceutical formulation, the process requires a great dimension to deliver a product of requisite quality. In the present work, the pharmaceutical study includes preparation of Aamalaki Mashi by Bahirdhuma and Antardhumaa method.

Expt no I] Preparation of Aamalaki Mashi by Bahirdhuma method.

Principle – Open heating

Date – 30/01/2015

Ingredients – Aamalaki Churna = 100 gms

Equipments – Rounded earthen pan, stirrer, glass thermometer, retort stand, L.P.G, weighing machine and watch.

Procedure –

1. An earthen pan containing Aamalaki Churna was kept on gas burner.
2. With the help of retort stand, glass thermometer was inserted in the Churna without touching the bottom of the pan.
3. The gas burner was ignited and the flame was adjusted to medium low.
4. Continuous stirring was done till the Churna became completely black colored and the fumes coming from the Churna were ceased.
5. At this stage, the pan was kept aside away from the flame and the final product was transferred to a clean plate.
6. During the process and at the end of the experiment, all the relevant observations were noted.

Expt no II] Title – Preparation of Aamalaki Mashi by Antardhumaa method.

Principle – Closed heating

Date – 15/02/2015

Ingredients – Aamalaki Churna = 100 gms

Equipments –

a. Preparation of Sharava samputa- Two Sharava, fuller's earth (multani mati), water and pieces of cloth.

b. Mashi preparation – Sharava samputa containing Aamalaki Churna, 4.5 cow dungs, Laghu Puta, Til Tailam, match box, weighing machine and watch.

Procedure –

1. A Sharava containing Aamalaki Churna was taken and another Sharava was placed on it upside down.
2. A mixture of fuller's earth and water was prepared and a piece of cloth was dipped in it so that the entire cloth was properly loaded with the mixture.
3. This mud cloth was wrapped around the area of contact of the Sharava.
4. After the mud cloth was completely dried, another mud cloth prepared in the similar manner was wrapped around it. Total seven mud cloths were used for wrapping and sealing.
5. Three cowdungs cut into small pieces were placed at the bottom of the Laghu Puta, the Sharava samputa was placed over it and the remaining one and a half cowdungs pieces were placed covering the sides and the top of Sharava samputa.
6. Some drops of Til Tailam were sprinkled over the cowdungs and with the help of a match stick the cowdungs were subjected to fire.
7. Sharava samputa was kept for Swangashita and the observations were noted.
8. Throughout the process, the internal temperature (inside closed Sharava) and external temperature (generated by burning cowdungs) were noted down.

III. Standardization and Physico-chemical characterization –

A. Standardization of Aamalaki Churna and Aamalaki Mashi –

Sample A = Aamalaki Churna

Sample B = Aamalaki Mashi prepared by Bahirdhuma method

Sample C = Aamalaki Mashi prepared by Antardhumaa method

This was done by following tests –

a. Organoleptic / Macroscopic evaluation –

In the present study, Aamalaki Churna and Aamalaki Mashi were investigated for its macroscopic characteristics. Macroscopic characters such as color, odor, taste, shape and texture were observed.

b. Physico-chemical analysis – All the methods used for these testings were as per the norms described in Indian Pharmacopoeia[62] and were done at IDRL and Accurate Laboratory, Pune –

1. Loss on drying
2. Total ash
3. Acid insoluble ash
4. Alcohol Soluble extractives
5. Water soluble extractives
6. pH (10%)
7. TLC
8. Preliminary Phytochemical tests (Water extract)
9. Estimation of Vitamin C and Total Organic Carbon (TOC)

c. Analytical tests –

1. Qualitative and Quantitative estimation of inorganic radicals in Aamalaki Churna and Mashi samples were done by ICP-AES[63]. Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) was carried out at Sophisticated Analytical Instrument Facility (RSIC), Indian Institute of Technology, Powai, Mumbai.
2. SEM analysis[64] of Aamalaki Churna and Mashi samples was done at Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Powai, Mumbai. A SEM is probably the most widespread analytical instrument available in analytical laboratories destined to characterize physical properties such as morphology, shape, size or size distribution of materials at the micro and nanoscale. The performance of a modern, high-resolution SEM, in particular its spatial resolution, can reach to enable identification and even morphology characterization of nanoparticles down to below 10 nm.

62. The Ayurvedic Pharmacopoeia of India, part – I, volume – VII, first edition, 2011Ministry of Health and Family Welfare Government of India, Department of AYUSH

63. www.rsic.iitb.ac.in/icp-aes.html

64. www.rsic.iitb.ac.in/feg-sem.html

IV. Toxicity studies –

Toxicological studies of the Aamalaki Mashi prepared by Bahirdhuma and Antardhumaa method were conducted as per the OECD guidelines and are internationally accepted. Permission to conduct the trial was obtained from the 'Animal ethics committee' of National Toxicological Center, Pune before initiation of the work.

A. Acute Toxicity[65,66] –

Acute toxicity testing was carried out as per OECD 423 to determine the effect of a single dose on a particular animal species. In acute toxicological testing, the investigational product was administered at different dose levels, and the effect was observed for 14 days. All mortalities caused by the investigational product during the experimental period were recorded and morphological, biochemical, pathological, and histological changes in the dead animals were investigated. Acute toxicity testing permits the 50% lethal dose (LD_{50}) of the investigational product to be determined.

Drug Details:

Test Substance	:	T1 (Aamalaki Mashi prepared by Bahirdhuma method)
		T2 (Aamalaki Mashi prepared by Antardhumaa method)
Physical Nature	:	Jet black colored fine Powder
Anupana (Vehicle)	:	Water.
Dose	:	2000 mg/ Kg

Test Animals:

Species/ strain	:	Albino Swiss mice
Number	:	6
Age	:	8-12 weeks old
Weight	:	18-22 gms
Sex	:	Female

65. Acute and sub-acute toxicity and efficacy studies of *Hippophaerhamnoides* based herbal antioxidant supplement-Rashid Aliet all. Indian J Pharmacol. 2012 Jul-Aug; 44(4): 504–508. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3469956/
66. Toxicity studies of the active fraction of stereospermumtetragonam dc -Renjitbinokingsley, Saminathan, Pemaiah Brindha, Appian Subramoniam International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 5, Suppl 3, 2013.

Housing condition:

Caging- Polypropylene rat cages covered with stainless steel grid top were used. Clean rice husk was used as the bedding material.

Water bottle - Each cage was supplied with propylene water bottles of capacity 250 ml each, with a stainless steel nozzle.

Housing - 3 animals per cage.

Room sanitation - Floor of the experimental room was swept and work-top and floor was mopped with disinfectant solution everyday.

Diet and water

Diet **-** Rodent pellet diet manufactured by Navmaharashtra Chakan Mills Ltd. was provided ad libitum.

Water - Drinking water filtered through 'Aqua guard' water filter system was provided ad libitum.

Animal Coding and experiment:

The animals were marked using picric acid. Appropriate labels were attached to the cages indicating the study number, test substance code, group number and sex, dose, and cage number.

Method: Animals were grouped and coded

Codes are as follows:

- H : Mark on Head
- B : Mark on Back
- T : Mark on Tail
- RF : Mark on Right front leg
- RH : Mark on Right hind leg
- RLS : Mark on Right leg side

Test conditions:

The animals were kept on fasting for 24 hours prior to dosing. Drug was administered orally using syringe and stainless steel feeding needle. Stock solution was prepared

just before dosing. Dosing volume was calculated as per weight of the animal. Dosing was done in the morning between 9.30 to 10.00 am.

Limit Test:

The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. In those situations, where there is little or no information about its toxicity, or in which the the test material is expected to be toxic, the main test should be performed. A limit test at one dose level of 2000 mg/kg body weight may be carried out with six animals (three animals per step).

General observations:

Each mouse was observed after the single dose; for first 30 minutes followed by an interval of 60 minutes, 90 minutes, 2 hours, 4 hours and 24 hour. Further all the animals were carefully observed for 14 days.

The animals were observed for general parameters like salivation, vomiting, urination, diarrhoea, respiration change, tremors, gait and convulsions; behavioral parameters like hyperactivity or decreased activity, restlessness, grooming, sleep and aggressiveness.

Histopathological Study: Gross Necropsy:

At the end of study period i.e. 14 days, the animals were anesthetized by using anesthetic ether. The body of the rat was cut open using stainless steel scissors and forceps. The organs viz. Stomach, Kidneys, Liver, Intestine, Heart Lung, Spleen, Brain, Uterus, and Trachea were collected very carefully and transferred into marked glass bottles containing 10 % formalin.

Photo No. 4 – Housing of Animals



Housing of animals in cage



Food and water *ad libitum*



Marking of animals



Weighing animals

B. Subacute Toxicity [65,66]

Sub acute toxicity testing was done according to OECD Guideline 407.

In the sub-acute toxicity study of test drugs T1 (Aamalaki Mashi prepared by Bahirdhuma method) and T2 (Aamalaki Mashi prepared by Antardhumaa method) the drug was administered to mice for a period of 28 days.

Objective of the study: The objective of the study was to determine whether there is cumulative toxicity. The test substance was orally administered daily in graduated doses to several groups of experimental animals, one dose level per group for a period of 28 days. During the period of administration, the animals were observed closely, each day for signs

Study Design: Randomized Control Design

Animal Assignment : Animals were allocated at random to the test groups as follows:

Table no. 2- Assignment of Albino Swiss Mice in Sub Acute Oral Toxicity

Groups	Test Group	Dose to Animals (mg/kg)	Number of animals
1	Control	Distilled water	6 (3 Males + 3 Females)
2	T1 Low	500 mg/ Kg body weight	6 (3 Males + 3 Females)
3	T1 Medium	1000 mg /Kg body weight	6 (3 Males + 3 Females)
4	T1 High	2000 mg / Kg body weight	6 (3 Males + 3 Females)
5	T2 Low	500 mg/ Kg body weight	6 (3 Males + 3 Females)
6	T2 Medium	1000 mg /Kg body weight	6 (3 Males + 3 Females)
7	T2 High	2000 mg / Kg body weight	6 (3 Males + 3 Females)

65. Acute and sub-acute toxicity and efficacy studies of *Hippophaerhamnoides* based herbal antioxidant supplement-Rashid Aliet all. Indian J Pharmacol. 2012 Jul-Aug; 44(4): 504–508. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3469956/

66. Toxicity studies of the active fraction of stereospermumtetragonam dc -Renjitbinokingsley, Saminathan, Pemaiah Brindha, Appian Subramoniam International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 5, Suppl 3, 2013.

Rationale for dose level selection –

Dose levels should be selected taking into account any existing toxicity and toxico-kinetic data available for the test compound or related materials. The highest dose level should be chosen with the aim of inducing toxic effects but not death or severe suffering. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and no-observed-adverse effects at the lowest dose level (NOAEL). Two to four fold intervals are frequently optimal for setting the descending dose levels and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages.

Accordingly, 1000 mg/kg = Intermediate dose, according to this half of this dose i.e 500 mg/kg was taken as low dose and double of this dose i.e 2000 mg/kg (which was proved to be safe in acute toxicity study) was taken as high dose.

Experimental Procedure:

1. Swiss Albino mice weighing between 18-22 gm were selected.
2. The animals were classified according to the above mentioned groups.
3. Each group contained 6 animals (3 male + 3 female). Each cage housed 6 animals.
4. Cages were labelled and the animals were marked for identification by using picric acid.
5. Drugs (T1 and T2) were weighed and prepared everyday for dosing.

Route of Administration:

The drug suspension was administered by oral route using a stainless steel feeding needle. The time of drug administration was maintained in morning between 10.00 am to 10.30 am throughout the study.

Food Consumption and compound intake

50 gm of standard rat food pellets were kept in feed tray every day. Next day remaining food pellets were weighed to find out daily food consumption by the animals. The time of filling the food tray was noted and kept constant through out the study. The animals had access to water and food ad libitum.

Observations:

General health of the animals in terms of body weight, behavior, skin, hair was carefully observed. Bedding material (paddy) of each cage was replaced twice a week.

Body weight -

Body weight chart was maintained throughout the study. The animals were weighed just before dosing i.e. 0 day, on 7^{th} day, 14^{th} day, 21^{st} day and on 28^{th} day.

Food consumption –

Food consumption chart was maintained throughout the study. The quantity of food consumed and remained were noted on 0 day, 7^{th} day, 14^{th} day, 21^{st} day and on 28^{th} day.

Haematology and Clinical Chemistry –

Blood collection was done at the end of study period i.e. 28th day. The animals were anesthetized by using anaesthetic ether 24 hours after the last dose. The blood samples were collected in two separate bottles, marked with animal number, group, haematology/ clinical chemistry. Anticoagulant (EDTA) was used in blood samples for haematological parameters. The blood samples were centrifuged at 2500 rpm in a cold centrifuge machine for 30 minutes and serum was separated to use for clinical chemistry.

Histopathological Studies:

At the end of '28-day study period', the animals were anesthetized by using anaesthetic ether after 24 hours of last dose. The body of the mice was cut open using stainless steel scissors and forceps. Liver, kidneys, stomach, intestine, heart, lung, spleen, brain, uterus, and trachea were carefully removed and transferred into glass bottles containing 10 % formalin. All the organs were weighed. Histopathology of liver and kidney was done; other organs were not considered for this study.

Results: The results were noted on the following basis-

- Effect on Body weight of male mice
- Effect on Food consumption
- Effect on Organ weight

- Effect on Haematological parameters
- Effect on Biochemical parameters
- Effect on Histopathology study

V. *In vivo* anti ulcer activity[67,68] –

Study design for Animal experiment to assess anti ulcer potential of Aamalaki Mashi using standard "Ethanol induced mucosal damage in Rats" animal model **-**

Purpose and Rationale: Intragastric application of absolute ethanol is a reproducible method to produce gastric lesions in experimental animals. The protective effect against various irritants has been called cytoprotective activity.

Aim and Objectives –

To assess anti-ulcer activity of drug T1 and T2 in ethanol induced ulcers in rats.

Animal Welfare –

This study was performed under the conditions recommended by the 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA). The protocol was approved by Institutional Animal Ethics Committee (IAEC) of NTC.

Table no. 3 - Reagents and Chemicals

Ethanol	1 ml/animal
Std. Marketed Drug (Ranitidine)	100 mg/kg
Saline	0.9 %

67. Dae-Kwon Bae et all., Different antiulcer activities of Pantoprazole in stress, alcohol, pylorus ligation induced ulcer models, Lab animal research 27 (1) 47-52.
68. Motawi TK, Hamed MA, Hashem RM, Shabana MH, Ahmed YR, Protective and therapeutic effects of Argyreiaspeciosa against ethanol induced gastric ulcer in rats. Z Naturforsch C. 2012 Jan-Feb;67(1-2):47-57.

Dose calculation based on standard Human Dose –

Mashi dosage form is described in the ancient texts of Ayurveda mainly for internal and external use. The dose of Mashi is clearly not mentioned in these texts. Mashi is in the form of Churna (powder), and if we consider the dose of churna as per Ayurvedic text, it is 1 tola[69] ≈ 10 gms. The dose[70] of powdered activated charcoal in gas troubles and hyperacidity problems is 10-20 gms in a day. In practice, Aamalaki Mashi is used in the dose of 5-10 gms in a day for treating hyperemesis, gaseous problems, etc. This dose is given in divided form for 3 to 4 times in a day. There is no standard reference regarding this dose. In this study, there was a need to optimize the dose of Mashi. Therefore, considering the above mentioned factors, 10 gms of Aamalaki Mashi in 24 hrs was taken as standard human dose for the study purpose. This could also help to determine the dose for efficacy study.

Human dose extrapolated to animal dose –

Dose of Aamalaki Mashi in 70 kg human = 10 gms = 10,000 mg

Dose conversion factor (human to rat) = 0.0180

x = dose in rat of 200 gm = 10000 X 0.0180 = 180 mg

180 mg in rat of 200 gm

We have to calculate dose per kg

Therefore,

For rat of 1000 gm (per kg) = 180 X 1000/200 = 900 mg/ kg ≈ 1000 mg/kg

Dose Preparation –

The test material was weighed and mixed with distilled water to achieve the final concentration.

69. Sharangdhar Samhita by Pandit Sharagdharacharya son of Pandit Damodar with the Commentary Adhamalla Dipika and Kashirams Gudartha Dipika edited by Pandit ParshuramShastri Vidyasagar, Chaukhamba Orientalia Varanasi, Madhyamkhanda 6/1.
70. https://www.drugs.com>Professionals>Natural Products (Pro)

Table no. 4 – Specifications of Test System

1	Species	*Rattus novegicus*
2	Strain	Wistar Rat
3	Source	National Toxicology Center, Pune
4	Sex	Male and Female
5	Number of animals in each group	3 Males + 3 Females = 6
6	Body weight range	150.0 – 180.0 gm
7	Age	10-12 weeks
8	Identification	By unique identification number marked by writing on cage tag and by corresponding color body markings
9	Environmental Conditions	Room temperature between 22-25° C, relative humidity 55-60 % and illumination cycle set to 12 hours light and 12 hours dark
10	Accommodation	3 animals per cage housed (as per CPCSEA guidelines) 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 Nav Maharashtra Chakan Oil Mills Ltd., Pune, ad libitum during acclimatization and during the study
12	Water	Potable water passed through 'Aquaguard' water filter ad libitum in plastic bottles with stainless steel sipper tubes.

Experimental Procedures –

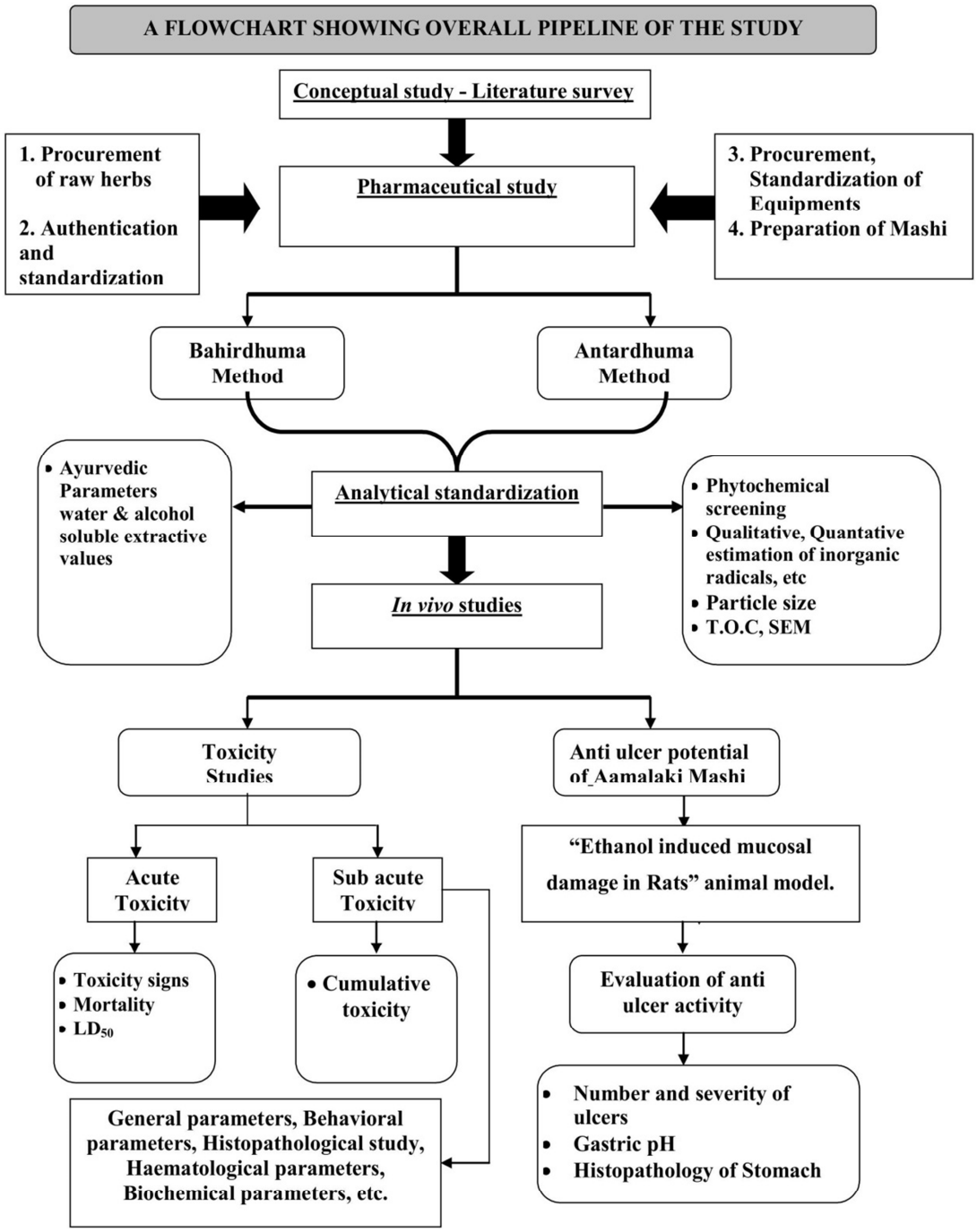
1. The rats were divided into respective groups of six animals each.

Table no. 5– Grouping of experimental animals- rats

Group I	Control
Group II	Disease Control
Group III	Treated with Ranitidine 100 mg/kg
Group IV	Treated with Drug T1
Group V	Treated with Drug T2

2. The rats were treated with the test and standard drugs prior to ulceration daily for seven days.
3. On the day of ulceration, the rats were kept for fasting for 24 hours and then administered 1 ml of absolute ethanol orally.
4. The animals were sacrificed after 1 hour of ulcerogen administration and their stomachs were excised and the gastric contents were aspirated.
5. The contents were subjected to 1000 rpm for 10 minutes and then analysed for pH (digital pH meter).
6. The stomachs were washed with normal saline and kept in 10 % formalin for the determination of ulcer index and histological studies.
7. Ulcer index was determined by the method of Andrade wherein,
   Level I ulcer Area =<1mm^2
   Level II Ulcer Area=1-3mm^2
   Level III Ulcer Area=>3mm^2
   The following parameters were determined
   Ulcerative lesion Index (ULI) = 1 X (No. Of Ulcers in Level I) + 2 X (No. Of Ulcers in Level II) + 3 X (No. Of Ulcers in Level III)
   Percentage Protective Ratio = 100-(ULI pretreated/ULI Control) *100
8. The results were expressed as Mean +_ SD and statistical significance between treated and control groups was analyzed using of one way analysis of variance (ANOVA), followed by Dunnett's t-test[71] where P<0.05 was considered statistically significant.

71. www.stat.purdue.edu/~xuanyaoh/stat350/xyMar23Lec22.pdf


A FLOWCHART SHOWING OVERALL PIPELINE OF THE STUDY
Conceptual study - Literature survey
1. Procurement of raw herbs
2. Authentication and standardization
Pharmaceutical study
3. Procurement, Standardization of Equipments
4. Preparation of Mashi
Bahirdhuma Method
Antardhuma Method
Ayurvedic Parameters water & alcohol soluble extractive values
Analytical standardization
Phytochemical screening
Qualitative, Quantative estimation of inorganic radicals, etc
Particle size
T.O.C, SEM
In vivo studies
Toxicity Studies
Anti ulcer potential of Aamalaki Mashi
Acute Toxicity
Sub acute Toxicity
"Ethanol induced mucosal damage in Rats" animal model.
Toxicity signs
Mortality
LD50
Cumulative toxicity
Evaluation of anti ulcer activity
Number and severity of ulcers
Gastric pH
Histopathology of Stomach
General parameters, Behavioral parameters, Histopathological study, Haematological parameters, Biochemical parameters, etc.

OBSERVATIONS

I. Physico-chemical characterization of Aamalaki Churna and Aamalaki Mashi

A. Aamalaki Churna yield = 1.8 kg

Table No. 6 – Yield of Aamalaki Churna

Material	Weight
Aamalaki Fruits	10 kg
Dried Aamalaki	2.0 kg
Fine Powder	1.8 kg

B. Standardization of Aamalaki Churna and aamalaki Mashi

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. Standardization is a tool in the quality control process.

All these tests are helpful for determination of quality and purity of the drug. The study drugs are abbreviated as follows-

Sample A = Aamalaki Churna

Sample B = Aamalaki Mashi prepared by Bahirdhuma method

Sample C = Aamalaki Mashi prepared by Antardhuma method

a. Organoleptic / Macroscopic evaluation –

In the present study, Aamalaki Churna and Aamalaki Mashi were investigated for its macroscopic characteristics. Macroscopic characters such as color, odor, taste, shape, texture were observed.

Table No. 7 – Organoleptic characters

Sr. No	Organoleptic characters	Sample A	Sample B	Sample C
1	Shabda	-	-	-
2	Sparsha	soft	very soft	soft
3	Rupa	brown colored	jet black	Jet black
4	Rasa	Panchrasa = Amla, Tikta, Madhur, Kashaya, Katu	characteristic	characteristic
5	Gandha	characteristic	Non specific	Non specific

The texture of Aamalaki Mashi is soft as compared with the Churna.

b. Physico-chemical analysis –

Table No. 8 – Physico-chemical analysis

Sr. No	Physico -chemical tests	Sample A	Sample B	Sample C
1	Loss on drying	10.53 %	2.70 %	2.17 %
2	Total Ash	2.08 %	3.36 %	5.52 %
3	Acid Insoluble Ash	0.15 %	0.18 %	0.16 %
4	Alcohol Soluble Extractives	16.23 %	0.18 %	0.71 %
5	Water Soluble Extractives	4.06 %	0.67 %	0.33 %
6	pH (10 %)	2.80	5.86	7.46

1. The percentage of loss on drying is significantly less in sample B and sample C than sample A which signifies less moisture content which is helpful in maintaining the stability of the drug and less chances of fungal or bacterial contamination and yeast growth.
2. Contents of total ash were found to be lower in sample A and B which may be due to low content of carbonates, phosphates, silicates and silica. All the three samples are low content of acid insoluble ash.
3. Alcohol soluble extractive values were found to be greater than water soluble extractive value in Sample A, indicating this sample contains maximum alcohol soluble compounds that can be extractable maximum into alcohol than the water. There was no significant difference in the alcohol and water extractive values in sample B and C.
4. pH signifies Sample A is the most acidic of all the three samples, sample B is slightly acidic and sample C is on neutral level.

Table No. 9 – Thin Layer Chromatography

Thin Layer Chromatography			
	Sample A	Sample B	Sample C
Extraction	Methanol extract		
Adsorbent	Silica gel $G_{60}F_{254}$		
Mobile phase	Toluene : Ethyl Acetate : Glacial Acetic Acid : Formic Acid (2.0:4.5:2.0:0.5)		
Detection			
UV – 254 nm	Four spots Rf - 0.23, 0.40, 0.58, 0.81 (all dark blue)	Four spots Rf - 0.53, 0.80, 0.85, 0.93 (all dark blue)	No separation was observed
UV – 365 nm	Two spots Rf – 0.35, 0.81 (all green)	Three spots Rf – 0.53, 0.80, 0.85 (all light blue)	
Iodine	Six spots Rf – 0.06, 0.21, 0.33, 0.41 (yellow) 0.56, 0.81 (blue)	Three spots Rf – 0.53, 0.80, 0.85 (all dark brown)	
Anisaldehyde Sulphuric acid	Four spots Rf – 0.11 (green) 0.41, 0.81 (brown) 0.95 (blue)	Five spots Rf – 0.25, 0.35 (green) 0.8 (brown) 0.85 (green) 0.93 (blue)	

TLC of Sample B denotes formation of new spots indicating new compounds which are not present in sample A and loss of few compounds which are present in sample A. This may be due to the heat treatment received in case of drug T1 due to which volatile and some organic molecules must be destroyed and there must be formation of new molecules.

c. Preliminary Phytochemical tests (Water extract)

Table No. 10 – Preliminary Phytochemical screening (Water extract)

Sr. No.	Tests	Sample A	Sample B	Sample C
1	Alkaloid	Negative	Negative	Negative
2	Flavonoid	Negative	Positive	Negative
3	Steroids	Negative	Negative	Negative
4	Tannin	Negative	Negative	Negative
5	Terpenoid	Negative	Negative	Negative
6	Saponin	Negative	Negative	Negative
7	Sugars	Positive	Negative	Negative

Sample A shows presence of sugars and sample B shows presence of flavonoid. There was absence of these phytochemicals in sample C.

d. Estimation of Vitamin C and Total Organic Carbon (TOC)

Table No. 11 – Estimation of Vitamin C and Total Organic Carbon (TOC)

Sr. No.	Tests	Sample A	Sample B	Sample C
1	Vitamin C	3.7742 %	0.0329 %	0.0153 %
2	TOC	-	42.712 %	3.2367 %

Sample B shows presence of high percentage of TOC.

e. Qualitative and Quantitative estimation of inorganic radicals by ICP-AES

Table No. 12 – Qualitative and Quantitative estimation of inorganic radicals by ICP-AES

Sr. No.	Elements	Sample A (%)	Sample B (%)	Sample C (%)
1	P	0.119	0.144	0.058
2	S	0.083	0.068	0.023
3	Ti	0.0042	ND	0.00004
4	Hg	0.0034	0.012	ND
5	Zr	ND	ND	ND
6	Ce	ND	ND	ND
7	Al	0.025	0.021	0.007
8	B	0.010	0.006	0.001
9	Ba	0.00079	0.00083	0.00049
10	Ca	0.365	0.435	0.306
11	Cr	0.00036	ND	ND
12	Cu	0.0016	0.0018	0.0009
13	Fe	0.013	0.017	0.0019
14	K	1.03	1.764	0.658
15	Pb	0.001	0.001	0.0007
16	Sr	0.0011	0.0014	0.00079
17	Zn	0.0029	0.0037	0.0035
18	Li	ND	ND	ND
19	Mg	0.098	0.104	0.061
20	Mn	0.00096	0.0011	0.0006
21	Na	0.018	0.02	0.016
22	Ni	0.00031	0.00073	ND

ND means less than 0.01 ppm

f. SEM analysis of Aamalaki Churna and Mashi samples –

Fig. 3 - SEM analysis of Sample A

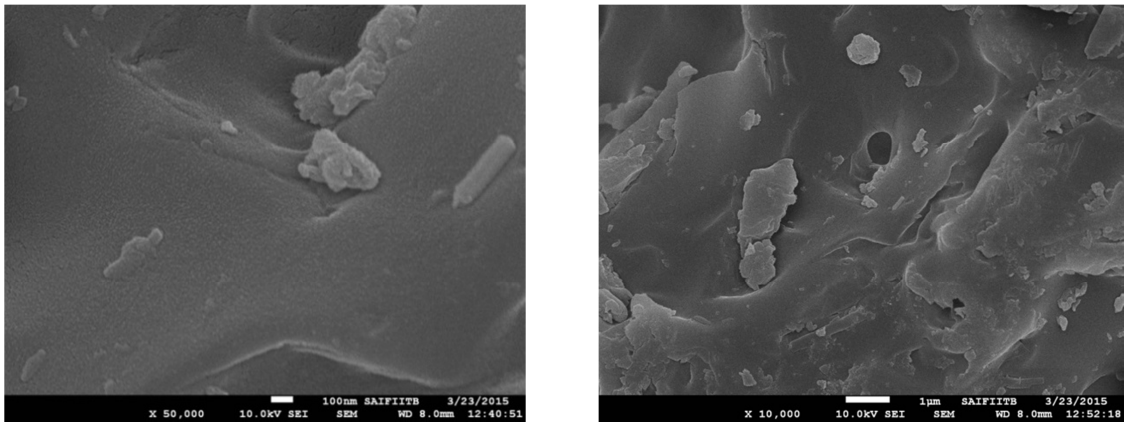


Fig. 4 - SEM analysis of Sample B

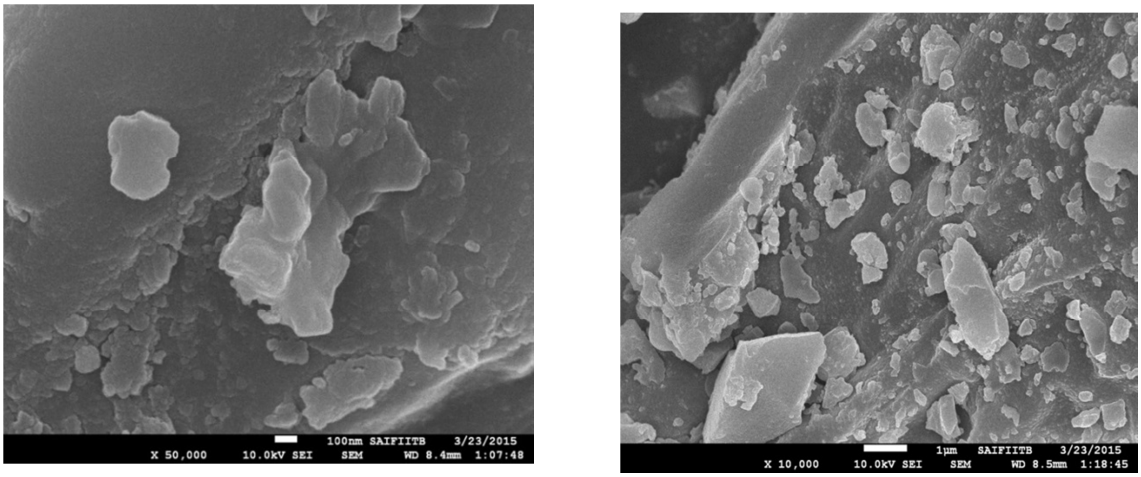


Fig. 5 - SEM analysis of Sample C

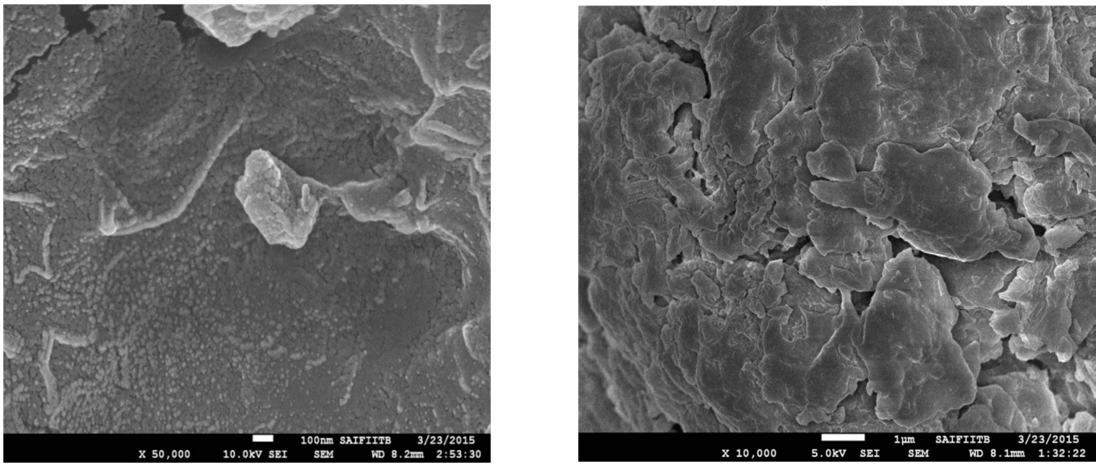


Table No. 13 – SEM analysis Interpretation

Sr. No.	Samples	Interpretation
1	Sample A	Formation of multilayer scaffolds, sheet like formation, there is no initiation of formation of nano particles, particles in agglomerated stage.
2	Sample B	Nano particles formation is seen, size of particles has reduced making the compound more penetrable/ absorbable Size of particle = 100 – 200 nm
3	Sample C	Particles are seen aggregated, there is initiation of nano particles but the particles are not separated.

II. Pharmaceutical Study

1. **Observations drawn from Pilot work**

A. Aamalaki Mashi prepared by Bahirdhuma method.

Expt no A 1- It was observed that the final product obtained contained few particles of white ash along with black ash.

Table No.14 (a) – Observations of Expt no A 1

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	50 gms
2	Starting time of expt	3.00 pm
3	End time of expt	3.45 pm
4	Initial temperature	31.5°C
5	Final temperature	162°C
6	Total yield	30 gms

Table No.14 (b) – Changes observed at various temperatures in Expt no A1

Sr. No	Temperature	Changes
1	65°C	Color started to change
2	90°C	Occurrence of pleasant smell
3	92°C	Appearance of fumes
4	104°C	Occurrence of burning smell
5	157°C	Formation of Angar (embers) and transformation of some part of black ash into white ash (Kshara formation)

Table No.14 (c) – Organoleptic characters of Aamalaki Mashi in Expt no A 1

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	soft	soft
3	Rupa	dark chrome yellow	black with white particles
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	characteristic
5	Gandha	non-specific	non-specific

Expt no A 2 -It was observed that the final product was completely black colored resembling a collyrium.

Table No.15 (a) – Observations of Expt no A 2

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	50 gms
2	Starting time of expt	2.30 pm
3	End time of expt	3.17 pm
4	Initial temperature	31.2°C
5	Final temperature	157°C
6	Total yield	32 gms

Table No.15 (b) – Changes observed at various temperatures in Expt no A 2

Sr. No	Temperature	Changes
1	60°C	Color started to change
2	89°C	Occurrence of pleasant smell
3	90°C	Appearance of fumes
4	104°C	Occurrence of burning smell
5	157°C	Formation of black ash and ceasing of fumes.

Table No.15 (c) – Organoleptic characters of Aamalaki Mashi in Expt no A 2

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	soft	soft
3	Rupa	dark chrome yellow	black
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	characteristic
5	Gandha	non-specific	non-specific

Expt No. A 3 -It was observed that the final product was completely black colored ash.

Table No.16 (a) – Observations of Expt no A 3

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	50 gms
2	Starting time of expt	12.30 pm
3	End time of expt	1.14 pm
4	Initial temperature	32.7°C
5	Final temperature	158°C
6	Total yield	33 gms

Table No.16 (b) – Changes observed at various temperatures in Expt no A 3

Sr. No	Temperature	Changes
1	63°C	Color started to change
2	92°C	Occurrence of pleasant smell
3	90°C	Appearance of fumes
4	105°C	Occurrence of burning smell
5	158°C	Formation of black ash and ceasing of fumes.

Table No.16 (c) – Organoleptic characters of Aamalaki Mashi in Expt no A 3

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	soft	soft
3	Rupa	Dark chrome yellow	Black
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	characteristic
5	Gandha	non-specific	non-specific

Expt No. A 4- It was observed that the final product was completely black colored ash.

Table No. 17 (a) – Observations of Expt no A 4

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	100 gms
2	Starting time of expt	2.30 pm
3	End time of expt	3.25 pm
4	Initial temperature	32.7°C
5	Final temperature	172°C
6	Total yield	56.2 gms

Table No. 17 (b) – Changes observed at various temperatures in Expt no A 4

Sr. No	Temperature	Changes
1	72.4°C	Color started to change
2	90°C	Occurrence of pleasant smell
3	92°C	Appearance of fumes
4	104°C	Occurrence of burning smell
5	172°C	Formation of black ash and ceasing of fumes.

Table No. 17 (c) – Organoleptic characters of Aamalaki Mashi in Expt no A 4

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	soft	soft
3	Rupa	dark chrome yellow	black
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	characteristic
5	Gandha	non-specific	non-specific

Expt No. A 5-It was observed that the final product was completely black colored ash.

Table No. 18 (a) – Observations of Expt no A 5

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	100 gms
2	Starting time of expt	1.45 pm
3	End time of expt	2.50 pm
4	Initial temperature	31.5°C
5	Final temperature	174°C
6	Total yield	58 gms

Table No.18 (b) – Changes observed at various temperatures in Expt no A 5

Sr. No	Temperature	Changes
1	74.4°C	Color started to change
2	96.8°C	Occurrence of pleasant smell
3	98.2°C	Appearance of fumes
4	105.7°C	Occurrence of burning smell
5	176°C	Formation of black ash and ceasing of fumes.

Table No. 18 (c) – Organoleptic characters of Aamalaki Mashi in Expt no A 5

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	soft	soft
3	Rupa	dark chrome yellow	black
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	characteristic
5	Gandha	non-specific	non-specific

Expt No. A 6 - It was observed that the final product was black colored ash with formation of some amount of white ash.

Table No. 19 (a) – Observations of Expt no A 6

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	100 gms
2	Starting time of expt	12.15 pm
3	End time of expt	1.20 pm
4	Initial temperature	32.5°C
5	Final temperature	185°C
6	Total yield	53 gms

Table No. 19 (b) – Changes observed at various temperatures in Expt no A 6

Sr. No	Temperature	Changes
1	70°C	Color started to change
2	93°C	Occurrence of pleasant smell
3	96°C	Appearance of fumes
4	108°C	Occurrence of burning smell
5	180°C	Formation of black ash and ceasing of fumes.
6	185°C	Beginning of Angar (embers) formation and transformation of some particles of black ash into white ash

Table No. 19 (c) – Organoleptic characters of Aamalaki Mashi in Expt no A 6

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	soft	soft
3	Rupa	dark chrome yellow	black ash mixed with white ash particles.
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	characteristic
5	Gandha	non-specific	non-specific

Expt No. A 7 - It was observed that the final product was completely black colored ash.

Table No.20 (a) – Observations of Expt no A 7

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	100 gms
2	Starting time of expt	2.30 pm
3	End time of expt	3.25 pm
4	Initial temperature	32.7°C
5	Final temperature	176°C
6	Total yield	57 gms

Table No.20 (b) – Changes observed at various temperatures in Expt no A 7

Sr. No	Temperature	Changes
1	72.4°C	Color started to change
2	90°C	Occurrence of pleasant smell
3	92°C	Appearance of fumes
4	104°C	Occurrence of burning smell
5	176°C	Formation of black ash and ceasing of fumes.

Table No.20 (c) – Organoleptic characters of Aamalaki Mashi in Expt no A 7

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	soft	very soft
3	Rupa	dark chrome yellow	black
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	characteristic
5	Gandha	non-specific	non-specific

B. Mashi preparation by Antardhuma method

Expt No. B 1 - It was observed that the final product was a mixture of black ash and white ash.

Table No.21 (a) – Observations of Expt no B 1

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	100 gms
2	Starting time of expt	10.00 am
3	End time of expt	7.30 pm
4	Time of disappearance of fumes from the Puta	11.45 am
5	Total yield	24 gms (black ash = 15 gms, white ash = 9 gms)

Table No.21 (b) – Organoleptic characters of Aamalaki Mashi in Expt no B 1

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	Soft	Soft
3	Rupa	Dark chrome yellow	Mixture of black and white ash.
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	Characteristic
5	Gandha	non-specific	Non-specific

Expt No. B 2 -It was observed that the final product was a mixture of black ash with small portions of white ash.

Table No.22 (a) – Observations of Expt no B 2

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	100 gms
2	Starting time of expt	10.30 am
3	End time of expt	8.30 pm
4	Time of disappearance of fumes from the Puta	12.05 pm
5	Total yield	33 gms (black ash = 29 gms, white ash = 4 gms)

Table No.22 (b) – Organoleptic characters of Aamalaki Mashi in Expt no B 2

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	soft	soft
3	Rupa	dark chrome yellow	mixture of black and white ash.
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	characteristic
5	Gandha	non-specific	non-specific

Expt No. B 3 -It was observed that the major portion of the final product was converted to black ash but there was a small part which was incompletely burnt.

Table No.23 (a) – Observations of Expt no B 3

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	100 gms
2	Starting time of expt	10.00 am
3	End time of expt	8.00 pm
4	Time of disappearance of fumes from the Puta	11.35 pm
5	Total yield	40 gms

Table No.23 (b) – Organoleptic characters of Aamalaki Mashi in Expt no B 3

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	Soft	Soft
3	Rupa	Dark chrome yellow	Mixture of black with remnants of incompletely burnt Aamalaki Churna
4	Rasa	Amla, tikta, madhur, kashaya, katu	Characteristic
5	Gandha	Non-specific	Non-specific

Expt No. B 4 - It was observed that the final product was black ash.

Table No.24 (a) – Observations of Expt no B 4

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	100 gms
2	Starting time of expt	10.45 am
3	End time of expt	8.15 pm
4	Time of disappearance of fumes from the Puta	12.10 pm
5	Total yield	35 gms

Table No.24 (b) – Organoleptic characters of Aamalaki Mashi in Expt no B 4

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	soft	soft
3	Rupa	dark chrome yellow	black ash
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	characteristic
5	Gandha	non-specific	non-specific

Expt No. B 5- It was observed that the final product was black ash.

Table No.25 (a) – Observations of Expt no B 5

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	100 gms
2	Starting time of expt	10.35 am
3	End time of expt	8.25 pm
4	Time of disappearance of fumes from the Puta	12.15 pm
5	Total yield	34 gms

Table No.25 (b) – Organoleptic characters of Aamalaki Mashi in Expt no B 5

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	soft	soft
3	Rupa	dark chrome yellow	black ash
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	characteristic
5	Gandha	non-specific	non-specific

2) **Observations drawn from actual pharmaceutical process-**

Expt no I] Preparation of Aamalaki Mashi by Bahirdhuma method.

It was observed that the final product was Mashi which was very soft and completely black colored resembling a collyrium.

Table No.26 (a) – Observations of Expt no I

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	100 gms
2	Starting time of expt	3.35 pm
3	End time of expt	4.35 pm
4	Initial temperature	31.5°C
5	Final temperature	172°C
6	Total yield	56.69 gms

Table No.26 (b) – Changes observed at various temperatures in Expt no I

Sr. No	Temperature	Changes
1	71.2°C	Color started to change
2	94.8°C	Occurrence of pleasant smell, formation of lumps which broke on continuous stirring
3	97.3°C	Appearance of fumes
4	103.7°C	Occurrence of burning smell
5	138°C	Proportion of fumes increased, freeness in stirring
5	152°C	Feeling of smoothness and freeness while stirring, raw material converting into black ash
6	172°C	Formation of complete black ash and ceasing of fumes.

Table No.26 (c) – Organoleptic characters of Aamalaki Mashi in Expt no I

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	Soft	soft
3	Rupa	dark chrome yellow	black
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	characteristic
5	Gandha	non-specific	non-specific

Table No.26 (d) – Color changes with temperature of Aamalaki Mashi in Expt no I

Sr. No	Temperature	Color changes
1	31.5°C	Dark chrome yellow
2	52°C	Original color getting darker
3	69°C	Light brown
4	113.5°C	Brown
5	126°C	Chocolate brown
6	148°C	Dark chocolate

7	154°C	Black
8	165°C	Dark back
9	172°C	Jet black

Expt no II] - Preparation of Aamalaki Mashi by Antardhuma method. It was observed that the final product obtained was Mashi which was soft and completely black colored ash.

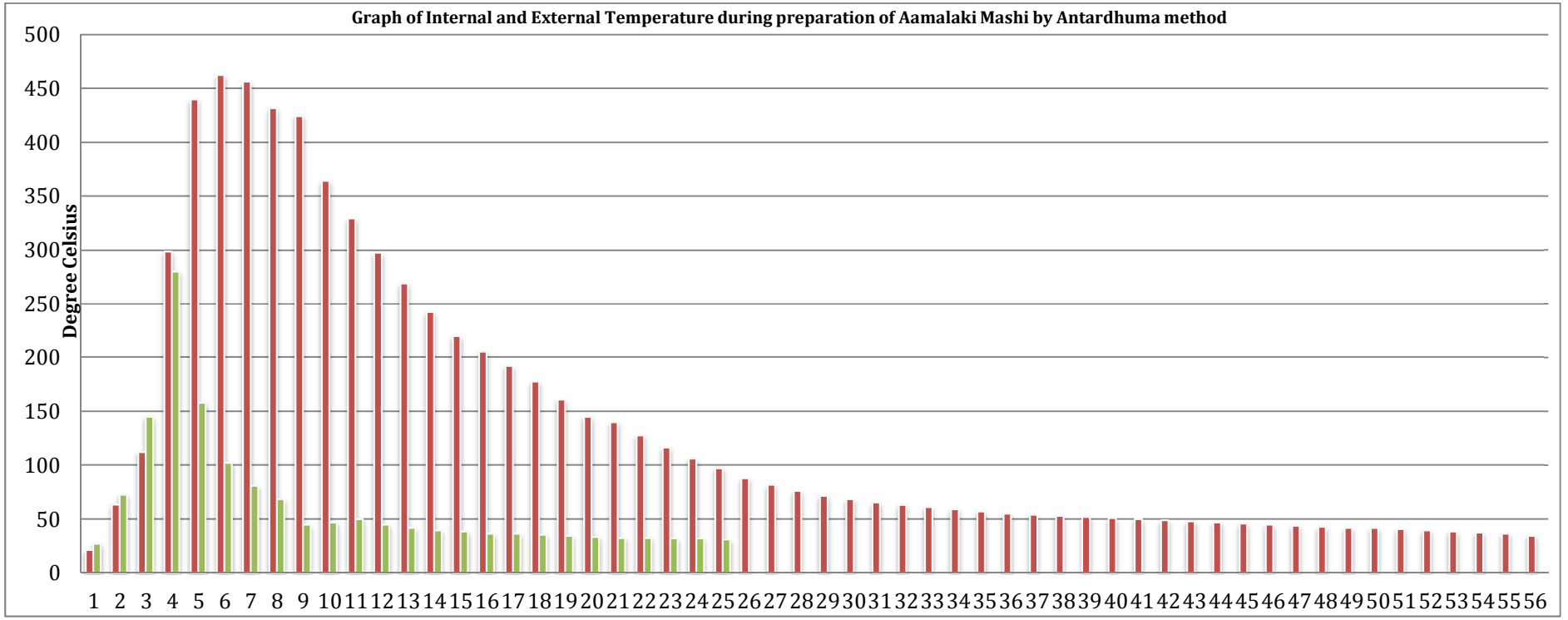
Table No.27 (a) – Observations of Expt no II

Sr. No	Observations	Findings
1	Weight of Aamalaki Churna	100 gms
2	Starting time of expt	10.55am
3	End time of expt	8.30 pm
4	Time of appearance of fumes from Puta	11.10 am
4	Time of disappearance of fumes from Puta	12.10 pm
5	Total yield	33 gms

Table No.27 (b) – Organoleptic characters of Aamalaki Mashi in Expt no II

Sr. No	Tests	Aamalaki Churna	Aamalaki Mashi
1	Shabda	--	--
2	Sparsha	soft	Soft
3	Rupa	dark chrome yellow	Black
4	Rasa	Amla, Tikta, Madhur, Kashaya, Katu	Characteristic
5	Gandha	non-specific	non-specific

Graph -1 Graph of Internal and External Temperature during preparation of Aamalaki Mashi by Antardhuma method of internal and external temp



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
Time	10.55	11.05	11.15	11.25	11.35	11.45	11.55	12.05	12.15	12.25	12.35	12.45	12.55	13.05	13.15	13.25	13.35	13.45	13.55	14.05	14.15	14.25	14.35	14.45	14.55	15.05	15.15	15.25	15.35	15.45	15.55	16.05	16.15	16.25	16.35	16.45	16.55	17.05	17.15	17.25	17.35	17.45	17.55	18.05	18.15	18.25	18.35	18.45	18.55	19.05	19.15	19.25	19.35	19.45	19.55	20.05
Internal temperature °C	21.3	63.2	112	299	440	462	456	432	425	364	329	298	269	242	220	205	192	178	161	145	140	128	116	106	97	88	82	76	71	68	65	63	61	59	57	55	54	53	52	51	50	49	48	47	46	45	44	43	42	42	41	40	39	38	37	35
External temperature °C	27	72	145	280	158	102	81	68	45	47	50	45	42	40	39	37	37	36	35	34	33	33	32	32	31																																			

III. Toxicity studies

1. **Observations of Acute Toxicity** – Mortality, functional testing, body weight and necropsy findings were observed in acute oral toxicity.

a. Mortality Chart:

Table No.28 (a)– Mortality chart of mice in Acute oral toxicity studies of drug T1

Test Sequence	Animal I.D code	Dose (mg/kg)	Mortality after 1st day	Mortality after 14th day
1	H	2000	None	None
2	B	2000	None	None
3	T	2000	None	None
4	RF	2000	None	None
5	RH	2000	None	None
6	RLS	2000	None	None

Table No.28 (b)– Mortality chart of mice in Acute oral toxicity studies of drug T2

Test Sequence	Animal I.D code	Dose (mg/kg)	Mortality after 1st day	Mortality after 14th day
1	H	2000	None	None
2	B	2000	None	None
3	T	2000	None	None
4	RF	2000	None	None
5	RH	2000	None	---
6	RLS	2000	None	---

On day 2, there was accidental death of two animals in the group receiving drug T2.

b. Functional Testing

Table No.29 – Functional parameters of mice in Acute oral toxicity studies in both groups T1 and T2

General parameters	Remarks	Behavioral parameters	Remarks
Salivation, Urination, Excretion	Normal	Hyperactivity or Decreased activity	Nil
Vomiting Diarrhea	Nil	Restlessness	Nil
Respiration change	Normal	Grooming	Normal

Tremors	Nil	Sleep	Normal
Convulsions	Nil	Aggressiveness	Nil
Gait	Normal		
Skin and fur	Normal texture and luster		
Secretions	No abnormal secretions		

c. Body weight – There is consistent increase in the body weight of the animals.

Table No.30 – Body weight of mice in Acute oral toxicity studies

Groups	**Bodyweight**		
	0 day	**7 day**	**14 day**
Control	22.0	22.5	24.0
	21.5	23.0	27.0
	23.0	25.0	27.0
Mean	**22.2**	**23.5**	**26.0**
SD	**0.8**	**1.3**	**1.7**
T1-A	19.5	23.5	15.0
	20.5	23.0	20.0
	20.0	22.0	20.0
Mean	**20.0**	**22.8**	**18.3**
SD	**0.5**	**0.8**	**2.9**
T2-A	19.5	22.0	23.0
	23.0	25.0	25.5
	21.5	24.5	26.5
Mean	**21.3**	**23.8**	**25.0**
SD	**1.8**	**1.6**	**1.8**
T1-B	19.5	20.0	21.5
	18.5	19.0	22.0
	20.0	16.5	20.0
Mean	**19.3**	**18.5**	**21.2**
SD	**0.8**	**1.8**	**1.0**
T2-B	22.0	15.0	22.0
	18.5	Dead	Dead
	18.0	Dead	Dead
Mean	**19.5**	**15.0**	**22.0**
SD	**2.2**	**-**	**-**

d. Necropsy findings / Histopathology findings

As per the OECD guidelines, histopathology is not conducted, those organs showing changes in gross necropsy can go for histopathological studies. In this study, there were no signs of changes seen grossly indicating no signs of toxicity.

Results - Since no mortality & no observable toxicity symptoms are recorded up to 2000 mg/kg. It is concluded that **LD 50 of drug T1 and T2 is greater than 2000 mg/kg**

2. Observations of Sub Acute Oral Toxicity

It was found that during this study there was accidental death of 1 animal each from the groups T1 I, T1 H, T2 I and T2 H on day 2, day 8, day 9 and day 15 respectively.

1. Effect on body weight of mice –

Table No.31 – Body weight of mice in Sub Acute Oral Toxicity studies

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
NC	29.0	28.5	35.5	37.0	38.0
	24.5	25.0	36.0	40.5	42.0
	23.0	23.0	33.0	37.0	37.5
	22.5	22.0	25.0	30.0	29.5
	26.5	27.0	33.0	38.5	39.0
	24.0	24.5	31.5	36.5	38.5
Mean	**24.9**	**25.0**	**32.3**	**36.6**	**37.4**
SD	**2.4**	**2.4**	**4.0**	**3.5**	**4.2**
T1 L	25.0	33.0	38.5	42.0	42.0
	28.5	33.0	35.5	42.5	43.5
	29.5	32.0	35.0	38.5	38.5
	30.5	33.0	34.0	37.5	37.0
	22.5	30.0	32.0	38.0	38.5
	28.5	34.0	33.5	36.5	35.5
Mean	**27.4**	**32.5**	**34.8**	**39.2**	**39.2**
SD	**3.0**	**1.4**	**2.2**	**2.5**	**3.0**
T1 I	22.5	24.5	28.0	32.5	30.0

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
	26.5	27.5	28.5	32.5	32.5
	27.0	Dead	Dead	Dead	Dead
	24.0	27.0	32.5	39.5	37.5
	30.5	32.0	30.0	34.0	32.5
	27.0	31.0	31.5	34.6	32.0
Mean	**26.3**	**28.4**	**30.1**	**34.6**	**32.9**
SD	**2.8**	**3.1**	**1.9**	**2.9**	**2.8**
T1 H	22.0	26.5	30.5	37.0	35.5
	20.5	27.0	30.5	39.0	37.0
	26.5	29.5	20.5	28.0	27.0
	23.5	27.5	29.0	34.0	32.0
	32.0	30.0	Dead	Dead	Dead
	22.5	27.0	29.5	36.0	35.0
Mean	**24.5**	**27.9**	**28.0**	**34.8**	**33.3**
SD	**4.2**	**1.5**	**4.2**	**4.2**	**4.0**
T2 L	24.0	24.5	26.0	28.5	26.5
	29.0	33.5	35.5	39.5	38.0
	30.5	32.5	32.5	35.0	30.0
	28.0	26.5	26.5	31.0	28.0
	22.5	30.5	34.0	35.0	29.0
	25.5	31.5	37.0	42.5	40.5
Mean	**26.6**	**29.8**	**31.9**	**35.3**	**32.0**
SD	**3.1**	**3.6**	**4.6**	**5.2**	**5.8**
T2 I	19.5	23.0	26.5	25.5	24.5
	21.5	27.0	33.5	40.0	40.0
	29.0	28.5	Dead	Dead	Dead
	30.0	30.5	30.5	32.0	30.0
	30.0	37.0	37.0	38.5	36.5
	30.5	34.0	34.0	38.5	35.5
Mean	**26.8**	**30.0**	**32.3**	**34.9**	**33.3**
SD	**4.9**	**5.0**	**4.0**	**6.1**	**6.1**

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
T2 H	29.0	30.5	29.0	Dead	Dead
	29.5	39.0	40.0	46.5	44.5
	23.0	32.5	37.5	42.0	38.5
	25.5	29.5	30.0	35.0	33.5
	28.0	32.0	32.5	37.0	35.0
	28.0	35.0	28.5	28.5	28.0
Mean	**27.2**	**33.1**	**32.9**	**37.8**	**35.9**
SD	**2.5**	**3.5**	**4.8**	**6.9**	**6.1**

Table No.32 – Statistical analysis of Body weight of mice in Sub Acute Oral Toxicity studies at Day 0

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-2.500	1.281	No	ns	-7.771 to 2.771
NC vs T1I	-1.333	0.6830	No	ns	-6.605 to 3.938
NC vs T1H	0.4167	0.2134	No	ns	-4.855 to 5.688
NC vs T2L	-1.667	0.8537	No	ns	-6.938 to 3.605
NC vs T2I	-1.833	0.9391	No	ns	-7.105 to 3.438
NC vs T2H	-2.250	1.152	No	ns	-7.521 to 3.021

Graph -2 : Body weight of mice in Sub Acute Oral Toxicity studies at Day 0

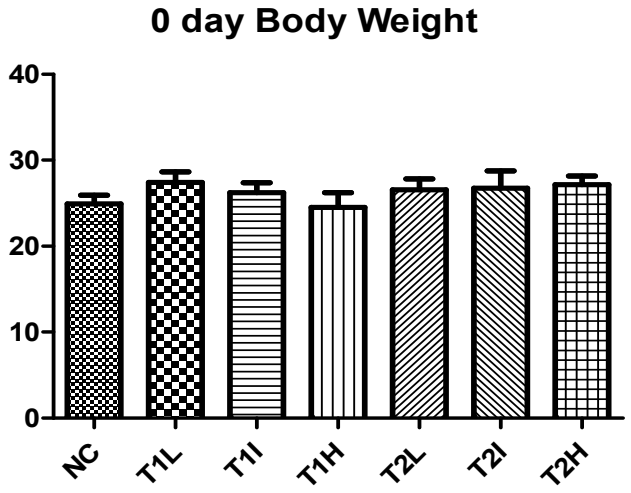
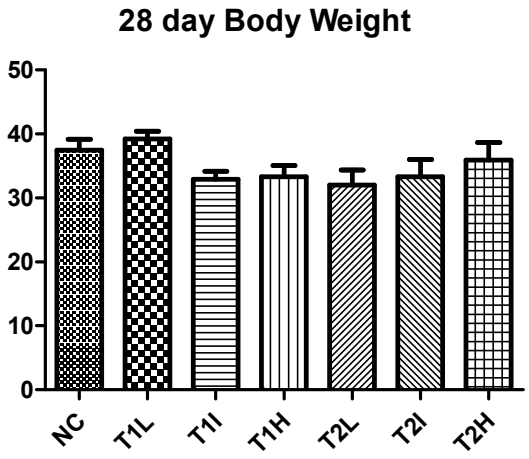


Table No.33– Statistical analysis of Body weight of mice in Sub Acute Oral Toxicity studies at Day 28

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-1.750	0.6413	No	ns	-9.161 to 5.661
NC vs T1I	4.517	1.578	No	ns	-3.257 to 12.29
NC vs T1H	4.117	1.438	No	ns	-3.656 to 11.89
NC vs T2L	5.417	1.985	No	ns	-1.995 to 12.83
NC vs T2I	4.117	1.438	No	ns	-3.656 to 11.89
NC vs T2H	1.517	0.5299	No	ns	-6.257 to 9.290

Graph – 3 :Body weight of mice in Sub Acute Oral Toxicity studies at Day 28



Body Weight

Non significant (p <0.05) increase in body weight was observed in all the groups.

2. Effect on Food Consumption **-**

Food Consumption Data in grams Total Quantity of Food = 50 gms

R= Remained food C = Consumed food C/A = Consumed per animals

Table No.34– Food consumption of mice in Sub Acute Oral Toxicity studies

	0 Day			7 Day			14 Day			21 Day			28 Day		
	R	C	C/A	R	C	C/A	R	C	C/A	R	C	C/A	R	C	C/A
NC	25.0	25.0	4.2	20.5	29.5	4.9	21.5	28.5	4.8	20.0	30.0	5.0	18.5	31.5	5.3
T1 L	6.0	44.0	7.3	19.5	30.5	5.1	22.5	27.5	4.6	18.5	31.5	5.3	9.5	40.5	6.8
T1 I	25.5	24.5	4.1	26.0	24.0	4.0	27.5	22.5	3.8	23.0	27.0	4.5	18.0	32.0	5.3
T1 H	26.0	24.0	4.0	24.5	25.5	4.3	24.0	26.0	4.3	27.0	23.0	3.8	30.0	20.0	3.3
T2 L	10.5	39.5	6.6	25.5	24.5	4.1	25.0	25.0	4.2	26.0	24.0	4.0	23.5	26.5	4.4
T2 I	20.0	30.0	5.0	29.0	21.0	3.5	27.0	23.0	3.8	23.5	26.5	4.4	25.0	25.0	4.2
T2 H	3.0	47.0	7.8	20.0	30.0	5.0	22.5	27.5	4.6	22.0	28.0	4.7	30.0	20.0	3.3

Daily food and water consumption was found to be normal in control group. In the treatment groups, quantity of food was maintained and normal in T1L and T1I whereas in the remaining groups i.e T1H, T2L, T2I and T2H the quantity of food decreased day by day.

3. Effect on Organ weight –

Table No.35 – Effect on organ weight of mice in Sub Acute Oral Toxicity studies

		Adrenals	Heart	Kidneys	Liver	Spleen	Lungs	Testies	Ovaries	Brain	Pancreas
Group 1	1	0.009	0.27	0.616	2.1	0.144	0.26	2.463	0.225	0.41	0.15
	2	0.01	0.19	0.578	2.246	0.186	0.306	2.67	0.211	0.423	0.193
	3	0.005	0.15	0.505	2.042	0.144	0.271	2.233	0.235	0.403	0.148
	4	0.008	0.156	0.327	1.464	0.168	0.226			0.394	0.155
	5	0.011	0.196	0.538	2.29	0.192	0.203			0.39	0.156
	6	0.005	0.188	0.662	1.796	0.186	0.207			0.428	0.158
	Mean	0.008	0.192	0.538	1.99	0.17	0.246	2.455	0.224	0.408	0.16
	SD	0.003	0.043	0.117	0.311	0.022	0.04	0.219	0.012	0.015	0.017
Group 2	1	0.008	0.191	0.548	2.436	0.208	0.24	2.463	0.256	0.46	0.152
	2	0.01	0.221	0.604	2.036	0.217	0.267	2.67	0.243	0.412	0.201
	3	0.006	0.204	0.564	1.99	0.186	0.26	2.233	0.25	0.372	0.165
	4	0.01	0.234	0.571	1.835	0.157	0.26			0.415	0.188
	5	0.01	0.174	0.463	1.83	0.211	0.253			0.394	0.158
	6	0.007	0.192	0.502	1.821	0.117	0.256			0.354	0.173
	Mean	0.009	0.203	0.542	1.991	0.183	0.256	2.455	0.25	0.401	0.173
	SD	0.002	0.022	0.051	0.236	0.039	0.009	0.219	0.007	0.037	0.019
Group 3	1	0.006	0.232	0.42	1.679	0.16	0.26	2.463	0.235	0.402	0.145
	2	0.008	0.263	0.443	2.019	0.158	0.27	2.67	0.262	0.407	0.168

		Adrenals	**Heart**	**Kidneys**	**Liver**	**Spleen**	**Lungs**	**Testies**	**Ovaries**	**Brain**	**Pancreas**
	3	Dead	Dead	Dead	Dead	Dead	Dead	Dead	0.218	Dead	Dead
	4	0.01	0.178	0.525	2.312	0.199	0.274			0.372	0.168
	5	0.009	0.161	0.51	1.198	0.175	0.227			0.38	0.177
	6	0.007	0.155	0.479	1.653	0.16	0.209			0.394	0.164
	Mean	0.008	0.198	0.475	1.772	0.17	0.248	1.711	0.238	0.391	0.164
	SD	0.002	0.047	0.044	0.42	0.017	0.029	1.485	0.022	0.015	0.012
Group 4	1	0.008	0.191	0.511	2.295	0.173	0.269	2.463	0.242	0.402	0.177
	2	0.009	0.185	0.43	2.27	0.2	0.254	2.67	Dead	0.374	0.196
	3	0.01	0.168	0.4	1.44	0.182	0.238	2.233	0.256	0.392	0.154
	4	0.011	0.182	0.504	1.718	0.18	0.234			0.388	0.143
	5	Dead	Dead	Dead	Dead	Dead	Dead			Dead	Dead
	6	0.009	0.174	0.523	1.895	0.117	0.203			0.4	0.138
	Mean	0.009	0.18	0.474	1.924	0.17	0.24	2.455	0.166	0.391	0.162
	SD	0.001	0.009	0.055	0.366	0.031	0.025	0.219	0.144	0.011	0.024
Group 5	1	0.009	0.12	0.375	1.89	0.112	0.245	2.463	0.237	0.356	0.157
	2	0.008	0.159	0.509	1.775	0.125	0.22	2.67	0.215	0.38	0.141
	3	0.007	0.212	0.426	1.88	0.199	0.235	2.233	0.24	0.364	0.151
	4	0.009	0.186	0.456	1.652	0.158	0.245			0.45	0.134
	5	0.01	0.19	0.424	1.448	0.19	0.255			0.28	0.143
	6	0.011	0.2	0.59	2.766	0.236	0.275			0.425	0.188
	Mean	0.009	0.178	0.463	1.902	0.17	0.246	2.455	0.231	0.376	0.152
	SD	0.001	0.033	0.076	0.454	0.047	0.019	0.219	0.014	0.059	0.019
Group 6	1	0.005	0.15	0.425	1.263	0.209	0.27	2.463	0.233	0.387	0.112
	2	0.007	0.192	0.556	2.369	0.194	0.275	2.67	0.227	0.394	0.173
	3	Dead	Dead	Dead	Dead	Dead	Dead	Dead	0.248	Dead	Dead
	4	0.009	0.184	0.427	1.598	0.18	0.185			0.419	0.149
	5	0.009	0.186	0.656	1.715	0.195	0.25			0.39	0.183
	6	0.01	0.193	0.472	1.996	0.172	0.255			0.401	0.155
	Mean	0.008	0.181	0.507	1.788	0.19	0.247	1.711	0.236	0.398	0.154
	SD	0.002	0.018	0.099	0.418	0.014	0.036	1.485	0.011	0.013	0.027
Group 7	1	Dead	Dead	Dead	Dead	Dead	Dead	Dead	0.25	Dead	Dead
	2	0.009	0.26	0.504	2.107	0.188	0.258	2.463	0.236	0.427	0.177
	3	0.008	0.191	0.535	2.077	0.152	0.223	2.67	0.248	0.375	0.185
	4	0.008	0.184	0.4	1.798	0.193	0.246			0.421	0.193
	5	0.01	0.186	0.482	1.81	0.188	0.271			0.382	0.16
	6	0.01	0.173	0.453	1.182	0.217	0.231			0.4	0.181
	Mean	0.009	0.199	0.475	1.795	0.188	0.246	1.711	0.245	0.401	0.179
	SD	0.001	0.035	0.051	0.372	0.023	0.02	1.485	0.008	0.023	0.012

Table No.36 – Statistical analysis of weight of heart of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-0.01100	0.5876	No	ns	-0.06184 to 0.03984
NC vs T1I	-0.006133	0.3124	No	ns	-0.05945 to 0.04719
NC vs T1H	0.01167	0.5943	No	ns	-0.04165 to 0.06499
NC vs T2L	0.01383	0.7390	No	ns	-0.03701 to 0.06467
NC vs T2I	0.01067	0.5433	No	ns	-0.04265 to 0.06399
NC vs T2H	-0.007133	0.3633	No	ns	-0.06045 to 0.04619

Graph – 4 : Weight of heart of mice in Sub Acute Oral Toxicity studies

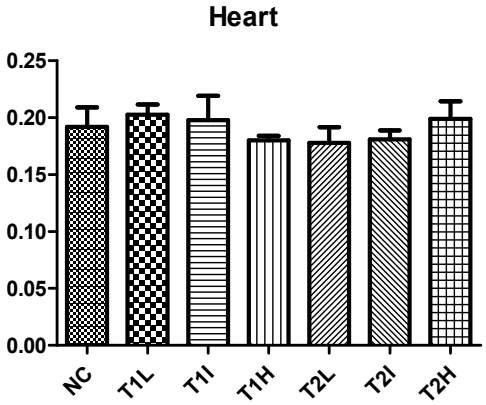


Table No. 37 – Statistical analysis of weight of kidney of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	0.03850	0.4119	No	ns	-0.2154 to 0.2924
NC vs T1I	-0.02080	0.2122	No	ns	-0.2871 to 0.2455
NC vs T1H	-0.005200	0.05304	No	ns	-0.2715 to 0.2611
NC vs T2L	-0.02867	0.3067	No	ns	-0.2825 to 0.2252
NC vs T2I	-0.1100	1.122	No	ns	-0.3763 to 0.1563
NC vs T2H	0.08280	0.8446	No	ns	-0.1835 to 0.3491

Graph – 5 : Weight of kidney of mice in Sub Acute Oral Toxicity studies

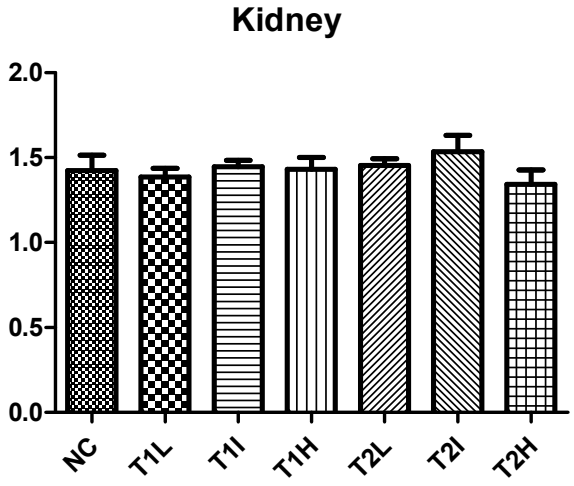


Table No. 38 – Statistical analysis of weight of adrenals of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-0.0005000	0.5008	No	ns	-0.003212 to 0.002212
NC vs T1I	-0.000000000931	0.0000008894	No	ns	-0.002844 to 0.002844
NC vs T1H	-0.0014	1.337	No	ns	-0.004244 to 0.001444
NC vs T2L	-0.0010	1.002	No	ns	-0.003712 to 0.001712
NC vs T2I	0.0000	0.0000	No	ns	-0.002844 to 0.002844
NC vs T2H	-0.0010	0.9550	No	ns	-0.003844 to 0.001844

Graph – 6: Weight of adrenals of mice in Sub Acute Oral Toxicity studies

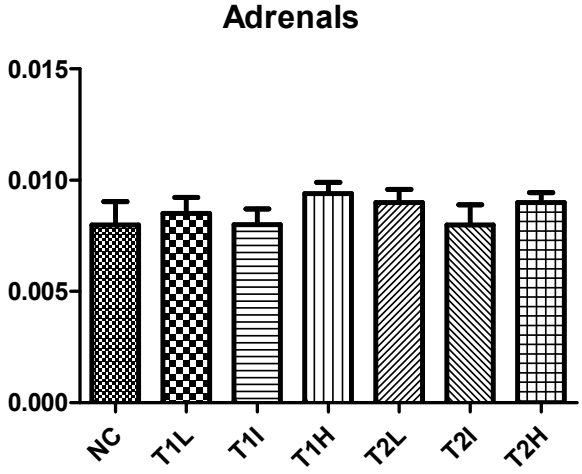


Table No. 39 – Statistical analysis of weight of Liver of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-0.001667	0.007763	No	ns	-0.5847 to 0.5814
NC vs T1I	0.2175	0.9658	No	ns	-0.3941 to 0.8290
NC vs T1H	0.06607	0.2934	No	ns	-0.5455 to 0.6776
NC vs T2L	0.08783	0.4091	No	ns	-0.4952 to 0.6709
NC vs T2I	0.2015	0.8948	No	ns	-0.4101 to 0.8130
NC vs T2H	0.1949	0.8655	No	ns	-0.4167 to 0.8064

Graph – 7 : Weight of Liver of mice in Sub Acute Oral Toxicity studies

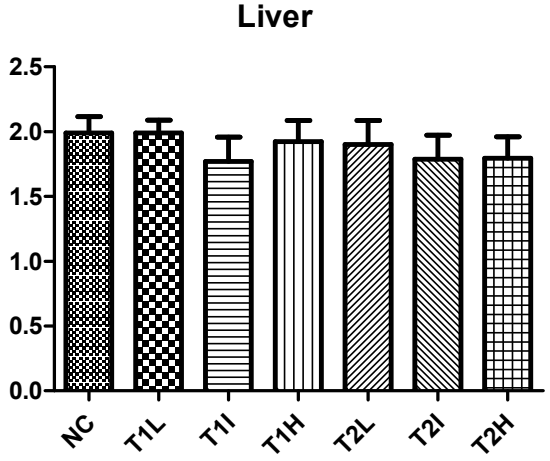


Table No.40– Statistical analysis of weight of Spleen of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-0.01267	0.7143	No	ns	-0.06083 to 0.03550
NC vs T1I	-0.0004000	0.02151	No	ns	-0.05091 to 0.05011
NC vs T1H	-0.0004000	0.02151	No	ns	-0.05091 to 0.05011
NC vs T2L	0.0000	0.0000	No	ns	-0.04816 to 0.04816
NC vs T2I	-0.0200	1.075	No	ns	-0.07051 to 0.03051
NC vs T2H	-0.0176	0.9463	No	ns	-0.06811 to 0.03291

Graph – 8 : Weight of Spleen of mice in Sub Acute Oral Toxicity studies

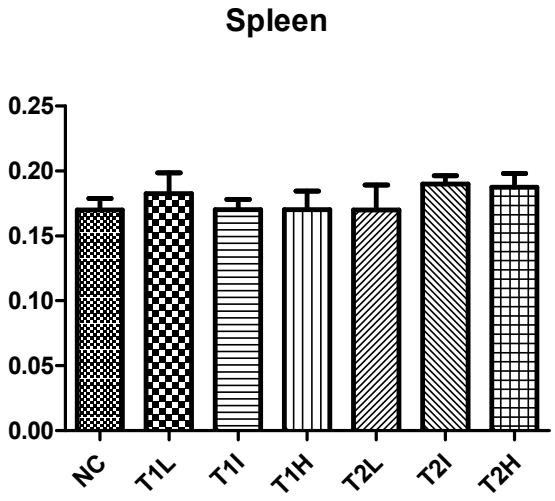


Table No.41 – Statistical analysis of weight of lungs of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-0.02883	1.683	No	ns	-0.07535 to 0.01769
NC vs T1I	-0.02083	1.160	No	ns	-0.06962 to 0.02796
NC vs T1H	-0.01243	0.6921	No	ns	-0.06122 to 0.03636
NC vs T2L	-0.01867	1.090	No	ns	-0.06519 to 0.02785
NC vs T2I	-0.01983	1.104	No	ns	-0.06862 to 0.02896
NC vs T2H	-0.01863	1.037	No	ns	-0.06742 to 0.03016

Graph – 9 : Weight of Lungs of mice in Sub Acute Oral Toxicity studies

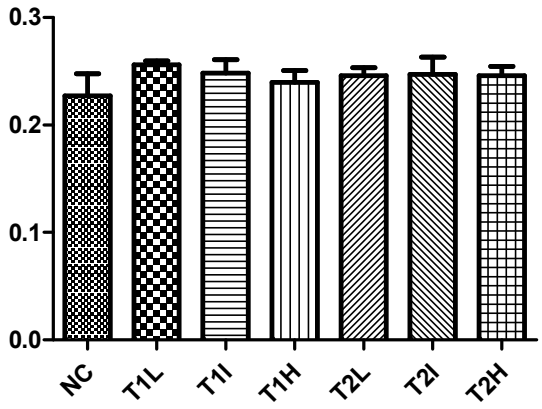


Table No.42 – Statistical analysis of weight of testies of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-0.004833	0.4625	No	ns	-0.03322 to 0.02355
NC vs T1I	-0.001067	0.09732	No	ns	-0.03083 to 0.02870
NC vs T1H	-0.01507	1.375	No	ns	-0.04483 to 0.01470
NC vs T2L	-0.003333	0.3190	No	ns	-0.03172 to 0.02505
NC vs T2I	-0.01127	1.028	No	ns	-0.04103 to 0.01850
NC vs T2H	-0.006467	0.5900	No	ns	-0.03623 to 0.02330

Graph – 10: Weight of Testies of mice in Sub Acute Oral Toxicity studies

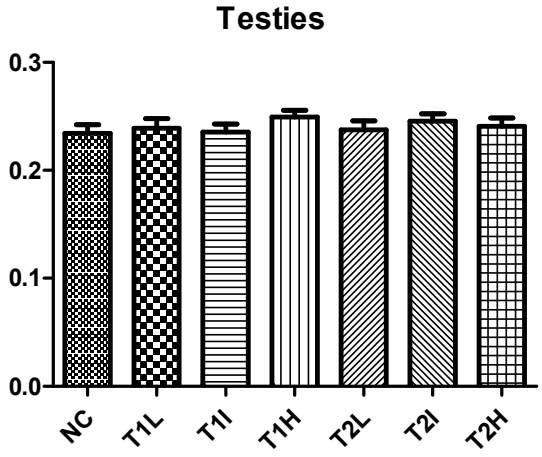


Table No.43 – Statistical analysis of weight of brain of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	0.006833	0.3814	No	ns	-0.04183 to 0.05550
NC vs T1I	0.01700	0.9046	No	ns	-0.03404 to 0.06804
NC vs T1H	0.01680	0.8940	No	ns	-0.03424 to 0.06784
NC vs T2L	0.03217	1.795	No	ns	-0.01650 to 0.08083
NC vs T2I	0.009800	0.5215	No	ns	-0.04124 to 0.06084
NC vs T2H	0.007000	0.3725	No	ns	-0.04404 to 0.05804

Graph – 11 : Weight of Brain of mice in Sub Acute Oral Toxicity studies

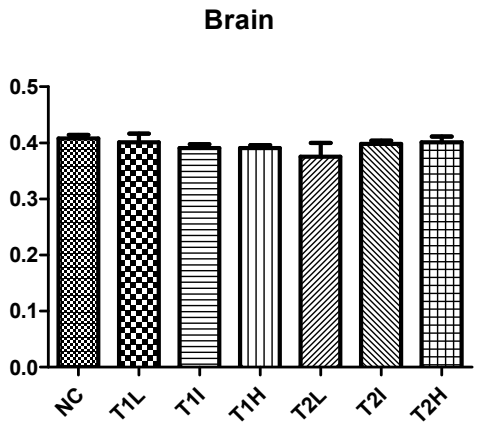
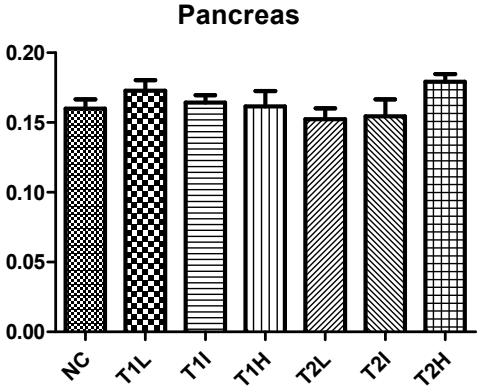


Table No.44 – Statistical analysis of weight of pancreas of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-0.01283	1.154	No	ns	-0.04303 to 0.01737
NC vs T1I	-0.0044	0.3773	No	ns	-0.03607 to 0.02727
NC vs T1H	-0.001600	0.1372	No	ns	-0.03327 to 0.03007
NC vs T2L	0.007667	0.6895	No	ns	-0.02253 to 0.03787
NC vs T2I	0.005600	0.4802	No	ns	-0.02607 to 0.03727
NC vs T2H	-0.0192	1.646	No	ns	-0.05087 to 0.01247

Graph – 12: Weight of Pancreas of mice in Sub Acute Oral Toxicity studies



4. Effect on Haematological parameters –

Table No.45 – Effect on Haematological parameters of mice in Sub Acute Oral Toxicity studies

WBC	LYM	MON	GRAN	LYM%	MON%	GRA%	RBC	HGB	HCT	MCV	MCH	MCHC	RDW	PLT	MPV	PDW	PCT
							Group 1										
7.40	5.70	0.20	1.50	76.70	3.30	20.00	10.61	14.60	43.90	41.40	13.70	33.20	20.60	582.00	5.10	14.10	0.27
9.50	16.90	0.40	2.20	86.60	2.20	11.20	9.62	14.20	43.40	45.20	14.70	32.70	12.90	558.00	5.20	14.50	0.29
6.00	4.20	0.20	1.60	69.50	4.60	25.90	9.34	12.90	39.40	42.20	13.80	32.70	13.90	643.00	5.30	14.50	0.34
7.10	4.80	0.30	2.00	67.90	4.30	27.80	8.19	11.50	36.10	44.10	14.00	31.80	13.30	544.00	4.90	14.20	0.27
7.10	8.30	0.50	4.30	63.20	4.30	32.50	9.79	13.20	40.90	41.80	13.40	32.20	19.30	751.00	5.00	14.20	0.38
7.70	5.30	0.30	2.10	69.40	3.90	26.70	9.26	13.00	40.00	43.30	14.00	32.50	14.80	769.00	5.10	14.40	0.39
7.47	**7.53**	**0.32**	**2.28**	**72.22**	**3.77**	**24.02**	**9.47**	**13.23**	**40.62**	**43.00**	**13.93**	**32.52**	**15.80**	**641.17**	**5.10**	**14.32**	**0.32**
1.15	**4.80**	**0.12**	**1.03**	**8.27**	**0.89**	**7.45**	**0.79**	**1.09**	**2.86**	**1.47**	**0.44**	**0.48**	**3.30**	**98.25**	**0.14**	**0.17**	**0.05**
							Group 2										
9.80	11.10	0.40	2.30	80.50	3.00	16.50	9.96	13.20	40.70	40.90	13.20	32.40	14.60	583.00	5.00	14.20	0.29
9.60	7.90	0.50	3.20	68.00	4.50	27.50	10.85	14.70	45.10	41.60	13.60	32.50	15.00	791.00	5.20	14.40	0.41
10.80	13.20	0.70	3.90	74.10	3.80	22.10	9.98	12.70	39.10	39.20	12.70	32.40	19.10	473.00	5.00	14.20	0.24
10.20	10.90	0.50	2.80	76.30	3.40	19.90	10.05	12.60	39.20	39.10	12.50	32.10	20.40	533.00	5.00	14.10	0.27
9.90	9.90	0.60	2.40	76.30	4.80	18.90	10.42	14.20	43.00	41.30	13.60	33.00	13.80	458.00	5.10	14.40	0.23
9.70	10.60	1.00	10.10	48.80	4.70	46.50	9.26	10.20	32.20	34.80	11.00	31.60	20.40	672.00	4.80	13.90	0.37
10.00	**10.60**	**0.62**	**4.12**	**70.67**	**4.03**	**25.23**	**10.09**	**12.93**	**39.88**	**39.48**	**12.77**	**32.33**	**17.22**	**585.00**	**5.02**	**14.20**	**0.30**
0.44	**1.73**	**0.21**	**2.99**	**11.46**	**0.74**	**11.06**	**0.53**	**1.58**	**4.42**	**2.53**	**0.98**	**0.46**	**3.07**	**127.58**	**0.13**	**0.19**	**0.07**

19.20	14.80	0.60	3.80	76.80	3.30	19.90	10.96	14.20	43.60	39.80	12.90	32.50	17.50	433.00	5.20	14.30	0.23
9.30	4.90	0.50	3.90	52.50	5.50	42.00	9.31	13.00	39.80	42.80	13.90	32.60	12.80	767.00	5.60	15.10	0.43
Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
11.90	10.00	0.30	1.60	83.70	3.00	13.30	9.81	12.90	40.50	41.30	13.10	31.80	15.10	641.00	5.20	14.40	0.33
6.50	3.80	0.40	2.30	58.40	6.10	35.50	9.90	13.70	43.70	44.20	13.80	31.30	14.90	649.00	5.20	14.70	0.34
14.60	10.00	0.60	4.00	68.20	4.60	27.20	10.42	12.90	40.40	38.80	12.30	31.90	22.50	457.00	5.10	14.20	0.23
12.30	**8.70**	**0.48**	**3.12**	**67.92**	**4.50**	**27.58**	**10.08**	**13.34**	**41.60**	**41.38**	**13.20**	**32.02**	**16.56**	**589.40**	**5.26**	**14.54**	**0.31**
4.89	**4.45**	**0.13**	**1.10**	**12.81**	**1.35**	**11.55**	**0.63**	**0.59**	**1.89**	**2.19**	**0.66**	**0.54**	**3.71**	**141.20**	**0.19**	**0.36**	**0.08**
							Group 3										
7.20	9.00	0.50	2.70	73.80	4.10	22.10	9.20	12.80	40.20	43.80	13.90	31.80	13.60	841.00	5.40	14.70	0.44
7.90	8.30	0.70	4.90	59.60	5.40	35.00	9.79	11.80	37.50	38.40	12.00	31.40	17.10	533.00	5.00	14.40	0.27
8.30	5.90	0.30	2.10	71.10	3.70	25.20	10.27	13.30	41.10	40.10	12.90	32.30	17.80	640.00	5.40	14.50	0.35
9.40	8.10	0.50	2.80	7.60	4.50	24.90	9.76	14.20	44.20	45.30	14.50	32.10	14.90	574.00	5.20	14.70	0.30
Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
8.50	8.60	0.30	1.60	81.50	3.00	15.50	10.39	13.60	42.80	41.20	13.00	31.70	17.80	458.00	5.00	14.30	0.23
8.26	**7.98**	**0.46**	**2.82**	**58.72**	**4.14**	**24.54**	**9.88**	**13.14**	**41.16**	**41.76**	**13.26**	**31.86**	**16.24**	**609.20**	**5.20**	**14.52**	**0.32**
0.81	**1.21**	**0.17**	**1.26**	**29.64**	**0.90**	**7.03**	**0.47**	**0.90**	**2.56**	**2.79**	**0.97**	**0.35**	**1.90**	**145.42**	**0.20**	**0.18**	**0.08**
							Group 4										
7.30	4.90	0.30	2.10	67.80	4.00	28.20	8.55	12.50	38.60	45.20	14.60	32.30	13.70	563.00	5.10	14.60	0.29
9.50	6.30	0.50	2.70	66.20	5.10	28.70	8.60	10.90	33.90	39.50	12.60	32.10	13.70	469.00	5.20	14.60	0.24
8.20	8.30	0.70	4.20	93.10	5.10	31.80	8.84	12.40	39.30	44.50	14.30	32.30	14.90	576.00	5.40	14.60	0.31
9.00	6.60	0.40	2.00	72.90	4.90	22.20	9.13	12.60	39.50	43.30	13.80	31.80	13.60	462.00	4.80	14.30	0.22
9.60	9.30	0.40	1.90	79.90	3.60	16.50	10.26	14.10	42.90	41.90	13.70	32.80	12.20	647.00	5.10	14.50	0.33

8.50	9.10	0.30	2.10	78.80	3.20	18.00	9.89	11.30	35.90	36.40	11.40	31.40	26.30	660.00	5.30	14.40	0.35
8.68	**7.42**	**0.43**	**2.50**	**76.45**	**4.32**	**24.23**	**9.21**	**12.30**	**38.35**	**41.80**	**13.40**	**32.12**	**15.73**	**562.83**	**5.15**	**14.50**	**0.29**
0.87	**1.76**	**0.15**	**0.88**	**9.87**	**0.83**	**6.26**	**0.71**	**1.13**	**3.12**	**3.33**	**1.19**	**0.48**	**5.25**	**84.46**	**0.21**	**0.13**	**0.05**
							Group 5										
8.50	6.70	0.40	2.40	69.70	4.70	25.60	9.45	12.60	39.50	41.80	13.30	31.80	12.60	530.00	5.20	14.50	0.28
5.80	3.90	0.30	1.60	67.70	4.50	27.80	11.18	12.80	41.70	37.30	11.40	30.60	22.70	569.00	5.50	14.40	0.31
Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
9.50	8.80	0.50	3.20	70.30	3.80	25.90	11.35	16.20	51.40	45.30	14.20	31.50	16.70	843.00	5.40	14.80	0.46
8.40	9.20	0.30	1.90	80.30	2.90	16.80	8.63	12.20	37.90	44.00	14.10	32.10	15.90	777.00	5.20	14.60	0.40
8.90	8.90	0.60	3.40	68.70	5.10	26.20	9.39	12.20	38.20	40.70	12.90	31.90	15.50	572.00	5.00	14.40	0.29
8.22	**7.50**	**0.42**	**2.50**	**71.34**	**4.20**	**24.46**	**10.00**	**13.20**	**41.74**	**41.82**	**13.18**	**31.58**	**16.68**	**658.20**	**5.26**	**14.54**	**0.35**
1.42	**2.24**	**0.13**	**0.79**	**5.11**	**0.87**	**4.37**	**1.20**	**1.70**	**5.60**	**3.10**	**1.13**	**0.59**	**3.70**	**141.50**	**0.19**	**0.17**	**0.08**
							Group 6										
Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
6.20	4.30	0.30	1.60	70.10	4.50	25.40	8.60	11.20	35.70	41.60	13.00	31.30	16.70	544.00	5.40	14.70	0.29
8.00	11.30	0.50	2.20	80.60	4.00	15.40	10.89	13.60	42.70	39.30	12.40	31.80	16.70	537.00	5.10	14.20	0.27
9.20	10.00	0.60	3.60	70.50	4.20	25.30	9.06	13.30	42.10	46.50	14.60	31.50	14.30	674.00	5.20	14.80	0.35
7.60	10.80	0.50	3.30	74.00	3.10	22.90	10.72	14.10	45.30	42.30	13.10	31.10	18.50	604.00	5.30	14.40	0.32
7.80	7.30	1.10	10.40	38.80	5.70	55.50	10.40	13.40	41.90	40.30	12.80	31.90	16.30	459.00	5.40	14.60	0.25
7.76	**8.74**	**0.60**	**4.22**	**66.80**	**4.30**	**28.90**	**9.93**	**13.12**	**41.54**	**42.00**	**13.18**	**31.52**	**16.50**	**563.60**	**5.28**	**14.54**	**0.30**
1.07	**2.92**	**0.30**	**3.55**	**16.21**	**0.94**	**15.42**	**1.04**	**1.12**	**3.54**	**2.77**	**0.84**	**0.33**	**1.50**	**80.39**	**0.13**	**0.24**	**0.04**
								Group 7									
Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead

6.20	4.30	0.30	1.60	70.10	4.50	25.40	8.60	11.20	35.70	41.60	13.00	31.30	16.70	544.00	5.40	14.70	0.29
8.00	11.30	0.50	2.20	80.60	4.00	15.40	10.89	13.60	42.70	39.30	12.40	31.80	16.70	537.00	5.10	14.20	0.27
9.20	10.00	0.60	3.60	70.50	4.20	25.30	9.06	13.30	42.10	46.50	14.60	31.50	14.30	674.00	5.20	14.80	0.35
7.60	10.80	0.50	3.30	74.00	3.10	22.90	10.72	14.10	45.30	42.30	13.10	31.10	18.50	604.00	5.30	14.40	0.32
7.80	7.30	1.10	10.40	38.80	5.70	55.50	10.40	13.40	41.90	40.30	12.80	31.90	16.30	459.00	5.40	14.60	0.25
7.76	**8.74**	**0.60**	**4.22**	**66.80**	**4.30**	**28.90**	**9.93**	**13.12**	**41.54**	**42.00**	**13.18**	**31.52**	**16.50**	**563.60**	**5.28**	**14.54**	**0.30**
1.07	**2.92**	**0.30**	**3.55**	**16.21**	**0.94**	**15.42**	**1.04**	**1.12**	**3.54**	**2.77**	**0.84**	**0.33**	**1.50**	**80.39**	**0.13**	**0.24**	**0.04**

Table No.46 – Statistical analysis of parameter WBC of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-1.367	2.029	No	ns	-3.196 to 0.4626
NC vs T1I	-1.233	1.746	No	ns	-3.152 to 0.6852
NC vs T1H	-0.7933	1.123	No	ns	-2.712 to 1.125
NC vs T2L	-1.217	1.806	No	ns	-3.046 to 0.6126
NC vs T2I	-0.7533	1.066	No	ns	-2.672 to 1.165
NC vs T2H	-0.2933	0.4153	No	ns	-2.212 to 1.625

Graph – 13 : WBC of mice in Sub Acute Oral Toxicity studies

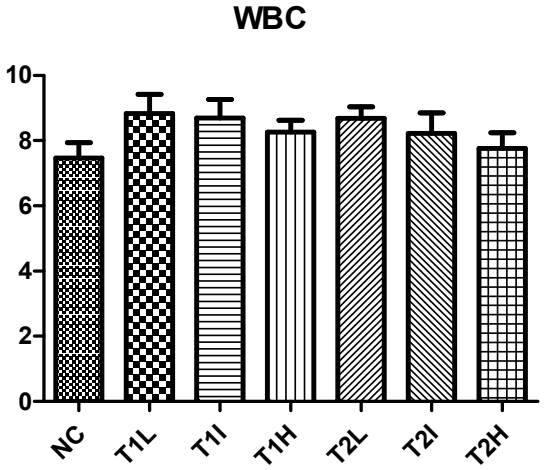


Table No. 47 – Statistical analysis of parameter RBC of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-0.6183	1.347	No	ns	-1.865 to 0.6282
NC vs T1I	-0.6117	1.271	No	ns	-1.919 to 0.6957
NC vs T1H	-0.4137	0.8594	No	ns	-1.721 to 0.8937
NC vs T2L	0.2567	0.5592	No	ns	-0.9898 to 1.503
NC vs T2I	-0.5317	1.105	No	ns	-1.839 to 0.7757
NC vs T2H	-0.4657	0.9674	No	ns	-1.773 to 0.8417

Graph – 14 : RBC of mice in Sub Acute Oral Toxicity studies

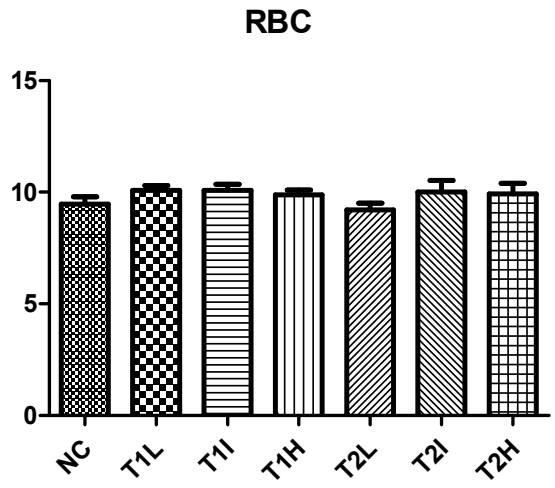


Table No. 48 – Statistical analysis of parameter Platelets of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	39.50	0.5608	No	ns	-151.8 to 230.8
NC vs T1I	51.77	0.7008	No	ns	-148.9 to 252.4
NC vs T1H	31.97	0.4327	No	ns	-168.7 to 232.6
NC vs T2L	78.33	1.112	No	ns	-113.0 to 269.6
NC vs T2I	-17.03	0.2306	No	ns	-217.7 to 183.6
NC vs T2H	77.57	1.050	No	ns	-123.1 to 278.2

Graph – 15 : Platelets of mice in Sub Acute Oral Toxicity studies

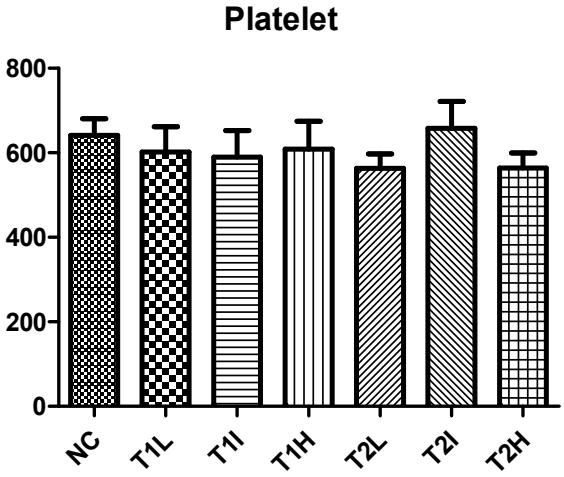


Table No. 49 – Statistical analysis of parameter Haemoglobin of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	0.3000	0.4270	No	ns	-1.608 to 2.208
NC vs T1I	-0.1067	0.1448	No	ns	-2.108 to 1.894
NC vs T1H	0.09333	0.1267	No	ns	-1.908 to 2.094
NC vs T2L	0.9333	1.329	No	ns	-0.9747 to 2.841
NC vs T2I	0.03333	0.04524	No	ns	-1.968 to 2.034
NC vs T2H	0.1133	0.1538	No	ns	-1.888 to 2.114

Graph – 16 : Haemoglobin of mice in Sub Acute Oral Toxicity studies

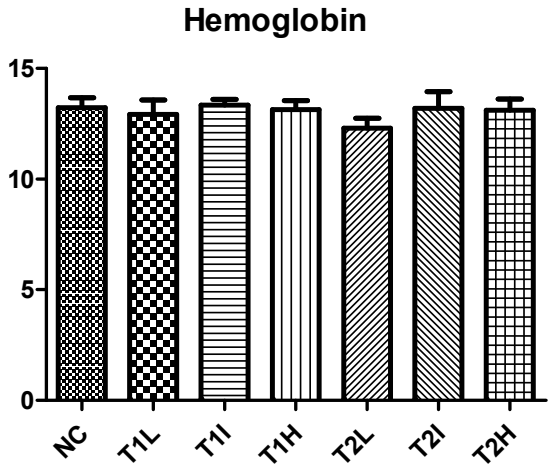
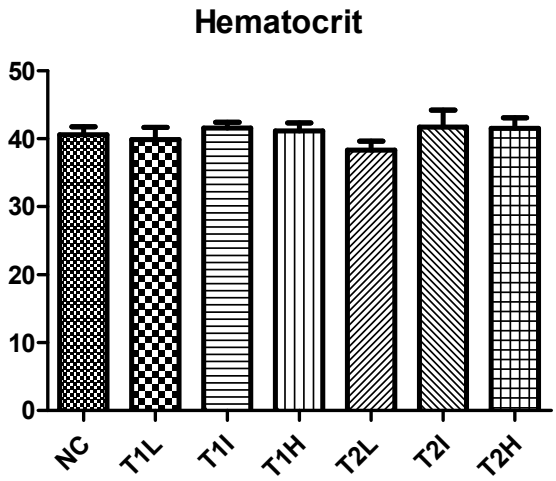


Table No.50 – Statistical analysis of parameter Hematocrit of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	0.7333	0.3520	No	ns	-4.924 to 6.391
NC vs T1I	-0.9833	0.4501	No	ns	-6.917 to 4.950
NC vs T1H	-0.5433	0.2487	No	ns	-6.477 to 5.390
NC vs T2L	2.267	1.088	No	ns	-3.391 to 7.924
NC vs T2I	-1.123	0.5142	No	ns	-7.057 to 4.810
NC vs T2H	-0.9233	0.4226	No	ns	-6.857 to 5.010

Graph – 17 : Hematocrit of mice in Sub Acute Oral Toxicity studies



5. Effect on Biochemistry parameters

Table No.51 – Effect on Biochemistry of mice in Sub Acute Oral Toxicity studies

	Animal No.	Glu	Urea	SGOT	SGPT	CHOL	TRI	HDL	PROTEIN	LDL	VLDL
NC	**1**	77.0	31.6	120.0	86.0	79.0	59.0	22.0	4.9	45.2	11.8
	2	71.0	40.2	129.0	72.9	84.0	31.0	21.0	4.9	56.8	6.2
	3	84.0	28.8	158.0	76.9	76.0	56.0	21.0	4.7	43.8	11.2
	4	87.0	34.5	162.0	81.6	96.0	104.0	27.0	5.4	48.2	20.8
	5	91.0	27.1	145.0	72.0	81.0	54.0	22.0	4.6	48.2	10.8
	6	93.0	35.3	112.0	70.0	80.0	75.0	22.0	5.1	43.0	15
	Mean	83.8	32.9	137.7	76.6	82.7	63.2	22.5	4.9	47.5	12.6
	SD	8.4	4.8	20.5	6.2	7.0	24.5	2.3	0.3	5.0	4.9
T1 L	**7**	95.0	29.7	273.0	76.4	96.0	72.0	27.0	5.0	54.6	14.4
	8	116.0	28.1	375.0	74.9	103.0	100.0	31.0	5.8	52.0	20
	9	108.0	39.0	158.0	85.0	70.0	93.0	16.0	5.1	35.4	18.6
	10	95.0	38.0	162.0	75.1	69.0	49.0	20.0	4.8	39.2	9.8
	11	82.0	35.4	135.0	81.0	73.0	59.0	18.0	4.8	43.2	11.8
	12	65.0	27.2	126.0	86.0	62.0	56.0	15.0	6.1	35.8	11.2
	Mean	93.5	32.9	204.8	79.7	78.8	71.5	21.2	5.3	43.4	14.3

	Animal No.	Glu	Urea	SGOT	SGPT	CHOL	TRI	HDL	PROTEIN	LDL	VLDL
	SD	18.3	5.2	98.7	5.0	16.6	20.9	6.4	0.6	8.2	4.2
T1 I	**13**	89.0	22.0	153.0	87.7	72.0	110.0	19.0	5.0	31.0	**22**
	14	100.0	45.9	277.0	108.2	82.0	99.0	22.0	5.4	40.2	**19.8**
	15	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
	16	83.0	40.3	142.0	86.0	82.0	106.0	27.0	4.7	33.8	**21.2**
	17	88.0	28.9	158.0	89.0	74.0	47.0	19.0	4.8	45.6	**9.4**
	18	78.0	38.0	380.0	99.7	85.0	123.0	23.0	4.6	37.4	**24.6**
	Mean	87.6	35.0	222.0	94.1	79.0	97.0	22.0	4.9	37.6	19.4
	SD	8.2	9.5	104.0	9.5	5.7	29.3	3.3	0.3	5.7	5.9
T1 H	**19**	91.0	24.2	167.0	91.2	74.0	51.0	24.0	4.4	39.8	**10.2**
	20	72.0	51.7	154.0	84.9	76.0	60.0	27.0	5.6	37.0	**12**
	21	81.0	51.8	138.0	89.0	69.0	57.0	19.0	5.3	38.6	**11.4**
	22	77.0	53.2	104.0	90.5	79.0	45.0	16.0	5.1	54.0	**9**
	23	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
	24	90.0	38.2	188.0	93.0	79.0	130.0	25.0	5.2	28.0	**26**
	Mean	82.2	43.8	150.2	89.7	75.4	68.6	22.2	5.1	39.5	13.7
	SD	8.2	12.6	31.7	3.0	4.2	34.8	4.5	0.4	9.3	7.0

	Animal No.	Glu	Urea	SGOT	SGPT	CHOL	TRI	HDL	PROTEIN	LDL	VLDL
T2 L	**25**	62.0	19.0	126.0	89.0	77.0	61.0	22.0	6.1	42.8	**12.2**
	26	94.0	30.8	221.0	129.8	77.0	99.0	25.0	5.4	32.2	**19.8**
	27	85.0	30.5	140.0	105.6	71.0	64.0	22.0	5.5	36.2	**12.8**
	28	40.0	36.6	185.0	97.0	63.0	45.0	14.0	5.8	40.0	**9**
	29	34.0	31.0	287.0	167.2	96.0	64.0	18.0	5.7	65.2	**12.8**
	30	74.0	38.6	139.0	109.0	78.0	81.0	31.0	5.9	30.8	**16.2**
T2 I	**Mean**	64.8	31.1	183.0	116.3	77.0	69.0	22.0	5.7	41.2	13.8
	SD	24.2	6.8	62.1	28.5	10.9	18.6	5.8	0.3	12.6	3.7
	31	84.0	30.3	169.0	108.4	67.0	43.0	24.0	4.6	34.4	**8.6**
	32	49.0	37.3	233.0	91.6	64.0	68.0	20.0	5.0	30.4	**13.6**
	33	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
	34	24.0	32.3	309.0	204.5	80.0	62.0	19.0	5.2	48.6	**12.4**
	35	70.0	28.2	138.0	86.0	93.0	82.0	16.0	5.8	60.6	**16.4**
	36	63.0	36.2	146.0	120.0	71.0	50.0	28.0	5.3	33.0	**10**
	Mean	58.0	32.9	199.0	122.1	75.0	61.0	21.4	5.2	41.4	12.2
	SD	22.8	3.9	71.9	48.0	11.7	15.3	4.7	0.4	12.9	3.1
T2 H	**37**	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead
	38	58.0	35.4	168.0	86.0	88.0	82.0	21.0	4.7	50.6	**16.4**
	39	72.0	28.3	113.0	107.6	83.0	85.0	29.0	4.8	37.0	**17**
	40	74.0	22.0	236.0	246.2	88.0	91.0	26.0	4.8	43.8	**18.2**

	Animal No.	Glu	Urea	SGOT	SGPT	CHOL	TRI	HDL	PROTEIN	LDL	VLDL
	41	53.0	34.3	113.0	127.3	98.0	101.0	27.0	5.2	50.8	**20.2**
	42	25.0	28.5	156.0	111.0	61.0	67.0	33.0	5.5	14.6	**13.4**
	Mean	56.4	29.7	157.2	135.6	83.6	85.2	27.2	5.0	39.4	17.0
	SD	19.7	5.4	50.6	63.5	13.8	12.5	4.4	0.3	15.0	2.5

Table No.52 – Statistical analysis of parameter Glucose of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-9.667	0.9765	No	ns	-36.55 to 17.22
NC vs T1I	-3.767	0.3628	No	ns	-31.97 to 24.43
NC vs T1H	1.633	0.1573	No	ns	-26.57 to 29.83
NC vs T2L	19.00	1.919	No	ns	-7.887 to 45.89
NC vs T2I	25.83	2.488	No	ns	-2.366 to 54.03
NC vs T2H	27.43	2.642	No	ns	-0.7660 to 55.63

Graph – 18 : Glucose of mice in Sub Acute Oral Toxicity studies

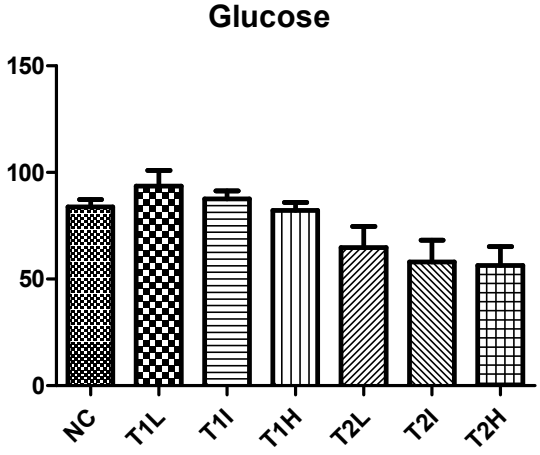


Table No.53 – Statistical analysis of parameter Urea of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	0.01667	0.003956	No	ns	-11.43 to 11.46
NC vs T1I	-2.103	0.4760	No	ns	-14.10 to 9.897
NC vs T1H	-10.90	2.468	No	ns	-22.90 to 1.097
NC vs T2L	1.833	0.4352	No	ns	-9.608 to 13.28
NC vs T2I	0.05667	0.01283	No	ns	-11.94 to 12.06
NC vs T2H	3.217	0.7280	No	ns	-8.784 to 15.22

Graph – 19 : Urea of mice in Sub Acute Oral Toxicity studies

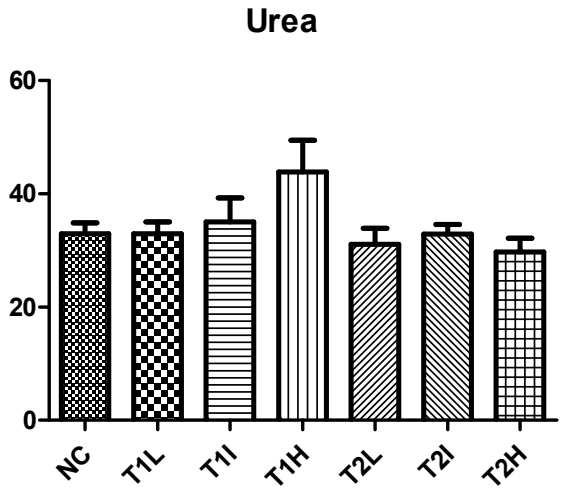


Table No.54 – Statistical analysis of parameter SGOT of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-67.17	1.682	No	ns	-175.6 to 41.29
NC vs T1I	-84.33	2.014	No	ns	-198.1 to 29.42
NC vs T1H	-12.53	0.2993	No	ns	-126.3 to 101.2
NC vs T2L	-45.33	1.135	No	ns	-153.8 to 63.12
NC vs T2I	-61.33	1.464	No	ns	-175.1 to 52.42
NC vs T2H	-19.53	0.4664	No	ns	-133.3 to 94.22

Graph – 20 : SGOT of mice in Sub Acute Oral Toxicity studies

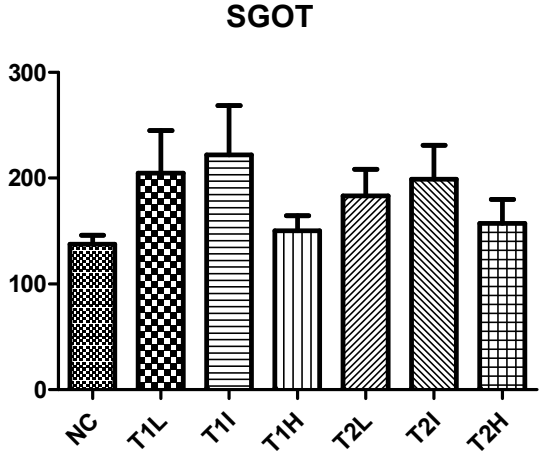


Table No.55 – Statistical analysis of parameter SGPT of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-3.167	0.1759	No	ns	-52.06 to 45.73
NC vs T1I	-17.55	0.9297	No	ns	-68.83 to 33.73
NC vs T1H	-13.15	0.6966	No	ns	-64.43 to 38.13
NC vs T2L	-39.70	2.205	No	ns	-88.59 to 9.194
NC vs T2I	-45.53	2.412	No	ns	-96.81 to 5.747
NC vs T2H	-59.05	3.128	Yes	*	-110.3 to -7.773

Graph – 21 : SGPT of mice in Sub Acute Oral Toxicity studies

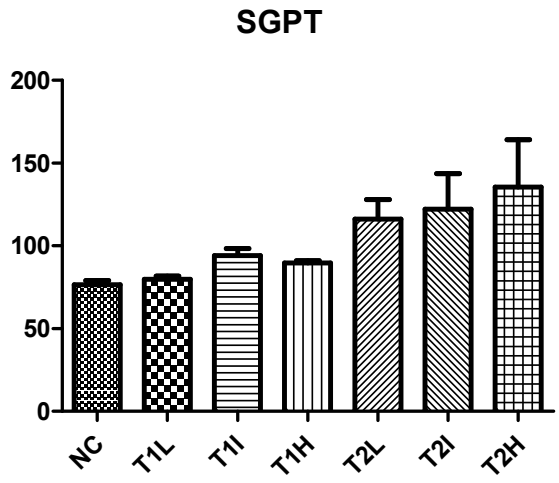


Table No.56 – Statistical analysis of parameter Cholesterol of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	3.833	0.6064	No	ns	-13.34 to 21.00
NC vs T1I	3.667	0.5530	No	ns	-14.34 to 21.67
NC vs T1H	7.267	1.096	No	ns	-10.74 to 25.27
NC vs T2L	5.667	0.8964	No	ns	-11.50 to 22.84
NC vs T2I	7.667	1.156	No	ns	-10.34 to 25.67
NC vs T2H	-0.9333	0.1408	No	ns	-18.94 to 17.07

Graph – 22 : Cholesterol of mice in Sub Acute Oral Toxicity studies

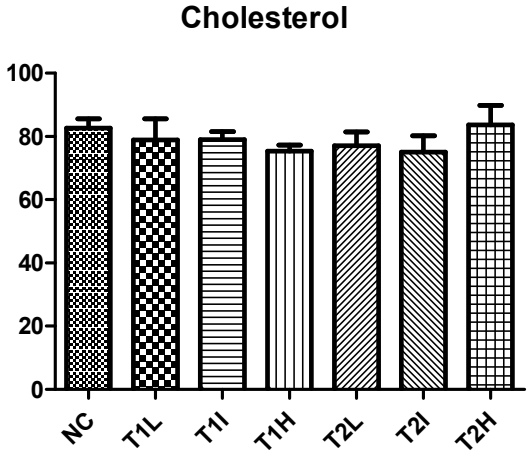


Table No.57 – Statistical analysis of parameter Triglycerides of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-8.333	0.6211	No	ns	-44.77 to 28.11
NC vs T1I	-33.83	2.404	No	ns	-72.05 to 4.386
NC vs T1H	-5.433	0.3861	No	ns	-43.65 to 32.79
NC vs T2L	-5.833	0.4348	No	ns	-42.27 to 30.61
NC vs T2I	2.167	0.1540	No	ns	-36.05 to 40.39
NC vs T2H	-22.03	1.566	No	ns	-60.25 to 16.19

Graph – 23 : Triglyceride of mice in Sub Acute Oral Toxicity studies

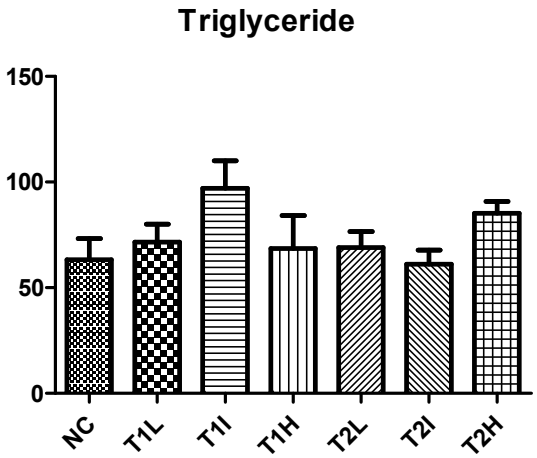


Table No.58 – Statistical analysis of parameter HDL Cholesterol of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	1.333	0.4884	No	ns	-6.081 to 8.748
NC vs T1I	0.5000	0.1746	No	ns	-7.276 to 8.276
NC vs T1H	0.3000	0.1048	No	ns	-7.476 to 8.076
NC vs T2L	0.5000	0.1831	No	ns	-6.915 to 7.915
NC vs T2I	1.100	0.3842	No	ns	-6.676 to 8.876
NC vs T2H	-4.700	1.641	No	ns	-12.48 to 3.076

Graph – 24 : HDL Cholesterol of mice in Sub Acute Oral Toxicity studies

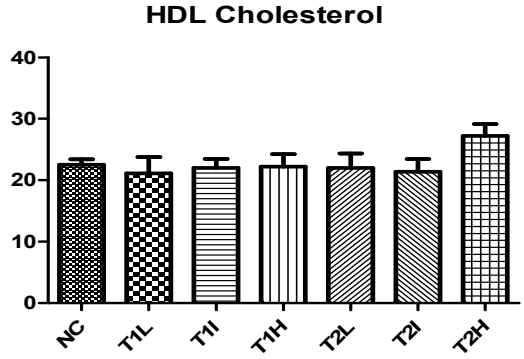
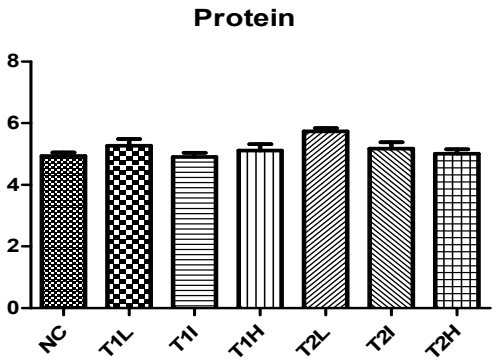


Table No.59 – Statistical analysis of parameter Protein Cholesterol of mice in Sub Acute Oral Toxicity studies

Dunnett's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
NC vs T1L	-0.3333	1.487	No	ns	-0.9423 to 0.2757
NC vs T1I	0.03333	0.1417	No	ns	-0.6054 to 0.6721
NC vs T1H	-0.1867	0.7937	No	ns	-0.8254 to 0.4521
NC vs T2L	-0.8000	3.568	Yes	**	-1.409 to -0.1910
NC vs T2I	-0.2467	1.049	No	ns	-0.8854 to 0.3921
NC vs T2H	-0.06667	0.2835	No	ns	-0.7054 to 0.5721

Graph – 25 : Protein of mice in Sub Acute Oral Toxicity studies



6. EFFECT ON HISTOPATHOLOGY -

Histopathological studies of liver and kidney were done. The results are as follows

Fig. No. 6 - HPR of Liver of Normal Control

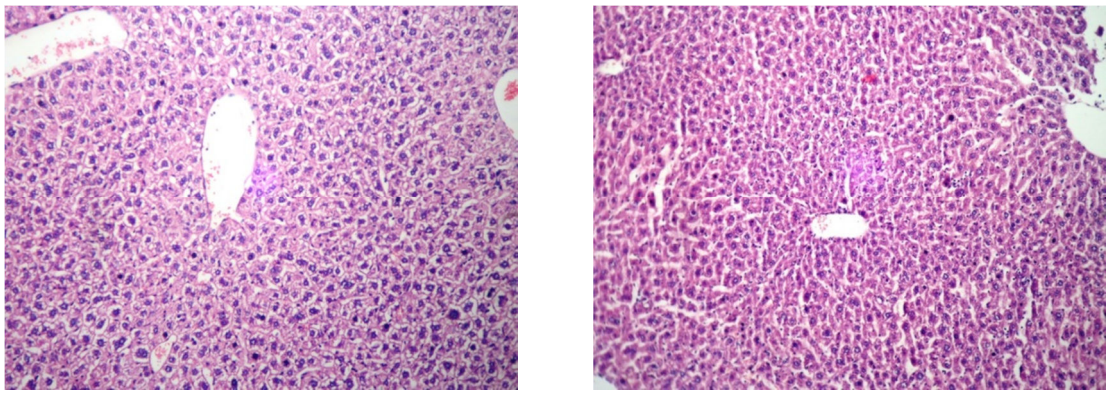


Fig. No. 7 - HPR of Liver of T1 Low dose level

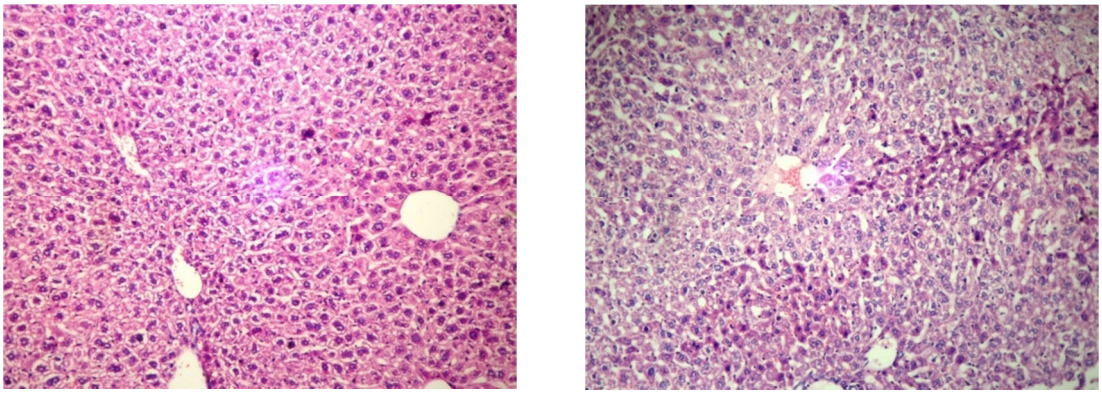


Fig. No. 8 - HPR of Liver of T1 Intermediate dose level

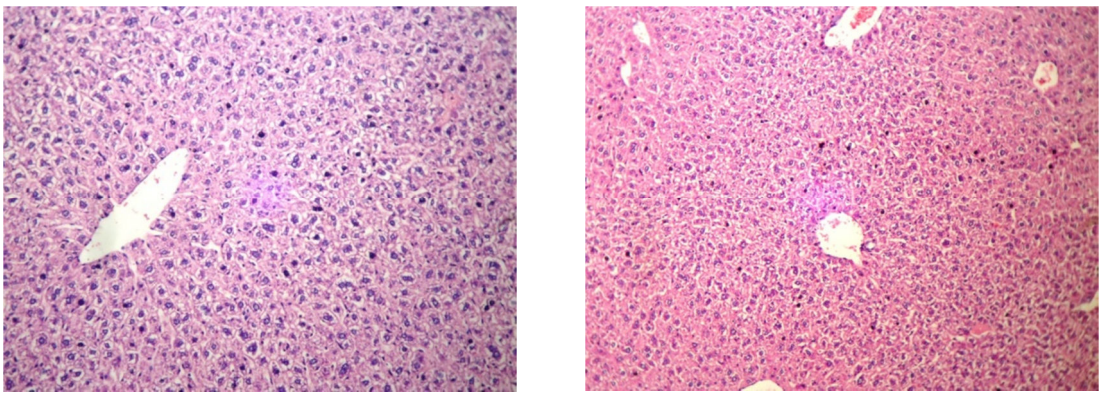


Fig. No. 9 - HPR of Liver of T1 High dose level

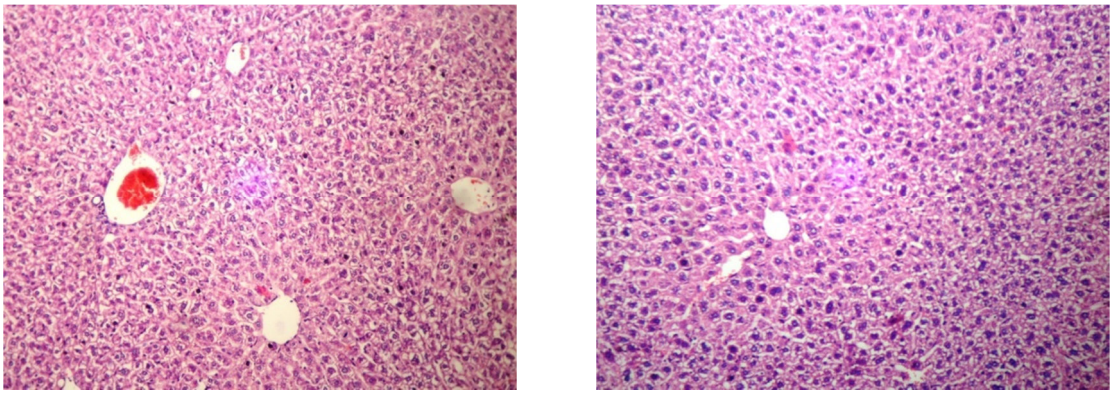


Fig. No. 10 - HPR of Liver of T2 Low dose level

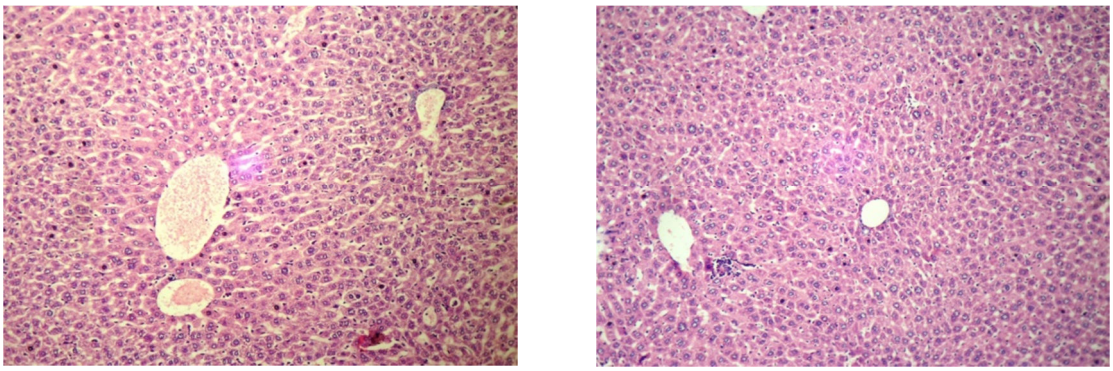


Fig. No. 11 - HPR of Liver of T2 Intermediate dose level

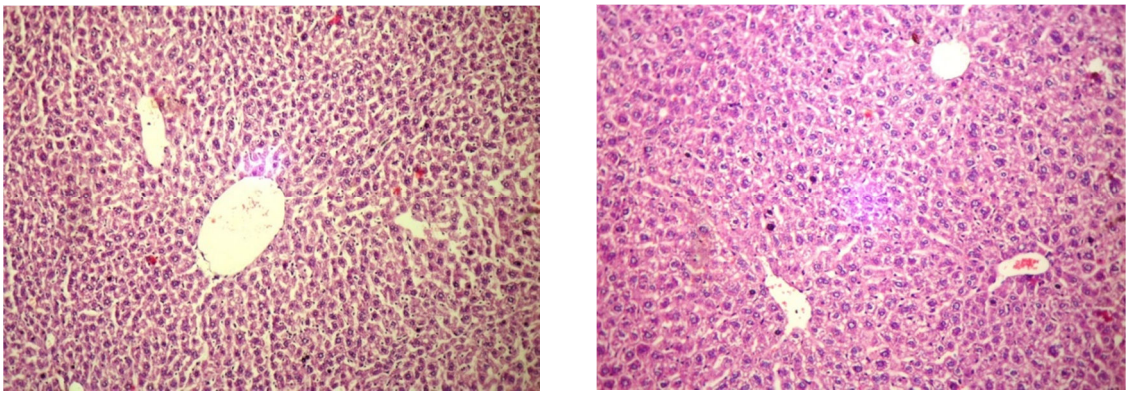
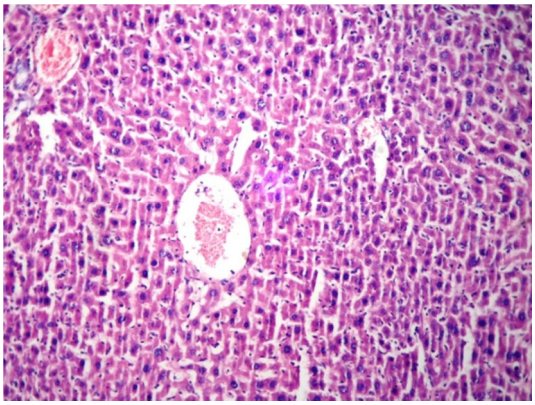


Fig. No. 12 - HPR of Liver of T2 High dose level



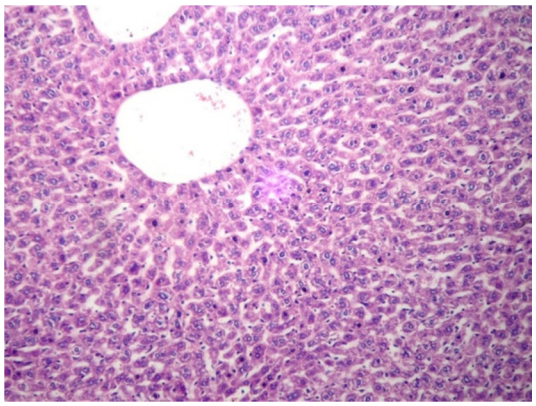


Fig. No. 13 - HPR of Kidney of Normal control

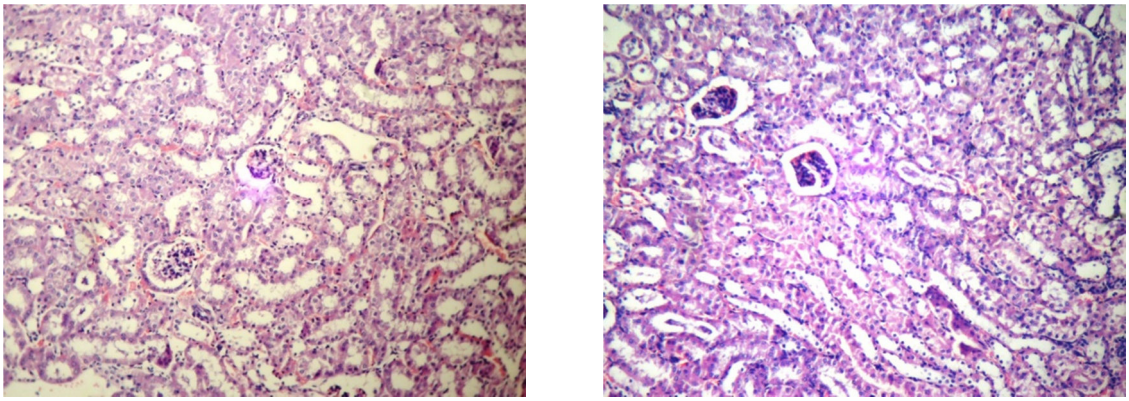


Fig. No. 14 - HPR of Kidney of T1 at Low dose level

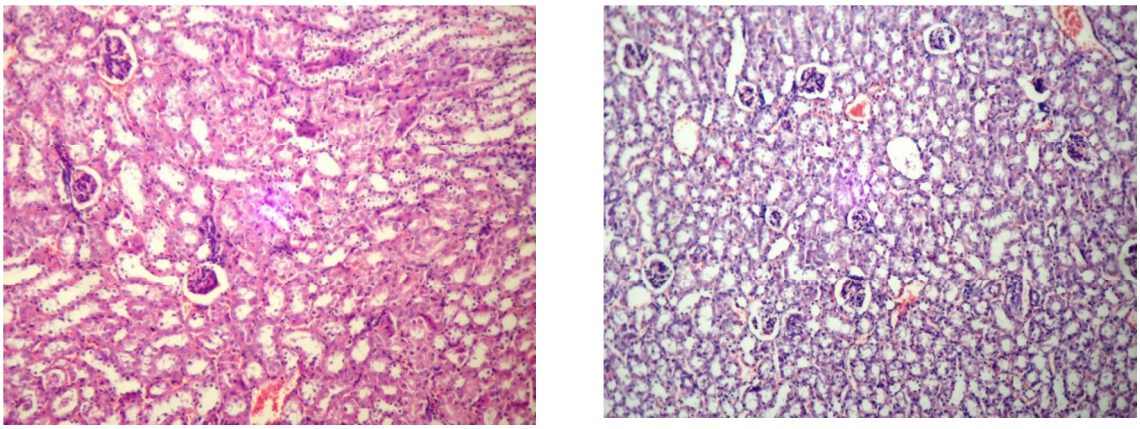


Fig. No. 15 - HPR of Kidney of T1 at Intermediate dose level

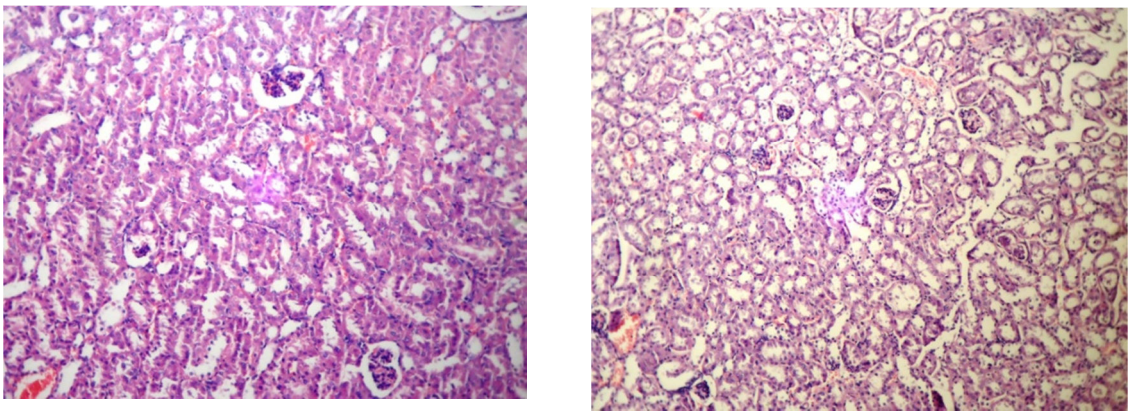


Fig. No. 16 - HPR of Kidney of T1 at High dose level

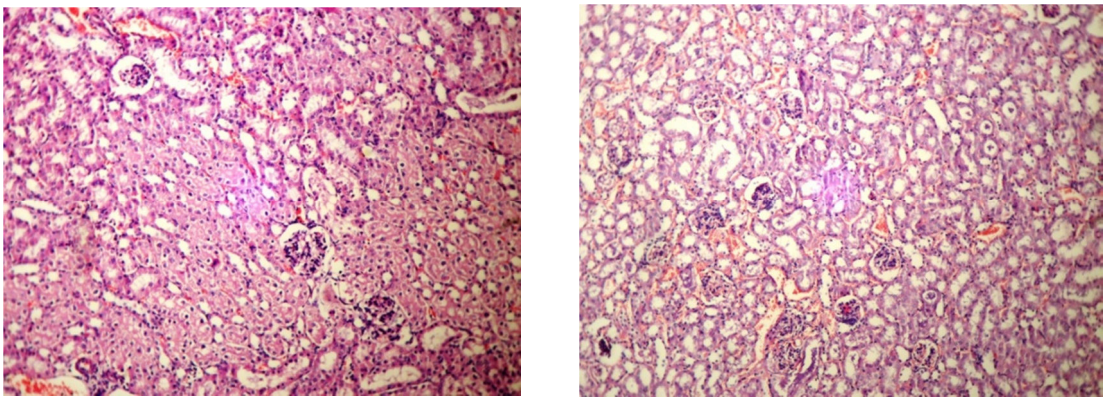


Fig. No. 17 - HPR of Kidney of T2 at Low dose level

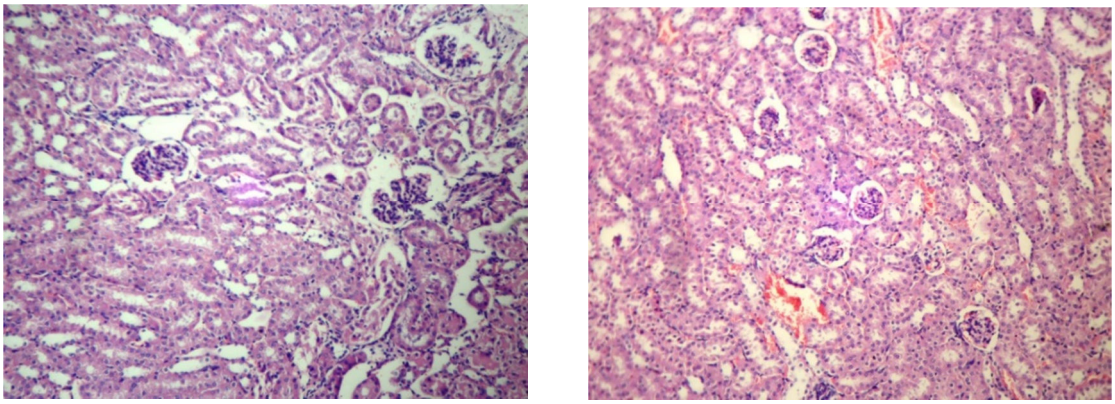


Fig. No. 18 - HPR of Kidney of T2 at Intermediate dose level

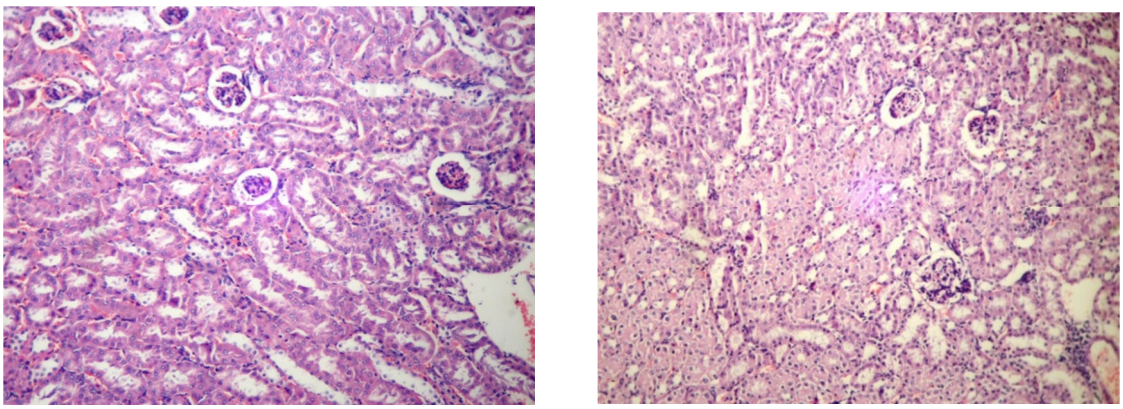


Fig. No. 19 - HPR of Kidney of T2 at High dose level

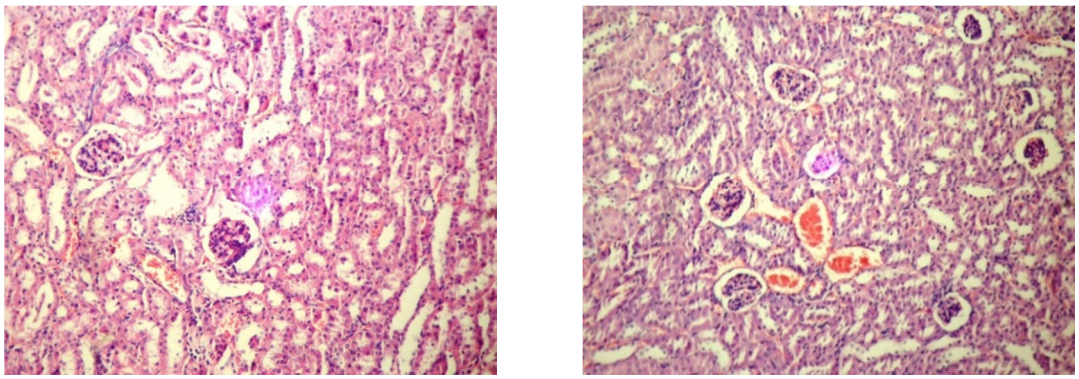


Table No.60 – HPR of Liver of drug T1 and T2 at low, intermediate and high dose levels in Sub Acute Oral Toxicity studies

1. Liver		**Referred by: APT Research Foundation, PUNE**								
Sr No	**Code No :**	Sinusoidal Congestion and hemorrhages	Focal cellular swelling , granular and vacuuolar changes in cytoplasm of hepatocytes	Degenerative changes in hepatocytes	Necrotic changes in hepatocyte, loss of nuclei etc.	Infiltration of mononuclear cells (MNC), Microgranuloma, lymphoid aggregation	Bile duct hyperplasia, etc	Fatty changes in hepatocytes	Interstitial fibrosis, Chronic Inflammatory changes etc	**OVERALL PATHOLOGICAL GRADE (LESION SCORE)**
1	**NC-H**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
2	**NC –T**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
3	**T1 Low, RLS**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
4	**T1 Low, LLS**	+ (focal)	+	+	+ (focal)	+	NAD	NAD	NAD	**Minimal (+)**
5	**T1 – Int-HBT**	NAD	NAD	+ (focal)	NAD	NAD	NAD	NAD	NAD	**NAD**
6	**T1 – Int-HT**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
7	**T1 High, B**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**

8	**T1 High, BT**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
9	**T 2 Low, H-LH**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
10	**T 2 Low, W**	+	+	+	NAD	+ (focal)	NAD	NAD	NAD	**Minimal (+)**
11	**T 2 Int T-LH**	NAD	+	+	NAD	NAD	NAD	NAD	NAD	**Minimal (+)**
12	**T 2 Int T-RH**	NAD	NAD	+	NAD	NAD	NAD	NAD	NAD	**Minimal (+)**
13	**T 2 High LH**	NAD	+ (focal)	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
14	**T 2 High B-RF**	+ (focal)	+	+	+ (focal)	NAD	NAD	NAD	NAD	**Minimal (+)**

Note : Overall Grade score as-NAD =No Abnormality Detected , **Minimal changes** (+), **Mild changes** (++), **Moderate changes** (+++), **Severe changes** (++++).

Focal changes may not be significant for alteration of functional capacity of the organ.

Table No.61 – HPR of Kidney of drug T1 and T2 at low, intermediate and high dose levels in Sub Acute Oral Toxicity studies

2.Kidney		**Referred by: APT Research Foundation, PUNE**							
Sr No	**Code No**	Congestion and hemorrhages	Focal tubular swelling , granular and vacuuolar changes in cytoplasm	Tubular degeneration, Sloughing of tubular epithelium	Necrotic changes of tubular epithelium	Infiltration of mononuclear cells (MNC)	Glomerular changes (Size, Atrophy, hypercellularity, etc)	Interstitial fibrosis	**OVERALL PATHOLOGICAL GRADE (LESION SCORE)**
1	**NC-Gr I-H**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
2	**NC-Gr I-T**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
3	**T1 Low, Gr 2-RLI**	+	+	NAD	NAD	++	NAD	NAD	**Minimal (+)**
4	**T1 Low, Gr 2-LL5**	+ (focal)	+	+	NAD	NAD	NAD	NAD	**Minimal (+)**
5	**T1 – Int-Gr 3- HBT**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
6	**T1 – Int-Gr 3- HT**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
7	**T1 High, B 4**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**

8	**T1 High, BT 4**	+ (focal)	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
9	**T 2 Low, H-LH 5**	+ (focal)	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
10	**T 2 Low, W- 5**	+ (focal)	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
11	**T 2 Int T-LH 6**	+ (focal)	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
12	**T 2 Int T-RH 6**	++	+	NAD	NAD	NAD	NAD	NAD	**Minimal(+)**
13	**T 2 High LH 7**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**
14	**T 2 High D-RF 7**	NAD	NAD	NAD	NAD	NAD	NAD	NAD	**NAD**

Note : Overall Grade score as-NAD =No Abnormality Detected , **Minimal changes** (+), **Mild changes** (++), **Moderate changes** (+++), **Severe changes** (++++).

IV. *In vivo* anti-ulcer activity -

The observations are as follows

A. Ulcer index –

It was observed that in the Ethanol induced ulcerated control group, the ulcer index was 42.83 +_ 10.02 and the maximum numbers of ulcers were of the score 2 and 3. Standard drug Ranitidine was found to produce a decrease in the ulcer index, the ulcer index being 33.50 +_ 7.23; protection percentage is 22 %. T1 was found to produce significant decrease in the ulcer index as compared to the standard drug, the ulcer index being 13.83 +_ 2.48 and there were less number of ulcers with score 2 and 3 as compared with standard drug, providing a protection of 68 %. The ulcer index in the group treated with T2 was higher than standard drug, the ulcer index being 37.50 +_ 18.03. In this group, there were maximum ulcers with score 2 and 3 and provided protection of 13 %.

Table No.62 – Effect of drug T1 and T2 on ulcer index and % protection in Ethanol induced ulcers in Rats.

		DC			Std (Ranitidine)			Test 1			Test 2		
	Intensity	**No. of Lesions**	**Score**	**Index**	**No. of Lesions**	**Score**	**Index**	**No. of Lesions**	**Score**	**Index**	**No. of Lesions**	**Score**	**Index**
Animal 1	1	0	0		6	6		2	2		4	4	
	2	12	24	36	7	14	38	3	6	17	7	14	33
	3	4	12		6	18		3	9		5	15	
Animal 2	1	5	5		4	4		5	5		4	4	
	2	17	34	54	8	16	41	3	6	14	8	16	68
	3	5	15		7	21		1	3		16	48	
Animal 3	1	2	2		3	3		4	4		4	4	
	2	2	4	57	4	8	41	2	4	14	15	30	49
	3	17	51		10	30		2	6		5	15	
Animal 4	1	5	5		6	6		1	1		2	2	
	2	12	24	35	8	16	28	2	4	11	6	12	32
	3	2	6		2	6		2	6		6	18	
Animal 5	1	4	4		0	0		0	0		1	1	
	2	6	12	40	4	8	26	1	2	11	4	8	21

	3	8	24		6	18		3	9		4	12	
Animal 6	1	6	6		2	2		1	1		4	4	
	2	7	14	35	2	4	27	3	6	16	3	6	22
	3	5	15		7	21		3	9		4	12	
Mean				42.83			33.50			13.83			37.50
SD				10.02829			7.231874			2.483277			18.03053
% Protection	--				22 %			68 %			13 %		

The values are expressed as Mean +_ SD. The test and standard groups were compared with the ulcerated control group. Statistical significance was analyzed using One way analysis if variance (ANOVA), followed by Dunnett's t-test where $p<0.05$ was considered statistically significant.

B. Gastric pH

Table No.63 – Effect of drug T1 and T2 on Gastric pH in Ethanol induced ulcers in Rats.

	NC	DC	Std (Ranitidine)	Test 1	Test 2
Animal 1	3	2.8	4	3.5	3.2
Animal 2	3.5	2.5	3	4.5	2.5
Animal 3	2.5	3	4.5	4	3
Animal 4	X	X	X	X	X
Animal 5	X	X	X	X	X
Animal 6	X	X	X	X	X
Mean	**3.00**	**2.77**	**3.83**	**4.00**	**2.90**
SD	**0.50**	**0.25**	**0.76**	**0.50**	**0.36**

The values are expressed as Mean +_ SD. The test and standard groups were compared with the ulcerated control group. Statistical significance were analysed using One way analysis if variance (ANOVA), followed by Dunnett's t-test where p 0.05 was considered statistically significant.

C. Organ weight –

In all the groups, there has been no significant changes in the organ weights.

Table No.64 – Effect of drug T1 and T2 on organ weight in Ethanol induced ulcers in Rats.

Animal No.	Sex	Adrenals	Heart	Kidneys	Liver	Spleen	Lungs	Testes	Ovaries	Pancreas
Group 1 Normal Control										
1	M	0.082	0.713	1.852	8.014	1.044	1.679	2.488	0.185	0.548
2	M	0.095	0.932	1.79	7.387	0.994	1.083	2.716	0.196	0.57
3	M	0.074	0.779	1.626	9.481	0.91	1.705	2.526	0.18	0.586
4	F	0.101	0.673	1.339	5.623	0.982	1.455			0.432
5	F	0.106	0.798	1.403	7.209	0.892	1.462			0.557
6	F	0.096	0.67	1.476	6.451	0.681	1.198			0.458
Mean		0.092	0.761	1.581	7.361	0.917	1.43	2.577	0.187	0.525
SD		0.012	0.099	0.21	1.326	0.129	0.25	0.122	0.008	0.064
Group 2 Disease Control										
1	M	0.044	0.772	1.702	8.767	0.979	1.243	2.427	0.15	0.577
2	M	0.07	0.78	1.786	8.375	0.81	1.536	2.6	0.134	0.591
3	M	0.074	0.983	1.775	7.164	0.737	1.429	2.64	0.15	0.593
4	F	0.106	0.633	1.333	7.007	0.413	1.178			0.406
5	F	0.063	0.798	1.282	5.189	0.687	1.047			0.701
6	F	0.133	0.629	1.39	5.127	0.408	1.063			0.776
Mean		0.082	0.766	1.545	6.938	0.672	1.249	2.556	0.145	0.607
SD		0.032	0.13	0.234	1.537	0.226	0.198	0.113	0.009	0.126
Group3 Standard Drug										
1	M	0.095	0.824	1.6	7.64	0.872	1.065	2.742	0.12	0.491
2	M	0.055	0.746	1.799	8.184	0.775	1.231	2.46	0.241	0.458

3	M	0.076	0.894	1.716	8.485	0.956	0.889	2.478	0.152	0.565
4	F	0.078	0.572	1.188	5.543	0.625	0.923			0.462
5	F	0.117	0.633	1.435	6.18	0.538	1.065			0.458
6	F	0.054	0.614	1.076	5.147	0.411	1.427			0.672
Mean		0.079	0.714	1.469	6.863	0.696	1.1	2.56	0.171	0.518
SD		0.024	0.128	0.29	1.424	0.208	0.201	0.158	0.063	0.086
Group 4 T1										
1	M	0.063	0.654	1.448	6.249	0.868	1.124	2.736	0.188	0.449
2	M	0.098	0.865	1.609	8.711	0.928	1.292	2.545	0.246	0.329
3	M	0.071	0.759	1.86	9.097	0.88	1.065	2.54	0.188	0.502
4	F	0.112	0.776	1.537	7.856	0.968	1.175			0.588
5	F	0.127	0.638	1.434	7.382	0.886	1.262			0.483
6	F	0.107	0.566	1.261	6.005	0.489	1.283			0.666
Mean		0.096	0.71	1.525	7.55	0.837	1.2	2.607	0.207	0.503
SD		0.025	0.109	0.202	1.26	0.174	0.094	0.112	0.033	0.116
Group 5 T2										
1	M	0.108	0.755	1.593	7.761	0.685	1.187	2.431	0.271	0.608
2	M	0.103	0.805	1.803	8.539	0.626	1.673	2.336	0.176	0.664
3	M	0.098	0.749	1.511	8.629	0.578	1.117	2.395	0.286	0.502
4	F	0.112	0.741	1.62	7.847	0.601	1.289			0.518
5	F	0.065	0.697	1.183	5.691	0.569	1.286			0.483
6	F	0.158	0.663	1.493	5.916	0.479	1.212			0.663
Mean		0.107	0.735	1.534	7.397	0.59	1.294	2.387	0.244	0.573
SD		0.03	0.049	0.204	1.285	0.068	0.197	0.048	0.06	0.082

The values are expressed as Mean +_ SD. The test and standard groups were compared with the ulcerated control group. Statistical significance was analyzed using One way analysis if variance (ANOVA), followed by Dunnett's t-test where $p<0.05$ was considered statistically significant.

D. Histopathology of Stomach

The sections of stomach in disease control group showed hyper-keratinization, degenerative changes in non-glandular and glandular part, degenerative and necrotic changes of mucosa, hemorrhages in glandular part, chronic inflammatory changes of basement membrane and eosinophilic infiltration. Standard drug Ranitidine was helpful in reducing the inflammation and hemorrhages in the gastric mucosa. Pre-treatment with Drug T1 was significant in reducing the inflammation, hemorrhages, degenerative and necrotic changes in the stomach tissues giving a better protection as compared with the standard drug. T2 drug imparted mild changes in the stomach mucosa and its protection activity was not as significant as that of drug T1.

Table No.65 – HPR of section of stomach of Rats treated with drug T1 and T2 in Ethanol induced ulcers in Rats.

Sr No	Group	Code No	Degenerative changes in non-glandular part, Hyper-keratinization	Degenerative changes in glandular part , vacuolar changes	Loss of glandular features of mucosa	Degenerative and necrotic changes of mucosa	Mucosal surface Hemorrhages in glandular part	Inflammatory changes with MNC infiltration, hemorrhages etc	Chronic inflammatory changes of basement membrane , Eosinophilic infiltration	OVERALL PATHO-LOGICAL GRADE (LESION SCORE)
1	Control	Stomach 1	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
2		Stomach 2	NAD	+	NAD	NAD	NAD	NAD	NAD	NAD
3	Disease control	Stomach 3	+	+	+	+	++	NAD	+	Mild (++)
4		Stomach 4	NAD	+	+	+	++	NAD	+	Mild (++)
5	Standard drug	Stomach 5	NAD	+	NAD	NAD	NAD	NAD	NAD	MINIMAL (+)
6		Stomach 6	NAD	+	+	+	+	NAD	+	Mild (++)
7	T1	Stomach 7	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
8		Stomach 8	+	NAD	NAD	NAD	NAD	NAD	NAD	NAD
9	T2	Stomach 9	+	+	+	+	NAD	NAD	+	Mild (++)
10		Stomach 10	+	+	+	+	+	NAD	+	Mild (++)

Note : Overall Grade score as-

NAD =No Abnormality Detected, Minimal changes (+), Mild changes (++), Moderate changes (+++), Severe changes (++++).

Fig. No. 20 - HPR of stomach of Normal Control treated Rats

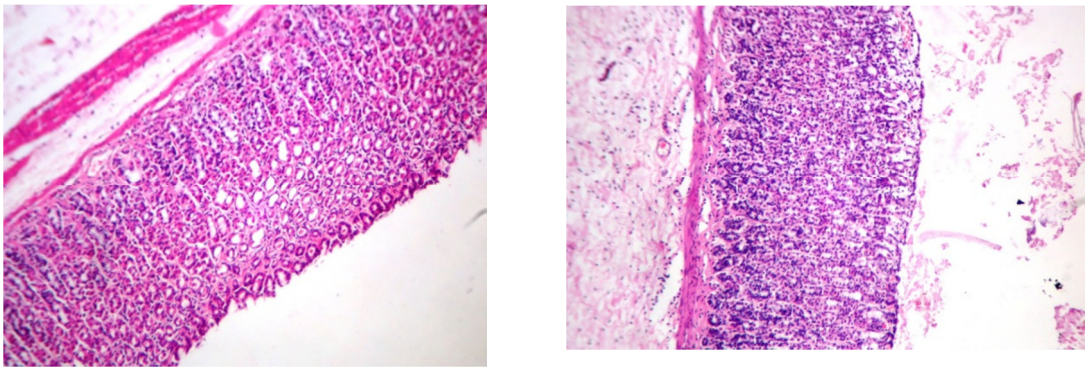


Fig. No. 21 - HPR of stomach of Disease Control treated Rats

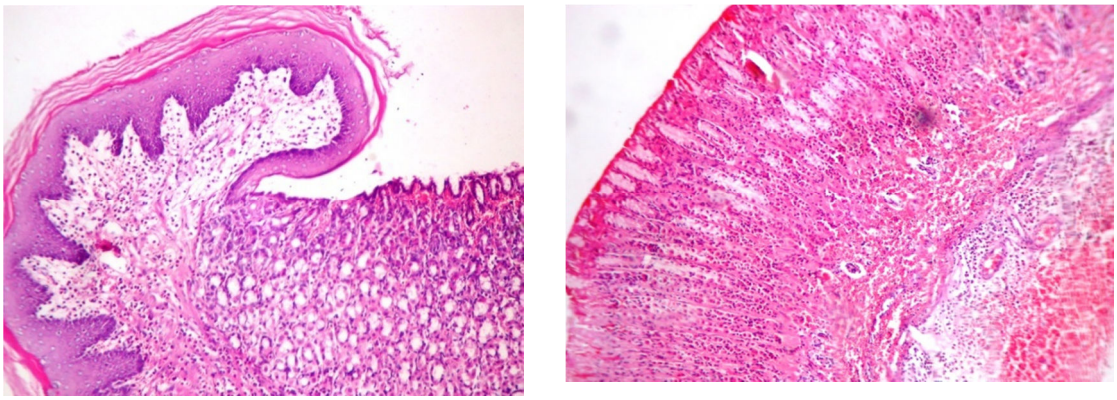


Fig. No. 22 - HPR of stomach of Standard Drug treated Rats

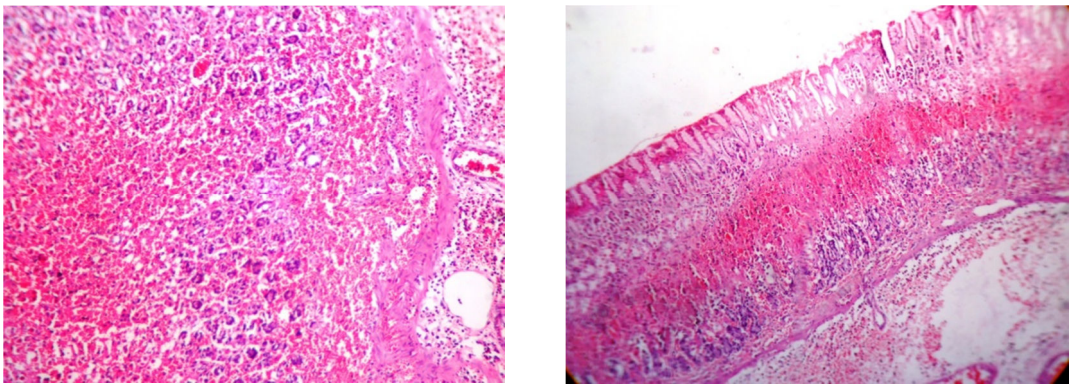


Fig. No. 23 - HPR of stomach of T1 treated Rats

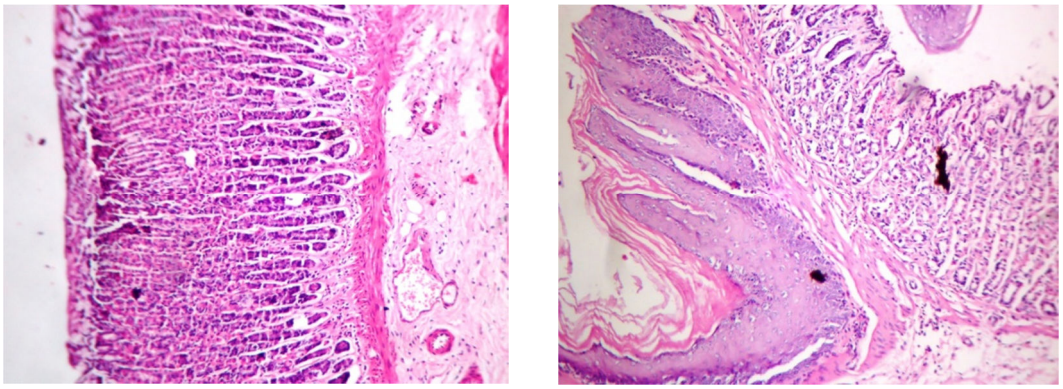


Fig. No. 24 - HPR of stomach of T2 treated Rats

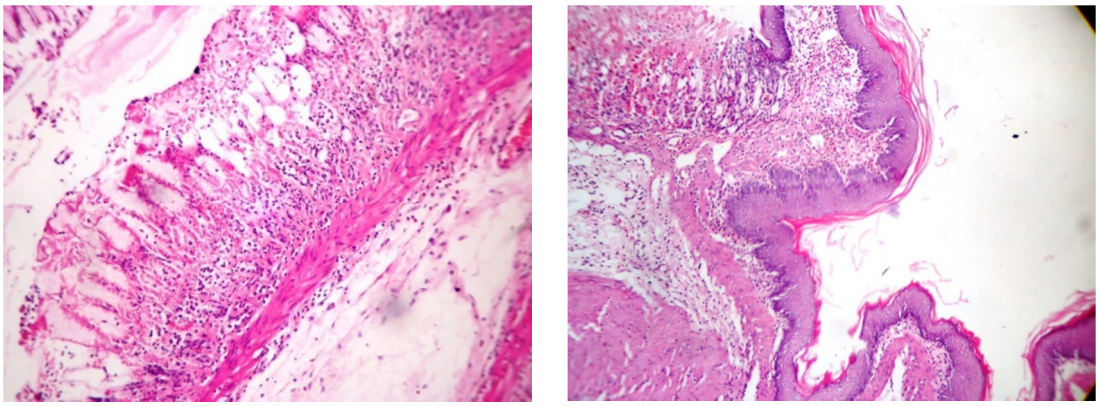
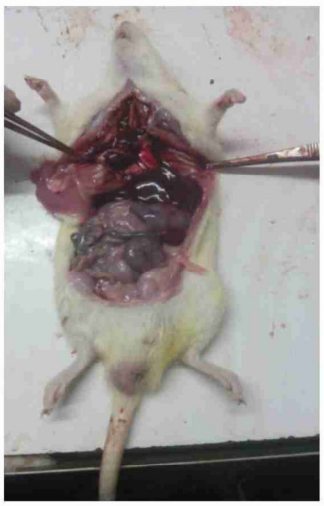


Photo No. 5 : Visceral study in Ethanol induced gastric ulcers in Rats

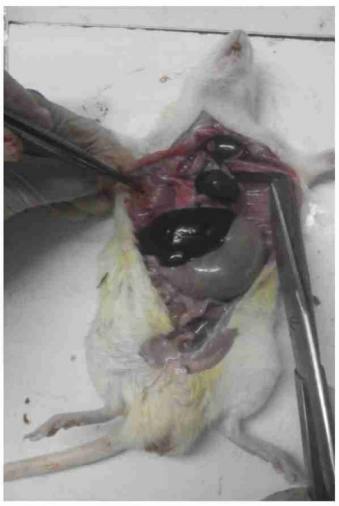




Normal Stomach **Inflamed ulcerated Stomach**







Normal Control **Disease Control** **Ranitidine (100mg/kg)**





T1 treated **T2 Treated**

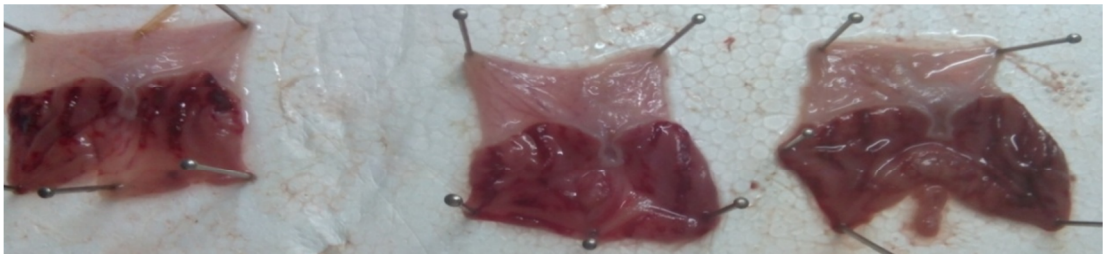
Photo 6- Macroscopic view of gastric ulcers induced by Ethanol in the groups - control, disease control, treated with standard drug, treated with T1 and treated with T2



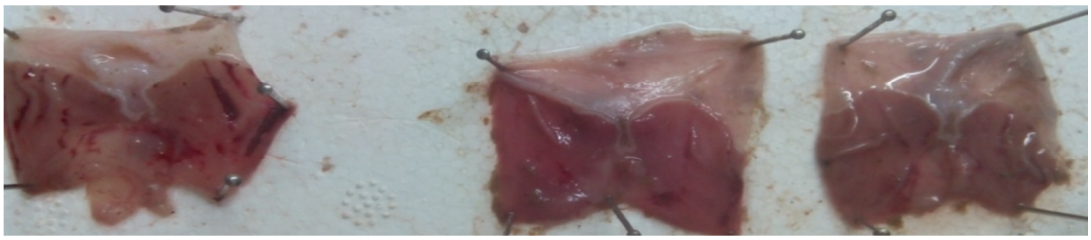
Control



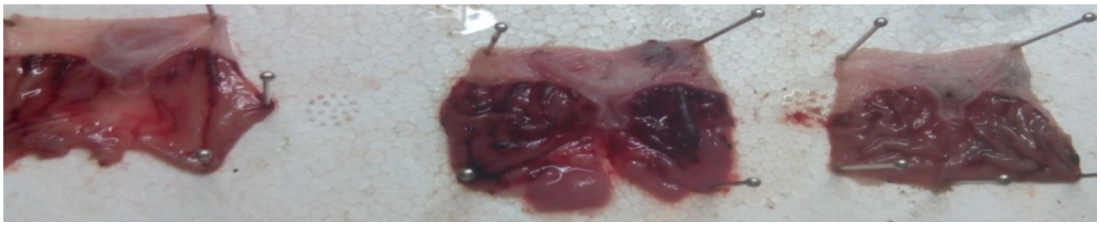
Disease Control (ulcers)



Std Drug (Ranitidine 100mg/kg)



Test Drug 1



Test Drug 2

Ethanol-induced severe damage to the gastric mucosa are shown as elongated bands of hemorrhage in the disease control group. As compared with the disease control group, in standard Ranitidine group, area of the elongated bands of hemorrhages was less. T1 produced an almost normal appearance of intact stomachs and very few elongated bands of hemorrhage were seen. T2 showed large numbers of elongated hemorrhagic bands as compared with the standard drug and T1.

DISCUSSION

This project was specially designed to investigate the pharmaceutical process, analytical testing, toxicity studies and to evaluate anti-ulcer activity of Aamalaki Mashi. Keeping this objective in mind, thorough literature research was done.

Mashi is a dosage form in which the raw drug material is burnt till it becomes completely black like a carbon. Use of Mashi Kalpana can be found since Samhita period. It is prepared by Bahirdhuma and Antardhuma method. There are three important Sanskara involved in Mashi preparation viz. Agni, Kala, Bhajana. They affect the physico-chemical parameters of the final product and need to be taken into consideration. Kapala or Kataha and Sarava samputa were used for Bahirdhuma and Antardhuma method respectively. Use of iron container in the preparation of Aamalaki Mashi, which is an amla dravya can degrade the quality of the final product. Nowadays, earthen pan, stainless steel utensils can be used its preparation. The main principle behind this is that there should not be any chemical reaction between the material of container and the medicinal drug during processing.

All plant materials are composed of carbon chains and hence are called organic. They also contain significant amount of inorganic constituents. By application of heat, the process of decomposition and degradation of organic matter begins resulting in the conversion of the plant material into organic charcoal. During this combustion process, smoke appears at the beginning of the process and material starts blackening. Due to Vayu and Akasha Mahabhut dominance, it can be used internally in the treatment of Vata-vimargagamana disorders. Therefore, it is seen in treating flatulence, dyspepsia, indigestion, nausea and vomiting. Also, as it is a form of organic charcoal, due to its adsorbent property, it can be also used in gastro-intestinal disorders where there is pain as pain symptom is associated with Vata Dosha[72].

वातादृते नास्ति रुजा न पाकः । पित्तादृते नास्ति कफाच्च पूयः ।। सु.सू.१७/७

72. Sushrut Samhita of Sushruta, Nibandhasangraha Commentary of Dalhanacharya, edited by Vaidya Yadavji Trikamji Acharya, Chaukhamba Surbharati Prakashana, Varanasi. Sutrasthan 17/7. pp 83.

During Pilot study, Aamalaki Mashi was prepared by Bahirdhuma and Antardhuma method. Pilot work was of help for making supportive frame work to do the pharmaceutical work and for assessment of approximate time, temperature, equipment, amount of raw material and physical changes during Mashi preparation. Kajjala like appearance and Nirdhuma were the endpoint of the preparation process. The endpoint 'Nirdhuma' was observed during Bahirdhuma method only. The temperature range for Bahirdhuma method ranged from 170°C - 180°C and for Antardhuma method, heat produced by 4.5 to 5 cow dungs. There was formation of Kshara when the temperature exceeded the given range. On the basis of pilot study, actual pharmaceutical work was done.

It was observed that the total yield obtained during the pilot work by Bahirdhuma method was nearly 55 % to 60 % and by Antardhuma method was nearly 35 % of the raw material used. In Bahirdhuma, the heating was done in an open vessel. The type of heating was open heating and unidirectional. Due to heating in open environment, presence of atmospheric oxygen can interfere with raw material, oxidation reaction takes place leading to the formation of oxides. In this process, the heating can be done in controlled manner. The organic constituents present in the Churna undergo decomposition on heating due to which loss of heat labile and volatile compounds can take place. Only those constituents which can sustain heat at the temperature of Mashi formation can be retained in the final product. Absence of alkaloids, steroids, saponins, terpenoid, sugar and presence of flavanoids and Vitamin C proves this analytically.

In Antardhuma, the heating was done in closed system. The type of heating was closed heating and from all sides. Due to heating in closed system, presence of oxygen can be very less so oxidation reaction is negligible as compared to Bahirdhuma method. So, the formation of oxides can be less resulting in fewer yield as compared to Bahirdhuma method.

Secondly as the system is closed, during heating process the pressure inside increases with the increase in the temperature and the volume decreases due to which the reaction takes place vigorously resulting in the weight loss. Heating takes place in

uncontrolled manner. Also, after the cowdungs are burnt completely and their temperature comes to normal room temperature, entrapped heat in the Sharava might cause the decomposition of organic constituents till the Sharava samputa attains Swangashitatva. Therefore, the time taken to prepare Aamalaki Mashi by Antardhuma method is more whereas in Bahirdhuma method due to direct contact with environment equilibrium is quickly maintained between the temperature of the system and environment so the time required is less.

In Darsan Shastra, some unique principals regarding Pakajotpatti have been described by Nyaya – Vaisesika viz. Pilu Paka[73]. In Pakajotpatti, the role of Agni plays an important role in transformation of one substance to another with complete change in their Panchamahabhautika composition. Due to which the final product is completely different form the original product. Due to Agni Sanskara, the particles in the raw drug start disintegrating leading to the breakdown of Sthula matter i.e. Parthiva and Aapya components. The Guru, Kathina, Sthira, Sthula guna are transformed into Laghu, Mrudu, Slakshna, Chala, Sukshma guna which are dominantly present in Vayu and Akash Mahabhut. So, Mashi is Laghu, Mrudu and its surface area is more than the Churna owing to the reduction in the size of Parthiva components. The Principle of Pilu Paka can be applied to Mashi dosage form. Due to Agni Sanskara, new interactions occur within the raw drug resulting in the formation of new chemical moieties due to which Mashi exhibits pharmacological action which is different from the original drug.

The quality control parameters for standardization of the Aamalaki Churna (sample A) and Aamalaki Mashi prepared by Bahirdhuma (sample B) and Antardhuma method (sample C) were established based on the organo-leptic characters, physic-chemical tests and analytical testing. The texture of Aamalaki Mashi is very soft which can be attributed to the Mrudu and Slakshna guna of Akash Mahabhoot. The heat application to plant material results into conversion to organic charcoal which is a form of carbon giving black color to Mashi. The burnt or charcoal like taste of Mashi indicates the presence of carbon. The Loss on Drying Test is designed to

73. Sulabha Padarthvidnyan by Dr.Prama Joshi, first edition,Aug2008,pp91-92.

measure the amount of water and volatile matters in a sample when the sample is dried under specified conditions. The % of loss on drying in significantly less in sample B and sample C than sample A which signifies less moisture content which is helpful in maintaining the stability of the drug and less chances of fungal or bacterial contamination and yeast growth. Ash value is useful in determining authenticity and purity of drugs and these values. Contents of total ash were found to be lower in sample A and B which may be due to low content of carbonates, phosphates, silicates and silica. All the three samples are low content of acid insoluble ash. The total ash is particularly important in the evaluation of purity of drugs, the presence of or absence of foreign inorganic matter such as metallic salts or silica. Extractive values are useful for evaluation of drugs and gives an idea about the nature of chemical constituents present in them. Alcohol soluble extractive value was found to be greater than water soluble extractive value in Sample A, indicating this sample contains maximum alcohol soluble compounds that can be extractable maximum into alcohol than the water. There was no significant difference in the alcohol and water extractive values in sample B and C. pH signifies Sample A is the most acidic of all the three samples, sample B is slightly acidic and sample C is on neutral level. These tests signify the stability of the drug.

Thin Layer Chromatography is useful for identifying compounds, their separation and determining their purity of the drug. TLC of Sample B denotes formation of new spots indicating new compounds which are not present in sample A and loss of few compounds which are present in sample A. This may be due to the heat treatment received in case of drug T1 due to which volatile and some organic molecules must be destroyed and there must be formation of new molecules.

The water soluble extracts obtained after extraction were characterized by preliminary phytochemical tests for rough idea of main constituents present in the drug like alkaloid, flavonoid, steroids, tannin, terpenoid, saponin and sugars. Sample A shows presence of sugars and sample B shows presence of flavonoid. There was absence of these phytochemicals in sample C. Flavanoids[74] are responsible for gastro protective

74. www.ncbi.nlm.nih.gov/pubmed/9741648. Indian J Physiol Pharmacol. 1998 Jul;42(3):343-51, Anti-ulcer potential of flavonoids -Parmar NS, Parmar S. PMID: 9741648.

and ulcer healing properties. Sample B shows presence of high percentage of TOC which may contribute to the adsorbent property of the drug.

Also, as per the elemental assay by ICP-AES, the percentage of sulphur[75], zinc[76] and copper[77] which are known for their wound healing properties in drug T1 is higher than drug T2. The percentage of Al[78], Ca[79] and Mg[80] whose compounds possess antacid property is higher in drug T1. The presence of these elements may also contribute to the anti-ulcer activity of drug T1. This helps to understand Mashi contains both organic and inorganic matter which is proved by the presence of phytochemicals like flavonoids, vitamin C as well inorganic elements.

SEM analysis of sample A showed there was a formation of multilayer scaffolds, sheet like formation, there was no initiation of formation of nano particles, particles were in agglomerated stage. In sample B, nano particles formation was seen, size of particles had reduced making the compound more penetrable/ absorbable and the size of particle ranged between 100 to 200 nm. In sample C, particles were seen aggregated, there was initiation of nano particles but the particles were not separated.

Toxicological screening is very important for the development of new drugs and for the extension of the therapeutic potential of existing molecules. Despite being an ancient and well documented systems of medicine, Ayurveda offers very little scientific data on the safety and efficacy of these medicines. New developments like the OECD guidelines which emphasis on toxicity studies in experimental animals and

75. https://www.sciencenews.org/archive/sulfur-aids-wound-healing
76. V. A. TrapkovÉ. A. ParfenovL. D. Smirnov, Synthesis and antiulcer activity of copper and zinc-containing coumarin antioxidants, Pharmaceutical Chemistry Journal , July 1996, Volume 30, Issue 7, pp 445–447 , DOI: 10.1007/BF02219679
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80. Sonia Narwal, Vipin Saini, Vichitra Kaushik , Evaluation Of Anti-Ulcer Activity Of Esomeprazole Magnesium Trihydrate Film In Wistar Rats With Gastric Ulceration Induced By Indomethacin , EJPMR - 2016,3(10), 443-447

represents a harmonized approach in the application of toxicological study protocols which are globally relied on. Thus procuring reliable toxicological data for Ayurvedic drugs and formulations would help us in a great manner for their wide spread acceptance.

In the present work, toxicity studies were restricted to only acute and sub acute studies. The guidelines followed were OECD 423 and OECD 407 respectively. The rationale behind this thought was that in humans, Aamalaki Mashi can be given continuously for two to three weeks as per the data available regarding its usage. In practice, there is no long term use of this drug, maximum 30 days may be considered as its higher limit of use in humans which can be equivalent to 2 to 3 weeks in experimental animal, mice. This medicine has been in use traditionally and there is no data recorded regarding its toxic effects in human till date. Also, the acute oral toxicity and sub acute oral toxicity studies conducted on this drug didn't show any signs of mortality or toxicity. Keeping this in mind, further chronic toxicity studies were not conducted for this drug.

In acute oral toxicity studies, dose level of 2000 mg/kg body weight was considered which is the limit test. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products. In those situations, where there is little or no information about its toxicity, or in which the the test material is expected to be toxic, the main test should be performed. A limit test at one dose level of 2000 mg/kg body weight may be carried out.

As per OECD 423 guidelines at first step, in case of T 1 drug, 3 animals were dosed (T1 A), as there was no mortality at this dose the remaining three animals were dosed with the same dose at second step (T 2 B). Similarly dosing with T2 drug was done.

In acute toxicity, there was accidental death of two animals in group T2 on day 2. There was a consistent increase in the body weight of the animals in both the groups. Cage side observations did not record any behavioral changes. As per the OECD 423

guidelines, histopathology was not conducted, those organs showing changes in gross necropsy can go for histopathological studies. In this study, there were no signs of changes seen grossly indicating no signs of toxicity. Since no mortality & no observable toxicity symptoms are recorded up to 2000 mg/kg LD50 of drug T1 and T2 is greater than 2000 mg/kg.

In sub acute oral toxicity studies, 1000 mg/kg was taken as Intermediate dose, according to this half of this dose i.e 500 mg/kg was taken as low dose and double of this dose i.e 2000 mg/kg which was proved to be safe in acute toxicity study was taken as high dose.

The rationale for dose level selection was that as per OECD 407, dose levels should be selected taking into account any existing toxicity and (toxico-) kinetic data available for the test compound or related materials. The highest dose level should be chosen with the aim of inducing toxic effects but not death or severe suffering. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and no-observed-adverse effects at the lowest dose level (NOAEL). Two to four fold intervals are frequently optimal for setting the descending dose levels and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages.

In subacute toxicity studies, all the animals fed with the treatment group were found to be in good health condition. There was one accidental death each in the groups T1I, T1H, T2I and T2H. There were no changes observed in their behavior and locomotor activity. There was absence of any visual sign of intoxication during this study. Morphological characteristics of skin, eyes, nose, and fur also revealed no abnormal changes. There was no significant increase in body weight was observed in all the groups. Daily food and water consumption were found to be normal in control group. In the treatment groups, quantity of food was maintained and normal in T1L and T1I whereas in the remaining groups i.e T1H, T2L, T2I and T2H the quantity of food decreased day by day. This can be attributed to the adsorbent property of carbon which is present in the test compound which might have caused reduced appetite. We can assume that drugs T1 and T2 have negligible role in stimulating appetite.

The organ weights in control and treatment group did not show any significant changes. When compared with the control, there was no significant change in the values of haemogram of treatment groups. There was no significant change in the biochemical parameters. Levels of blood sugar, urea, SGOT, Cholesterol, Triglycerides, HDL, proteins, LDL and VLDL were within the normal range in all the treated groups as compared with the control.

All animals in the study were subjected to a full, detailed gross necropsy which included careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. There were no detrimental changes observed grossly. Out of all the organs, liver being the chief organ of metabolism and kidney, the main organ for excretion, histopathology of these two organs were done. Also, the main constituent of Mashi is organic carbon which is non toxic in nature and doesn't have any toxic effects on the body, other organs were not considered for histopathology.

Histopathology of Liver in T1 group did not reveal any significant changes. In T1L group, there was some focal congestion, haemorrhage, cellular swelling, degenerative changes in hepatocytes and infiltration of mononuclear cells (MNC) which was very minimal in grade.

Histopathology of Liver in all the groups of T2 showed minimal congestion and haemorrhage, some focal cellular swelling and minimal degenerative changes in hepatocytes. There was focal infiltration of mononuclear cells (MNC) observed in T2L. All these signs were of minimal grade.

Histopathological examination of Kidney in low dose and intermediate dose of T1 group showed some focal congestion and haemorrhage, focal tubular swelling which were of minimal grade.

Histopathological examination of Kidney in low dose of T2 group showed minimal non significant changes like focal congestion and haemorrhage, focal tubular swelling and degeneration and infiltration of mononuclear cells (MNC).

The data available from acute and sub acute toxicity studies strongly suggest that Aamalaki Mashi prepared by both the methods is safe, non toxic and well tolerated at the tested oral doses since no detrimental changes were observed in animal macro-parameters, behavioral habits, haematology, serum biochemistry and histopathological parameters.

The symptom complex mentioned in acid peptic diseases[47] which are mostly seen in peptic ulcers like abdominal pain, nausea, water brash, vomiting, loss of appetite and weight loss. Complications include bleeding, perforation and obstruction in the digestive tract can be correlated with many such similar symptoms which are akin to those mentioned in ancient Ayurvedic texts like Pittaja Gulma, Parinam Shula, Urvadja Amlapitta, Chardi, Annadrava Shula, etc. Aamalaki Mashi in which the main ingredient is Aamalaki, inspite of no textual reference is in use since a long time in ethnomedical system in treating such gastric disorders where symptoms like flatulence, dyspepsia, indigestion, nausea and vomiting are particularly seen.

Presently Acid peptic diseases are very commonly found of which gastric ulcers are predominantly seen. Gastric ulcers are a type of peptic ulcer disease which can be a serious gastrointestinal disorder requiring a well targeted therapeutic strategy. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. There are number of drugs in Modern medicine available for the treatment of these ulcers, but clinical evaluation of these drugs show higher incidence of relapse, side-effects and drug interactions. This has been the rationale for the development of new anti-ulcer drugs in which the herbal drugs can offer a better protection and decreased relapse. Thus it is a logical approach to drug discovery by screening traditional herbal products instead of synthesized chemical molecules. So Aamalaki Mashi was taken as an archetype in treating acid peptic condition like gastric ulcer.

47. www.medindia.net/diseaseinfo

Mashi dosage form is described in the ancient texts of Ayurveda mainly for internal and external use. The dose of Mashi is clearly not mentioned in these texts. Mashi is in the form of churna (powder), and if we consider the dose of churna as per Ayurvedic text, it is 1 tola[69] ≈ 10 gms. The dose of powdered activated charcoal[70] in gas troubles and hyperacidity problems is 10-20 gms in a day. In practice, Aamalaki Mashi is used in the dose of 5-10 gms in a day for treating hyperemesis, gaseous problems, etc. This dose is given in divided form for 3 to 4 times in a day. There is no standard reference regarding this dose. In this study, there was a need to optimize the dose of Mashi. Therefore, considering the above mentioned factors, 10 gms of Aamalki Mashi in 24 hrs was taken as standard human dose for the study purpose. This was helpful to determine the dose for efficacy study. Accordingly, the human dose was extrapolated to animal dose and finally the animal dose was finalized as 1000 mg/kg body weight of rat. There are several models that are used to evaluate antiulcer medicines. Peptic ulcers can be induced by physiological, pharmacological or surgical manipulations in several animal species. 'Ethanol induced gastric mucosal damage in rats' animal model was used in the present study taking into consideration the prevention aspect. The ethanol-induced ulcer model is useful for studying the efficacy of potential drugs or testing agents that have cytoprotective and/or antioxidant activities. Acute exposure of the gastric mucosa of rats to ethanol can result in gastric lesions similar to those occurring in gastric ulcer; hence, ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity.

The ulcer index in group treated with T1 was less as compared with standard drug and drug T2 was less. Of all the groups, drug T2 had the least protection percentage and maximum ulcer index. Drug T1 has significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of lesion index offering a better protection as compared to standard control group and drug T2 suggesting its potent cytoprotective effect.

69. Sharangdhar Samhita by Pandit Sharagdharacharya son of Pandit Damodar with the Commentary Adhamalla Dipika and Kashirams Gudartha Dipika edited by Pandit ParshuramShastri Vidyasagar, Chaukhamba Orientalia Varanasi, Madhyamkhanda 6/1.

70. https://www.drugs.com>Professionals>Natural Products (Pro)

The gastric pH values of Standard drug Ranitidine and test drug T1 are 3.83 and 4.00 respectively whereas, the gastric pH of test drug T2 is 2.90 which is more acidic than the disease control i.e. 2.77. (normal control = 3). The increase in pH values indicates the effectiveness in reducing the gastric acidity.

In all the groups, there was no significant changes in the organ weights. On histopathology of stomach, the sections of stomach in disease control group showed hyper-keratinization, degenerative changes in non-glandular and glandular part, degenerative and necrotic changes of mucosa, hemorrhages in glandular part, chronic inflammatory changes of basement membrane and eosinophilic infiltration. Standard drug Ranitidine was helpful in reducing the inflammation and hemorrhages in the gastric mucosa. Pre-treatment with Drug T1 was significant in reducing the inflammation, hemorrhages, degenerative and necrotic changes in the stomach tissues giving a better protection as compared with the standard drug. T2 drug imparted mild changes in the stomach mucosa and its protection activity was not as significant as that of drug T1.

The ethanol administration to rats caused macroscopic gastric mucosal lesions consisting of elongated dark bands generally parallel to the long axis of the corpus of the stomach, along with loss of normal color and mucus along with presence of petechiae, hemorrhage and edema. These lesions are most likely related to mucus depletion and a constrictive effect on veins and arteries of the gastric mucosal, producing congestion, inflammation and tissue injury. The reduction of gastric mucosal blood flow can result in hemorrhage and necrosis in damaged tissue. As compared with the disease control group, in standard Ranitidine group, area of the elongated bands of hemorrhages was less. T1 produced an almost normal appearance of intact stomachs and very few elongated bands of haemorrhage were seen. T2 showed large numbers of elongated haemorrhagic bands as compared with the standard drug and T1.

All the above findings support the *in vivo* anti ulcer activity of drug T1 i.e Aamalaki Mashi prepared by Bahirdhuma method is considerably more effective than standard drug Ranitidine and also offer far better protection. It will be advisable not to use drug T2 i.e Aamalaki Mashi prepared by Antardhuma method as an anti ulcer agent as the

gastric pH in this group was nearly as that of disease control and also the percentage of protection was not significant as T1. Ethanol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane[81]. The gastric mucosal protection against oxidative injuries caused by ethanol and this protection is most likely due to antioxidant and gastro protective properties of drug T1. In addition, the presence of flavonoids in drug T1 certainly contribute to its antiulcerogenic activity. Flavanoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed. Also, as per the elemental assay by ICP-AES, the percentage of sulphur, zinc and copper which are known for their wound healing properties in drug T1is higher than drug T2. The percentage of Al, Ca and Mg whose compounds possess antacid property is higher in drug T1. The presence of these elements may also contribute to the anti-ulcer activity of drug T1. Also, taking into consideration, the pharmaceutical method, the Antardhuma method which is a closed heating method in which heating takes place in uncontrolled manner, can be responsible for incorporating Tikshna, Sukshma and Ushna guna in Mashi as compared to Bahirdhuma which may not be useful for anti-ulcer activity.

Hence newer therapeutic uses of Aamalaki Mashi can be evaluated by using different experimental models and its clinical efficacy can be tested in humans.

81. Acta Pharmaceutica Sinica B, volume 4, Issue 5, October 2014, Antiulcerogenic activity of *Scutiabuxifolia* on gastric ulcers induced by ethanol in rats- Aline Augusti Boligon et all,pp358–367.

CONCLUSION

The discussion held in the previous chapter leads to following conclusions –

1. Mashi is a dosage form in which bulk of raw material is reduced to a greater extent by application of certain quantum of energy. There are three important changes taking place during conversion of raw material to Mashi, viz.

a. Evaporation or loss of some constituents which are thermo-labile and volatile and are unfavorable in this dosage form.

b. Retention of some constituents which are therapeutically active organic and inorganic constituents or formations of new chemical moieties which are therapeutically active take place.

c. Conversion of some constituents into charcoal.

2. Aamalaki Mashi was prepared by Bahirdhuma and Antardhuma method, yield obtained from Bahirdhuma was more than Antardhuma. The total yield obtained by Bahirdhuma method was nearly 55 % to 60 % and by Antardhuma method was nearly 35 % of the raw material used.
3. In the pharmacognosy investigation, organoleptic characters of raw material and both Aamalaki Mashi samples as color, odor, taste, touch were studied. Standardization parameters such as loss on drying, total ash, acid insoluble ash, alcohol soluble extractives, water soluble extractives, pH, TLC, estimation of vitamin C and TOC were evaluated to obtain the qualitative information about purity and quality of raw material and Aamalaki Mashi prepared by both the methods.
4. Aamalaki Mashi prepared by Bahirdhuma method showed presence of flavonoids which contributes to its gastro-protective property and high percentage of TOC which contributes to adsorbent property of the drug.
5. The elemental assay by ICP-AES showed the presence of the inorganic elements present in raw material and both the Aamalaki Mashi samples.
6. Aamalaki Mashi contains both organic and inorganic matter which is proved by the presence of phytochemicals like flavonoids, vitamin C as well inorganic elements. Therefore retains its sendriyatva due to which its assimilation is easy in the body.
7. SEM analysis of Aamalaki mashi prepared by both the methods showed the characterization of the particles of the Mashi. In Aamalaki mashi prepared by Bahirdhuma method nano particles formation was seen, size of particles was reduced

making the compound more penetrable/ absorbable and the size of particle ranged between 100 to 200 nm.

8. Aamalaki Mashi in which the main ingredient is Aamalaki, inspite of no textual reference, is in use since a long time in ethnomedical system in treating gastric disorders like Amlapitta, Adhmana and Aanaha. The pharmacological screening of Aamalaki mashi prepared by both methods – namely drug T1 (Aamalaki Mashi prepared by Bahirdhuma method) and drug T2 (Aamalaki Mashi prepared by Antardhuma method) was performed. In this mainly acute toxicity, subacute toxicity studies and anti ulcer study were done. The acute oral toxicity study didn't show any signs of mortality upto a dose level of 2000 mg/kg in both the drugs. The subacute oral toxicity studies were conducted as per OECD guidelines 407 at three different dose levels – low dose (500 mg/kg), intermediate dose (1000 mg/kg) and high dose (2000 mg/kg). The study showed no signs of mortality or toxicity in both the treated group. Also statistically there was no significant change in the haematological, biochemical parameters and organ weights of the treated group as compared with the normal control.
9. For anti ulcer study, Ethanol-induced ulcer model was used for studying the efficacy of drug T1 and drug T2 to have cytoprotective and/or antioxidant activities.
10. Drug T1 has significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of lesion index as compared to control group suggesting its potent cytoprotective effect and also the protection percentage offered by this drug was maximum in comparison to other groups.
11. Drug T1 had higher pH values in comparison to other groups which indicates the effectiveness in reducing the gastric acidity.
12. The macroscopic appearance of rat stomachs in standard Ranitidine group showed fewer areas of the elongated bands of hemorrhages. T1 produced an almost normal appearance of intact stomachs and very few elongated bands of haemorrhage were seen. T2 showed large numbers of elongated haemorrhagic bands as compared with the standard drug and T1.
13. The gastric mucosal protection against oxidative injuries caused by ethanol and this protection is most likely due to antioxidant and gastro protective properties of drug T1. In addition, the presence of flavonoids in drug T1 certainly contribute to its antiulcerogenic activity. Also, as per the elemental assay by ICP-AES, the percentage of sulphur, zinc and copper which are known for their wound healing properties in

drug T1is higher than drug T2. The percentage of Al, Ca and Mg whose compounds possess antacid property is higher in drug T1. The presence of these elements may also contribute to the anti-ulcer activity of drug T1.

14. Thus, it can be concluded Aamalaki Mashi prepared by Bahirdhuma method has comparatively better efficacy than standard drug Ranitidine in anti-ulcer activity. It was also observed that of the two pharmaceutical process of preparing Aamalaki Mashi viz. 'Bahirdhuma' and 'Antardhuma' method, the Bahirdhuma method showed best results.
15. The additional benefits of Bahirdhuma were-

- the convenience of preparation;
- comparatively less time period required;
- yield is more;
- cost effectiveness.

FURTHER SCOPE OF STUDY

1. A study on the efficacy of external application of Aamalaki Mashi in external wounds or skin ulcers can be undertaken.
2. Application of higher standardization tools like HPTLC can be made to identify the chemical constituents in Aamalaki Mashi which can help to understand its various activities.
3. Various properties and effects of Aamalaki mashi in ulcer treatment can be studied by using different *in vivo* gastro-duodenal ulcer models.
4. Likewise, newer therapeutic uses of Aamalaki mashi can be understood by using disease experimental models.

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OECD/OCDE 423

Adopted:
17th December 2001

OECD GUIDELINE FOR TESTING OF CHEMICALS

Acute Oral Toxicity – Acute Toxic Class Method

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The original Guideline 423 was adopted in March 1996 as the second alternative to the conventional acute toxicity test, described in Test Guideline 401. Based on the recommendations of several expert meetings, revision was considered timely because: i) international agreement has been reached on harmonised LD50 cut-off values for the classification of chemical substances, which differ from the cut-offs recommended in the 1996 version of the Guideline, and ii) testing in one sex (usually females) is now considered sufficient.

2. The acute toxic class method (1) set out in this Guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods (Test Guidelines 420 and 425). The acute toxic class method is based on biometric evaluations (2)(3)(4)(5) with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment. The method as adopted in 1996 was extensively validated *in vivo* against LD50 data obtained from the literature, both nationally (6) and internationally (7).

3. Guidance on the selection of the most appropriate test method for a given purpose can be found in the Guidance Document on Acute Oral Toxicity Testing (8). This Guidance Document also contains additional information on the conduct and interpretation of Test Guideline 423.

4. Definitions used in the context of this Guideline are set out in Annex 1.

INITIAL CONSIDERATIONS

5. Test substances, at doses that are known to cause marked pain and distress due to corrosive or severely irritant actions, need not be administered. Moribund animals, or animals obviously in pain or showing signs of severe and enduring distress shall be humanely killed, and are considered in the interpretation of the test results in the same way as animals that died on test. Criteria for making the decision to kill moribund or severely suffering animals, and guidance on the recognition of predictable or impending death, are the subject of a separate Guidance Document (9).

6. The method uses pre-defined doses and the results allow a substance to be ranked and classified according to the Globally Harmonised System for the classification of chemicals which cause acute toxicity (10).

7. In principle, the method is not intended to allow the calculation of a precise LD_{50}, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test. The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%. The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

8. The testing laboratory should consider all available information on the test substance prior to conducting the study. Such information will include the identity and chemical structure of the substance; its physico-chemical properties; the result of any other *in vivo* or *in vitro* toxicity tests on the substance; toxicological data on the structurally related substances; and the anticipated use(s) of the substance. This information is necessary to satisfy all concerned that the test is relevant for the protection of human health and will help in the selection of the most appropriate starting dose.

PRINCIPLE OF THE TEST

9. It is the principle of the test that, based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex (normally females). Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.;

- no further testing is needed,
- dosing of three additional animals, with the same dose
- dosing of three additional animals at the next higher or the next lower dose level.

10. Details of the test procedure are described in Annex 2. The method will enable a judgement with respect to classifying the test substance to one of a series of toxicity classes defined by fixed LD50 cut-off values.

DESCRIPTION OF THE METHOD

Selection of animal species

11. The preferred rodent species is the rat, although other rodent species may be used. Normally females are used (9). This is because literature surveys of conventional LD50 tests show that, although there is little difference in sensitivity between the sexes, in those cases where differences are observed females are generally slightly more sensitive (11). However if knowledge of the toxicological or toxicokinetic properties of structurally related chemicals indicates that males are likely to be more sensitive, then this sex should be used. When the test is conducted in males adequate justification should be provided.

12. Healthy young adult animals of commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 8 and 12 weeks old and its weight should fall in an interval within $\pm$ 20 % of the mean weight of any previously dosed animals.

Housing and feeding conditions

13. The temperature in the experimental animal room should be 22ºC (± 3ºC). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals

14. The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions.

Preparation of doses

15. In general test substances should be administered in a constant volume over the range of doses to be tested by varying the concentration of the dosing preparation. Where a liquid end product or mixture is to be tested however, the use of the undiluted test substance, ie at a constant concentration, may be more relevant to the subsequent risk assessment of that substance, and is a requirement of some regulatory authorities. In either case, the maximum dose volume for administration must not be exceeded. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not normally exceed 1mL/100g of body weight: however in the case of aqueous solutions 2 mL/100g body weight can be considered. With respect to the formulation of the dosing preparation, the use of an aqueous solution/suspension/emulsion is recommended wherever possible, followed in order of preference by a solution/suspension/emulsion in oil (e.g. corn oil) and then possibly solution in other vehicles. For vehicles other than water the toxicological characteristics of the vehicle should be known. Doses must be prepared shortly prior to administration unless the stability of the preparation over the period during which it will be used is known and shown to be acceptable.

PROCEDURE

Administration of doses.

16. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours.

17. Animals should be fasted prior to dosing (e.g. with the rat, food but not water should be withheld over-night, with the mouse, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. Where a dose is administered in fractions over a period it may be necessary to provide the animals with food and water depending on the length of the period.

Number of animals and dose levels

18. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. The flow charts of Annex 2 describe the procedure that should be followed for each of the starting doses.

19. When available information suggests that mortality is unlikely at the highest starting dose level (2000 mg/kg body weight), then a limit test should be conducted. When there is no information on a substance to be tested, for animal welfare reasons it is recommended to use the starting dose of 300 mg/kg body weight.

20 The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs. Treatment of animals at the next dose, should be delayed until one is confident of survival of the previously dosed animals.

21. Exceptionally, and only when justified by specific regulatory needs, the use of additional upper dose level of 5000 mg/kg body weight may be considered (see Annex 3). For reasons of animal welfare concern, testing of animals in GHS Category 5 ranges (2000-5000mg/kg) is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health or the environment.

Limit test

22. The limit test is primarily used in situations where the experimenter has information indicating that the test material is likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. Information about the toxicity of the test material can be gained from knowledge about similar tested compounds or similar tested mixtures or products, taking into consideration the identity and percentage of components known to be of toxicological significance. In those situations where there is little or no information about its toxicity, or in which the the test material is expected to be toxic, the main test should be performed.

23. A limit test at one dose level of 2000 mg/kg body weight may be carried out with six animals (three animals per step). Exceptionally a limit test at one dose level of 5000 mg/kg may be carried out with three animals (see Annex 3). If test substance-related mortality is produced, further testing at the next lower level may need to be carried out.

OBSERVATIONS

24. Animals are 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, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed (12). All observations are systematically recorded with individual records being maintained for each animal.

25. Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep

and coma. The principles and criteria summarised in the Humane Endpoints Guidance Document (9) should be taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress should be humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible.

Body weight

26. Individual weights of animals should be determined shortly before the test substance is administered, and at least weekly thereafter. Weight changes should be calculated and recorded. At the end of the test surviving animals are weighed and humanely killed.

Pathology

27. All test animals (including those that die during the test or are removed from the study for animal welfare reasons) should be subjected to gross necropsy. All gross pathological changes should be recorded for each animal. Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours may also be considered because it may yield useful information.

DATA AND REPORTING

Data

28. Individual animal data should be provided. Additionally, all data should be summarised in tabular form, showing for each test group the number of animals used, the number of animals displaying signs of toxicity, the number of animals found dead during the test or killed for humane reasons, time of death of individual animals, a description and the time course of toxic effects and reversibility, and necropsy findings.

Test report

29. The test report must include the following information, as appropriate:

Test substance:

- physical nature, purity, and, where relevant, physico-chemical properties (including isomerisation);
- identification data, including CAS number.

Vehicle (if appropriate):

- justification for choice of vehicle, if other than water.

Test animals:

- species/strain used;
- microbiological status of the animals, when known;
- number, age, and sex of animals (including, where appropriate, a rationale for the use of males instead of females);
- source, housing conditions, diet etc.

Test conditions:

- details of test substance formulation including details of the physical form of the

material administered;
- details of the administration of the test substance including dosing volumes and time of dosing;
- details of food and water quality (including diet type/source, water source);
- the rationale for the selection of the starting dose.

Results:

- tabulation of response data and dose level for each animal (i.e. animals showing signs of toxicity including mortality; nature, severity, and duration of effects);
- tabulation of body weight and body weight changes;
- individual weights of animals at the day of dosing, in weekly intervals thereafter, and at the time of death or sacrifice;
- date and time of death if prior to scheduled sacrifice;
- time course of onset of signs of toxicity, and whether these were reversible for each animal;
- necropsy findings and histopathological findings for each animal, if available.

Discussion and interpretation of results.

Conclusions.

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ANNEX 1

DEFINITIONS

Acute oral toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours.

Delayed death means that an animal does not die or appear moribund within 48 hours but dies later during the 14-day observation period.

Dose is the amount of test substance administered. Dose is expressed as weight of test substance per unit weight of test animal (e.g. mg/kg).

GHS: Globally Harmonised Classification System for Chemical Substances and Mixtures. A joint activity of OECD (human health and the environment), UN Committee of Experts on Transport of Dangerous Goods (physical–chemical properties) and ILO (hazard communication) and co-ordinated by the Interorganisation Programme for the Sound Management of Chemicals (IOMC).

Impending death: when moribund state or death is expected prior to the next planned time of observation. Signs indicative of this state in rodents could include convulsions, lateral position, recumbence, and tremor (See the Humane Endpoint Guidance Document (9) for more details).

LD50 (median lethal oral dose) is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

Limit dose refers to a dose at an upper limitation on testing (2000 or 5000 mg/kg).

Moribund status: being in a state of dying or inability to survive, even if treated (See the Humane Endpoint Guidance Document (9) for more details).

Predictable death: presence of clinical signs indicative of death at a known time in the future before the planned end of the experimen ; for example: inability to reach water or food. (See the Humane Endpoint Guidance Document (9) for more details).

ANNEX 2

PROCEDURE TO BE FOLLOWED FOR EACH OF THE STARTING DOSES

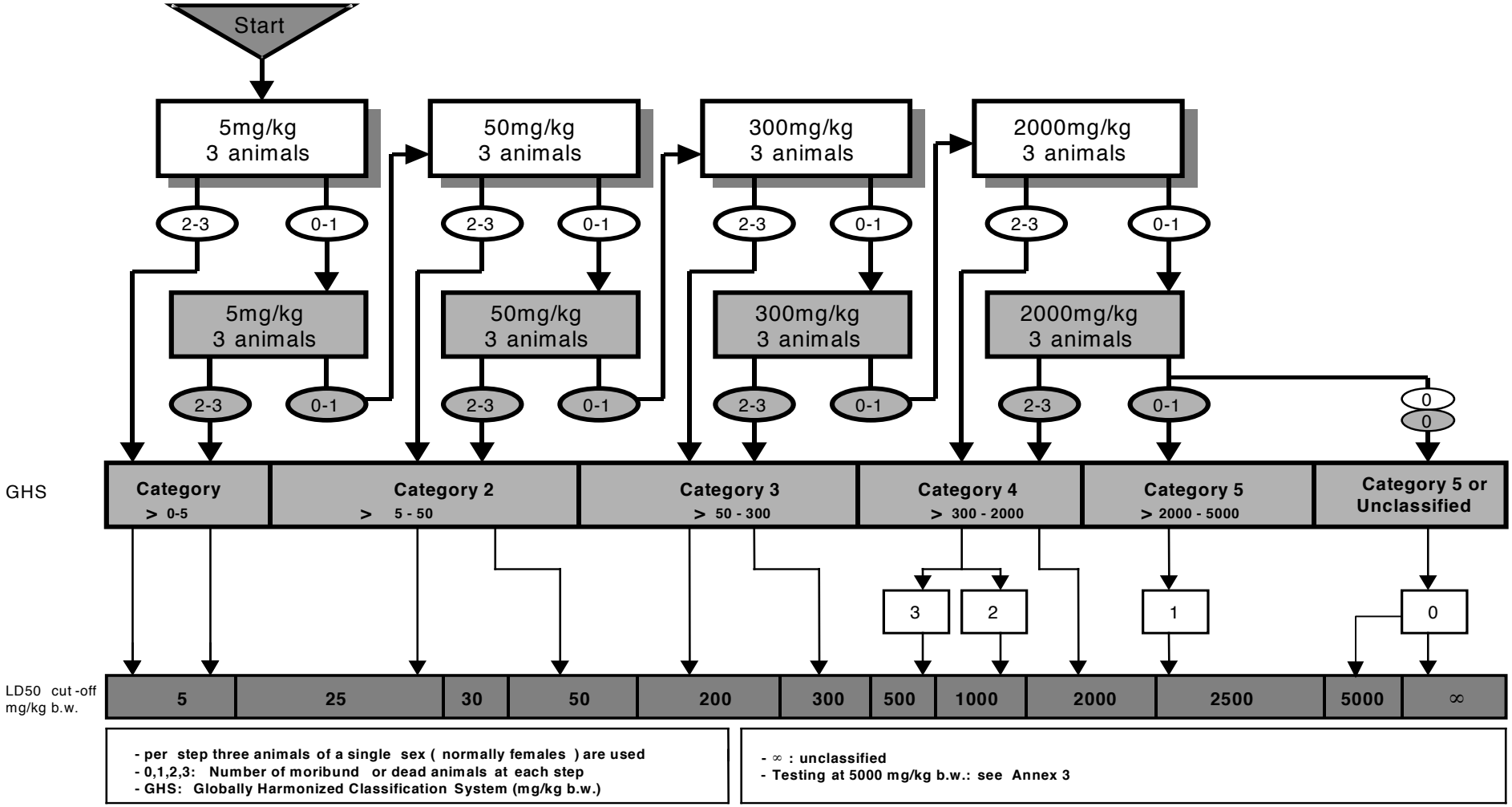
GENERAL REMARKS

1. For each starting dose, the respective testing schemes as included in this Annex outline the procedure to be followed.

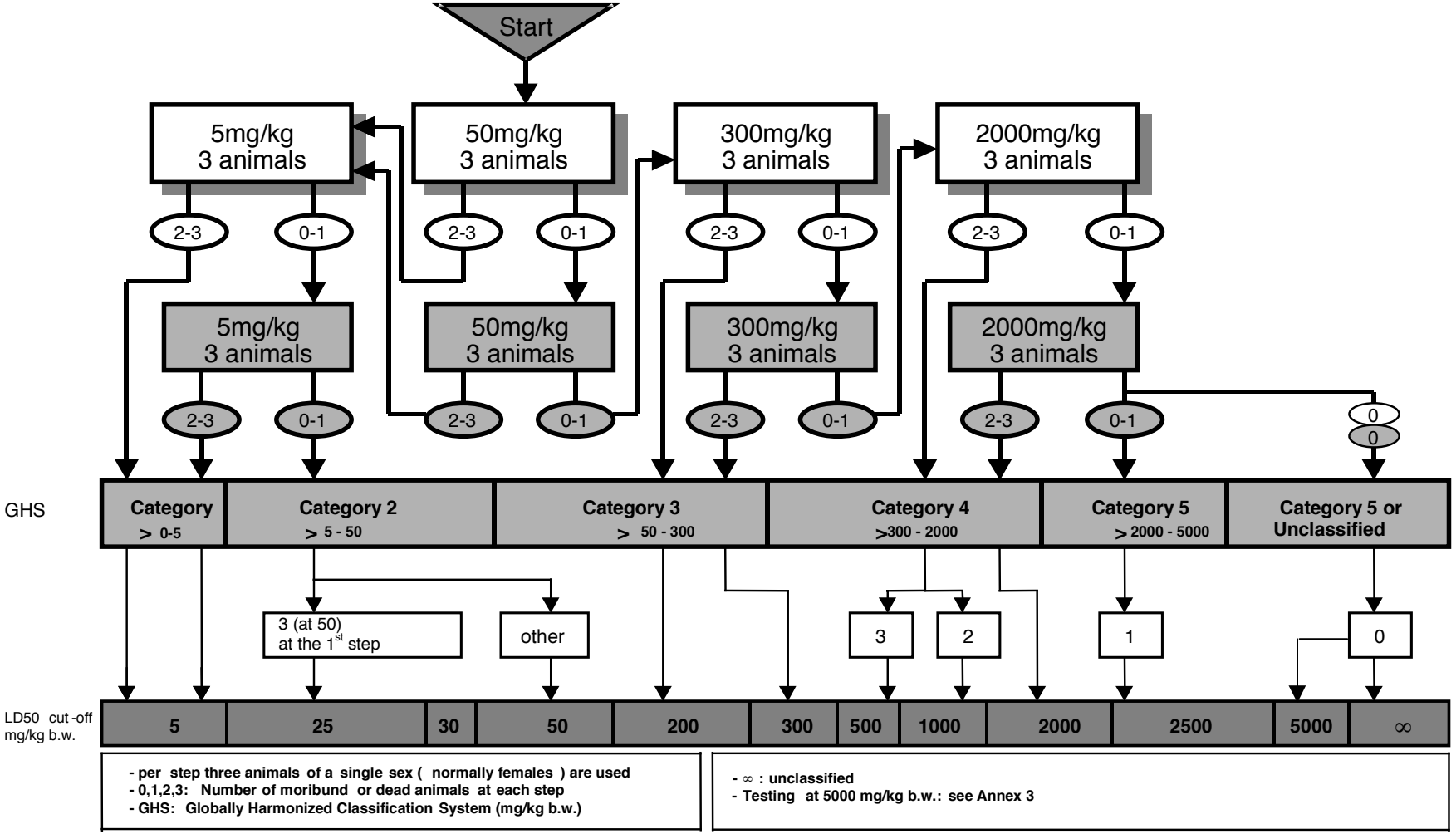
- Annex 2 a: Starting dose is 5 mg/kg bw
- Annex 2 b: Starting dose is 50 mg/kg bw
- Annex 2 c: Starting dose is: 300 mg/kg bw
- Annex 2 d: Starting dose is: 2000 mg/kg bw

Depending on the number of humanely killed or dead animals, the test procedure follows the indicated arrows.

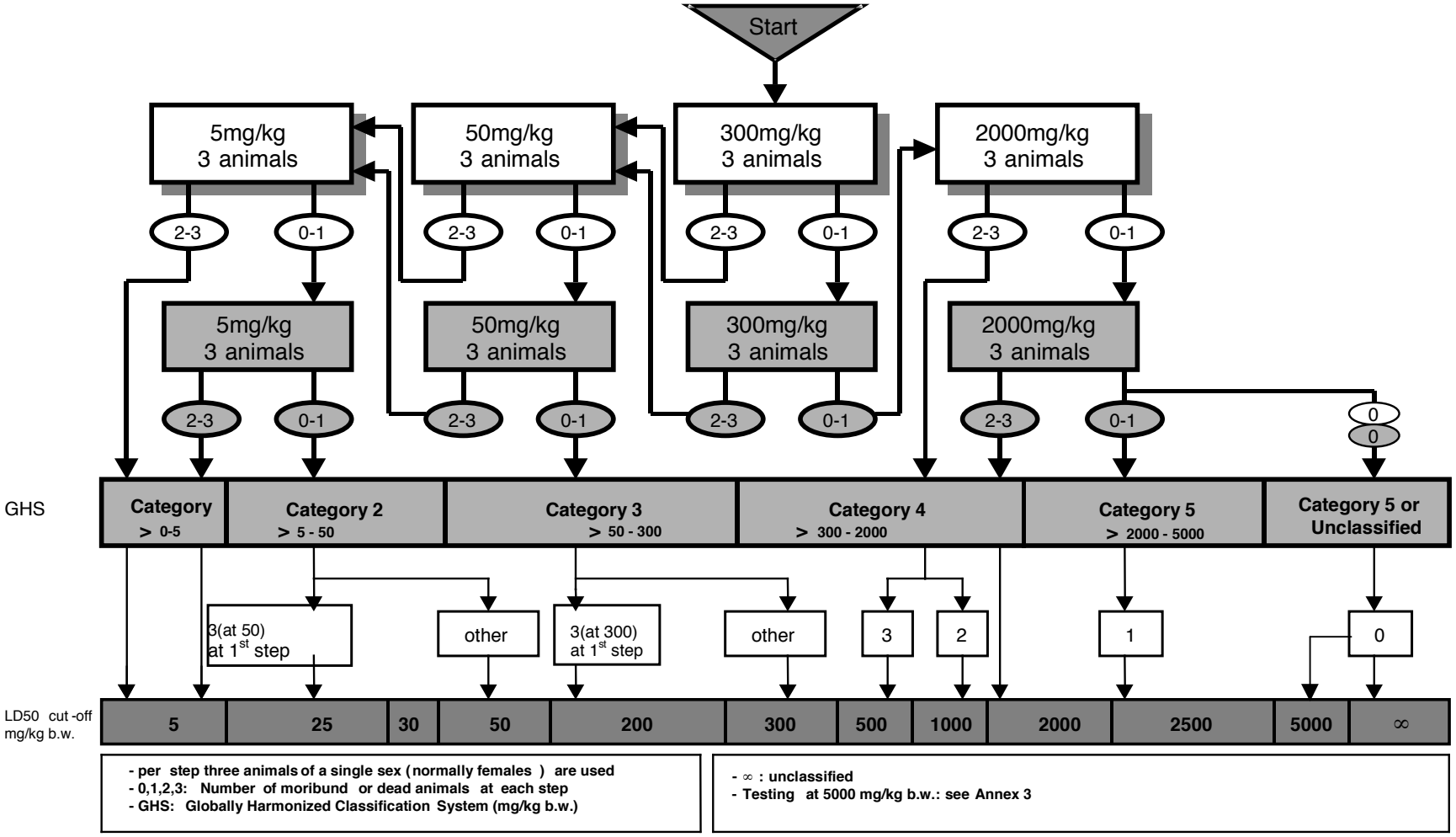
ANNEX 2a: TEST PROCEDURE WITH A STARTING DOSE OF 5 MG/KG BODY WEIGHT



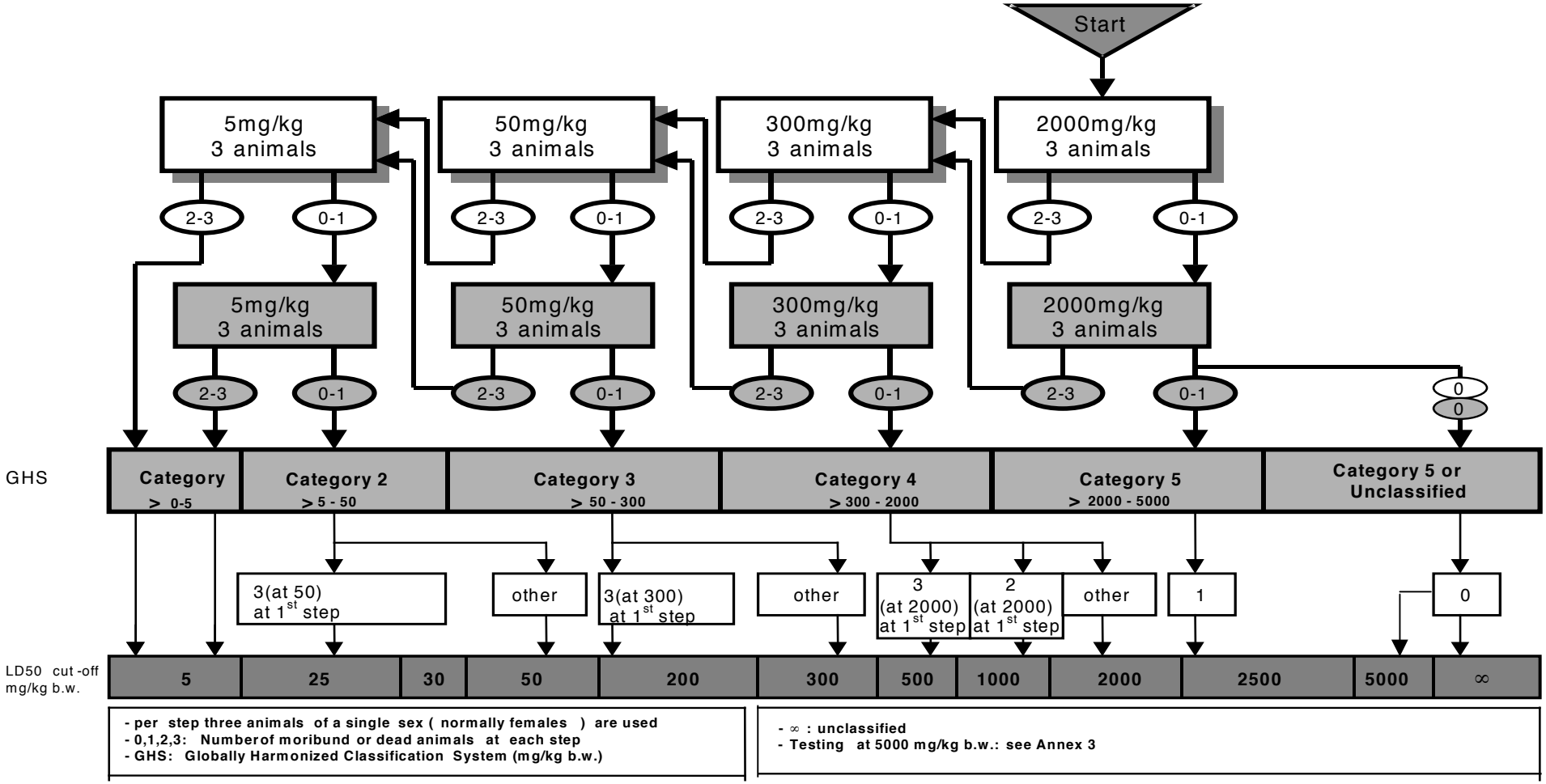
ANNEX 2b: TEST PROCEDURE WITH A STARTING DOSE OF 50 MG/KG BODY WEIGHT



ANNEX 2c: TEST PROCEDURE WITH A STARTING DOSE OF 300 MG/KG BODY WEIGHT



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



ANNEX 3

CRITERIA FOR CLASSIFICATION OF TEST SUBSTANCES WITH EXPECTED LD50 VALUES EXCEEDING 2000 MG/KG WITHOUT THE NEED FOR TESTING

1. Criteria for hazard Category 5 are intended to enable the identification of test substances which are of relatively low acute toxicity hazard but which, under certain circumstances may present a danger to vulnerable populations. These substances are anticipated to have an oral or dermal LD50 in the range of 2000-5000 mg/kg or equivalent doses for other routes. The test substance should be classified in the hazard category defined by: 2000mg/kg<LD50<5000mg/kg (Category 5 in the GHS) in the following cases:

a) If directed to this category by any of the testing schemes of Annex 2a-2d, based on mortality incidences;

b) if reliable evidence is already available that indicates the LD50 to be in the range of Category 5 values, or other animal studies or toxic effects in humans indicate a concern for human health of an acute nature.

c) Through extrapolation, estimation or measurement of data if assignment to a more hazardous category is not warranted, and

- reliable information is available indicating significant toxic effects in humans, or
- any mortality is observed when tested up to Category 4 values by the oral route, or
- where expert judgement confirms significant clinical signs of toxicity, when tested up to Category 4 values, except for diarrhoea, piloerection or an ungroomed appearance, or
- where expert judgement confirms reliable information indicating the potential for significant acute effects from the other animal studies.

TESTING AT DOSES ABOVE 2000 MG/KG

2. Recognising the need to protect animal welfare, testing of animals in Category 5 (5000 mg/kg) ranges is discouraged and should only be considered when there is a strong likelihood that results of such a test have a direct relevance for protecting human or animal health (10). No further testing should be conducted at higher dose levels.

3. When testing is required a dose of 5000mg/kg, only one step (i.e. three animals) is required. If the first animal dosed dies , then dosing procedes at 2000mg/kg in accordance with the flow charts in Annex 2. If the first animal survives, two further animals are dosed. If only one of the three animal dies , the LD50 value is expected to exceed 5000mg/kg. If both animals die, then dosing proceeds at 2000mg/kg.

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

Adopted by the Council on 27th July 1995

Repeated Dose 28-day Oral Toxicity Study in Rodents

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress. The original guideline 407 was adopted in 1981. In this revised version changes have been made with the objective of obtaining additional information from the animals used in the study.

2. This updated version of Guideline 407 is the outcome of a Consultation Meeting of an ad hoc Working Group of Experts on Systemic Short-term and (Delayed) Neurotoxicity, held in Paris in February 1992 (1). It is based on an earlier proposal by the United Kingdom, dated February 1991, and the comments received on that proposal from Member countries.

INITIAL CONSIDERATIONS

3. In the assessment and evaluation of the toxic characteristics of a chemical, the determination of oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained by acute testing. This study provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time. The method comprises the basic repeated dose toxicity study that may be used for chemicals on which a 90 day study is not warranted (e.g. when the production volume does not exceed certain limits) or as a preliminary to a long term study. The duration of exposure should normally be 28 days although a 14-day study may be appropriate in certain circumstances; justification for use of a 14-day exposure period should be provided.

4. The revised Guideline places more emphasis on neurological effects as a specific endpoint, and the need for careful clinical observations of the animals, so as to obtain as much information as possible, is stressed. The method should identify chemicals with neurotoxic potential, which may warrant further in-depth investigation of this aspect. In addition, the method may give an indication of immunological effects and reproductive organ toxicity.

5. Definitions used are given in the Annex.

PRINCIPLE OF THE TEST

6. The test substance is orally administered daily in graduated doses to several groups of experimental animals, one dose level per group for a period of 28 days. During the period of administration the animals are observed closely, each day for signs of toxicity. Animals which die or are killed during the test are necropsied and at the conclusion of the test surviving animals are killed and necropsied.

DESCRIPTION OF THE METHOD

Selection of animal species

7. The preferred rodent species is the rat, although other rodent species may be used. Commonly used laboratory strains of young healthy adult animals should be employed. The females should be nulliparous and non-pregnant. Dosing should begin as soon as possible after weaning and, in any case, before the animals are nine weeks old. At the commencement of the study the weight variation of animals used should be minimal and not exceed ± 20 % of the mean weight of each sex. Where a repeated dose oral study is conducted as a preliminary to a long term study, preferably animals from the same strain and source should be used in both studies.

Housing and feeding conditions

8. The temperature in the experimental animal room should be 22°C (± 3°C). Although the relative humidity should be at least 30% and preferably not to exceed 70% other than during room cleaning, the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. The choice of diet may be influenced by the need to ensure a suitable admixture of a test substance when administered by this method. Animals may be housed individually, or be caged in small groups of the same sex; for group caging, no more than five animals should be housed per cage.

Preparation of animals

9. Healthy young adult animals are randomly assigned to the control and treatment groups. Cages should be arranged in such a way that possible effects due to cage placement are minimized. The animals are identified uniquely and kept in their cages for at least five days prior to the start of the study to allow for acclimatisation to the laboratory conditions.

Preparation of doses

10. The test compound is administered by gavage or via the diet or drinking water. The method of oral administration is dependent on the purpose of the study, and the physical/chemical properties of the test material.

11. Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that, wherever possible, the use of an aqueous solution/suspension be considered first, followed by consideration of a solution/emulsion in oil (e.g. corn oil) and then by possible solution in other vehicles. For vehicles other than water the toxic characteristics of the vehicle must be known. The stability of the test substance in the vehicle should be determined.

PROCEDURE

Number and sex of animals

12. At least 10 animals (five female and five male) should be used at each dose level. If interim kills are planned, the number should be increased by the number of animals scheduled to be killed before the completion of the study. Consideration should be given to an additional satellite group of ten animals (five per sex) in the control and in the top dose group for observation of reversibility, persistence, or delayed occurrence of toxic effects, for at least 14 days post treatment.

Dosage

13. Generally, at least three test groups and a control group should be used, but if from assessment of other data, no effects would be expected at a dose of 1000mg/kg bw/d, a limit test may be performed. If there are no suitable data available, a range finding study may be performed to aid the determination of the doses to be used. Except for treatment with the test substance, animals in the control group should be handled in an identical manner to the test group subjects. If a vehicle is used in administering the test substance, the control group should receive the vehicle in the highest volume used.

14. Dose levels should be selected taking into account any existing toxicity and (toxico-) kinetic data available for the test compound or related materials. The highest dose level should be chosen with the aim of inducing toxic effects but not death or severe suffering. Thereafter, a descending sequence of dose levels should be selected with a view to demonstrating any dosage related response and no-observed-adverse effects at the lowest dose level (NOAEL). Two to four fold intervals are frequently optimal for setting the descending dose levels and addition of a fourth test group is often preferable to using very large intervals (e.g. more than a factor of 10) between dosages.

Limit test

15. If a test at one dose level of at least 1000 mg/kg body weight/day or, for dietary or drinking water administration, an equivalent percentage in the diet, or drinking water (based upon body weight determinations), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data from structurally related compounds, then a full study using three dose levels may not be considered necessary. The limit test applies except when human exposure indicates the need for a higher dose level to be used.

Administration of doses

16. The animals are dosed with the test substance daily seven days each week for a period of 28 days; use of a five-day per week dosing regime or a 14-day exposure period needs to be justified. When the test substance is administered by gavage, this should be done in a single dose to the animals using a stomach tube or a suitable intubation cannula. The maximum volume of liquid that can be administered at one time depends on the size of the test animal. The volume should not exceed 1ml/100g body weight, except in the case of aqueous solutions where 2ml/100g body weight may be used. Except for irritating or corrosive substances which will normally reveal exacerbated effects with higher concentrations, variability in test volume should be minimised by adjusting the concentration to ensure a constant volume at all dose levels.

17. For substances administered via the diet or drinking water it is important to ensure that the quantities of the test substance involved do not interfere with normal nutrition or water balance. When the test substance is administered in the diet either a constant dietary concentration (ppm) or a constant dose level in terms of the animals' body weight may be used; the alternative used must be specified. For a substance administered by gavage, the dose should be given at similar times each day, and adjusted as necessary to maintain a constant dose level in terms of animal body weight. Where a repeated dose study is used as a preliminary to a long term study, a similar diet should be used in both studies.

Observations

18. The observation period should be 28 days, unless the study duration is 14 days (see paragraph 3). Animals in a satellite group scheduled for follow-up observations should be kept for at least a further 14 days without treatment to detect delayed occurrence, or persistence of, or recovery from toxic effects.

19. General clinical observations should be made at least once a day, preferably at the same time(s) each day and considering the peak period of anticipated effects after dosing. The health condition of the animals should be recorded. At least twice daily, all animals are observed for morbidity and mortality.

20. Once before the first exposure (to allow for within-subject comparisons), and at least once a week thereafter, detailed clinical observations should be made in all animals. These observations should be made outside the home cage in a standard arena and preferably at the same time, each time. They should be carefully recorded, preferably using scoring systems, explicitly defined by the testing laboratory. Effort should be made to ensure that variations in the test conditions are minimal and that observations are preferably conducted by observers unaware of the treatment. Signs noted should include, but not be limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g. lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypies (e.g. excessive grooming, repetitive circling) or bizarre behaviour (e.g. self-mutilation, walking backwards) should also be recorded (2).

21. In the fourth exposure week sensory reactivity to stimuli of different types (2) (e.g. auditory, visual and proprioceptive stimuli) (3)(4)(5), assessment of grip strength (6) and motor activity assessment (7) should be conducted. Further details of the procedures that could be followed are given in the respective references. However, alternative procedures than those referenced could also be used.

22. Functional observations conducted in the fourth exposure week may be omitted when the study is conducted as a preliminary study to a subsequent subchronic (90-day) study. In that case, the functional observations should be included in this follow-up study. On the other hand, the availability of data on functional observations from the repeated dose study may enhance the ability to select dose levels for a subsequent subchronic study.

23. Exceptionally, functional observations may also be omitted for groups that otherwise reveal signs of toxicity to an extent that would significantly interfere with the functional test performance.

Body weight and food/water consumption

24. All animals should be weighed at least once a week. Measurements of food consumption should be made at least weekly. If the test substance is administered via the drinking water, water consumption should also be measured at least weekly.

Haematology

25. The following haematological examinations should be made at the end of the test period: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, platelet count and a measure of blood clotting time/potential.

26. Blood samples should be taken from a named site just prior to or as part of the procedure for killing the animals, and stored under appropriate conditions.

Clinical Biochemistry

27. Clinical biochemistry determinations to investigate major toxic effects in tissues and, specifically, effects on kidney and liver, should be performed on blood samples obtained of all animals just prior to or as part of the procedure for killing the animals (apart from those found moribund

and/or intercurrently killed). Overnight fasting of the animals prior to blood sampling is recommended[1]. Investigations of plasma or serum shall include sodium, potassium, glucose, total cholesterol, urea, creatinine, total protein and albumin, at least two enzymes indicative of hepatocellular effects (such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, and sorbitol dehydrogenase). Measurements of additional enzymes (of hepatic or other origin) and bile acids may provide useful information under certain circumstances.

28. Optionally, the following urinalysis determinations could be performed during the last week of the study using timed urine volume collection; appearance, volume, osmolality or specific gravity, pH, protein, glucose and blood/blood cells.

29. In addition, studies to investigate serum markers of general tissue damage should be considered. Other determinations that should be carried out if the known properties of the test substance may, or are suspected to, affect related metabolic profiles include calcium, phosphate, fasting triglycerides, specific hormones, methaemoglobin and cholinesterase. These need to be identified for chemicals in certain classes or on a case-by-case basis.

30. Overall, there is a need for a flexible approach, depending on the species and the observed and/or expected effect with a given compound.

31. If historical baseline data are inadequate, consideration should be given to determination of haematological and clinical biochemistry variables before dosing commences.

Pathology

Gross necropsy

32. All animals in the study shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The liver, kidneys, adrenals, testes, epididymides, thymus, spleen, brain and heart of all animals (apart from those found moribund and/or intercurrently killed) should be trimmed of any adherent tissue, as appropriate, and their wet weight taken as soon as possible after dissection to avoid drying.

33. The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination: all gross lesions, brain (representative regions including cerebrum, cerebellum and pons), spinal cord, stomach, small and large intestines (including Peyer's patches), liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea and lungs (preserved by inflation with fixative and then immersion), gonads, accessory sex organs (e.g. uterus, prostate), urinary bladder, lymph nodes (preferably one lymph node covering the route of administration and another one distant from the route of administration to cover systemic effects), peripheral nerve (sciatic or tibial) preferably in close proximity to the muscle, and a section of bone marrow (or, alternatively, a fresh mounted bone marrow aspirate). The clinical and other

[1] For a number of measurements in serum and plasma, most notably for glucose, overnight fasting would be preferable. The major reason for this preference is that the increased variability which would inevitably result from non-fasting, would tend to mask more subtle effects and make interpretation difficult. On the other hand, however, overnight fasting may interfere with the general metabolism of the animals and, particularly in feeding studies, may disturb the daily exposure to the test substance. If overnight fasting is adopted, clinical biochemical determinations should be performed after the conduct of functional observations in week 4 of the study.

findings may suggest the need to examine additional tissues. Also any organs considered likely to be target organs based on the known properties of the test substance should be preserved.

Histopathology

34. Full histopathology should be carried out on the preserved organs and tissues of all animals in the control and high dose groups. These examinations should be extended to animals of all other dosage groups, if treatment-related changes are observed in the high dose group.

35. All gross lesions shall be examined.

36. When a satellite group is used, histopathology should be performed on tissues and organs identified as showing effects in the treated groups.

DATA AND REPORTING

Data

37. Individual data should be provided. Additionally, all data should be summarised in tabular form showing for each test group the number of animals at the start of the test, the number of animals found dead during the test or killed for humane reasons and the time of any death or humane kill, the number showing signs of toxicity, a description of the signs of toxicity observed, including time of onset, duration, and severity of any toxic effects, the number of animals showing lesions, the type of lesions and the percentage of animals displaying each type of lesion.

38. When possible, numerical results should be evaluated by an appropriate and generally acceptable statistical method. The statistical methods should be selected during the design of the study.

Test report

39. The test report must include the following information:

Test substance:

- physical nature, purity and physicochemical properties;
- identification data.

Vehicle (if appropriate):

- justification for choice of vehicle, if other than water.

Test animals:

- species/strain used;
- number, age and sex of animals;
- source, housing conditions, diet, etc.;
- individual weights of animals at the start of the test.

Test conditions:

- rationale for dose level selection;

- details of test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation;
- details of the administration of the test substance;
- conversion from diet/drinking water test substance concentration (ppm) to the actual dose (mg/kg body weight/day), if applicable;
- details of food and water quality.

Results:

- body weight/body weight changes;
- food consumption, and water consumption, if applicable;
- toxic response data by sex and dose level, including signs of toxicity;
- nature, severity and duration of clinical observations (whether reversible or not);
- sensory activity, grip strength and motor activity assessments;
- haematological tests with relevant base-line values;
- clinical biochemistry tests with relevant base-line values;
- body weight at killing and organ weight data;
- necropsy findings;
- a detailed description of all histopathological findings;
- absorption data if available;
- statistical treatment of results, where appropriate.

Discussion of results.

Conclusions.

LITERATURE

(1) OECD (Paris, 1992). Chairman's Report of the Meeting of the ad hoc Working Group of Experts on Systemic Short-term and (Delayed) Neurotoxicity.

(2) IPCS (1986). Principles and Methods for the Assessment of Neurotoxicity Associated with Exposure to Chemicals. Environmental Health Criteria Document No. 60

(3) Tupper, D.E., Wallace, R.B. (1980). Utility of the Neurologic Examination in Rats. Acta Neurobiol. Exp., 40, 999-1003.

(4) Gad, S.C. (1982). A Neuromuscular Screen for Use in Industrial Toxicology. J. Toxicol Environ. Health, 9, 691-704.

(5) Moser, V.C., McDaniel, K.M., Phillips, P.M. (1991). Rat Strain and Stock Comparisons Using a Functional Observational Battery: Baseline Values and Effects of Amitraz. Toxicol. Appl. Pharmacol., 108, 267-283.

(6) Meyer O.A., Tilson H.A., Byrd W.C., Riley M.T. (1979). A Method for the Routine Assessment of Fore- and Hindlimb Grip Strength of Rats and Mice. Neurobehav. Toxicol., 1, 233-236.

(7) Crofton K.M., Howard J.L., Moser V.C., Gill M.W., Reiter L.W., Tilson H.A., MacPhail R.C. (1991). Interlaboratory Comparison of Motor Activity Experiments: Implication for Neurotoxicological Assessments. Neurotoxicol. Teratol. 13, 599-609.

ANNEX

DEFINITIONS

Dose is the amount of test substance administered. Dose is expressed as weight (g, mg) or as weight of test substance per unit weight of test animal (e.g. mg/kg), or as constant dietary concentrations (ppm).

Dosage is a general term comprising of dose, its frequency and the duration of dosing.

Evident toxicity is a general term describing clear signs of toxicity following administration of test substance. These should be sufficient for hazard assessment and should be such that an increase in the dose administered can be expected to result in the development of severe toxic signs and probable mortality.

NOAEL is the abbreviation for no-observed-adverse-effect level and is the highest dose level where no adverse treatment-related findings are observed.

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Ref. No. _____ Date _____

Report No. 250. 7-12-2014.

Name of the Party	Dr Nilambari Patil, (Ph.D.Student). Pune.
Your Ref.No.	Letter dated 4-12-2014.
Type of the Sample.	Fresh Awala.
Date of Receipt.	4-12-2014.
Batch No.	-
Quantity Received.	1 X 100 gm.

Sample Drawn by Party.

Authentification Certificate

The sample has been critically studied with macroscopic, microscopic and organoleptic characters . We here by authenticate that the sample of fresh Awala belongs to fruit of *Emblica officinalis* L. from the family Euphorbiaceae.

The certificate is issued on request and given only for Academic use.

AeDesai

For I.D.R.A.& L. Pune.

Indian Drugs Research Association & Laboratory

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Ref. No. _____ Date 13-04-15.

Report No. 331.

CERTIFICATE OF ANALYSIS
CONFIDENTIAL

Name of the Party.	Dr Nilambari Patil, Pune.
Your Ref.No.	Your letter dated 18-3-2015.
Type of the Sample.	Sample-A.
Date of receipt.	18-3-2015.
Quantity Received.	1 X 50gm. Sample Drawn by Party.

Description: Black coloured free flowing coarse Powder.

Physico Chemical test:-

1) Loss on Drying:	2.70 %
2) Total Ash:	3.36 %
3) Acid Insoluble Ash:	0.18 %
4) Alcohol Soluble Extractive:	0.18 %
5) Water Soluble Extractive:	0.67 %
6) pH (10%)	5.86
7) Thin Layer Chromatography:-	
Extraction -	Methanol Extract.
Adsorbent Used:	Silica gel $G_{60}F_{254}$.
Mobile Phase –	Toluene:Ethyl Acetate:Glacial Acetic Acid:Formic Acid (2:4.5:2:0.5)
Detection-	
UV-254 nm –	Four spots. Rf-0.53,0.80,0.85,09.93(All dark blue)
UV – 365 nm-	Three spots. Rf- 0.53, 0.80, 0.85 (All light blue).
Iodine-	Three spots. Rf-0.53,0.80,0.85(All dark brown).
Anisaldehyde Sulphuric acid: Reagent.	5 spots. 0.25,0.35,(Green)0.80,(Brown) 0.85,(Green),0.93(Blue).

Preliminary Phytochemical tests (Water extract).

1) Alkaloid	Negative.
2) Flavonoid:	Positive.
3) Steroids:	Negative.
4) Tannin:	Negative..
5) Terpenoid:	Negative
6) Saponin:	Negative
7) Sugars:	Negative.

13/4/2015

For I.D.R.A.& L. Pune.

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E-mail : idralpune@gmail.com, Website - www.idra.in

Ref. No. _____ Date _____

Report No. 332. 13-04-15.

CERTIFICATE OF ANALYSIS
CONFIDENTIAL

Name of the Party.	Dr Nilambari Patil, Pune.
Your Ref.No.	Your letter dated 18-3-2015.
Type of the Sample.	Sample-B.
Date of receipt.	18-3-2015.
Quantity Received.	1 X 50gm.
	Sample Drawn by Party.

Description: Black coloured free flowing coarse Powder.

Physico Chemical test:-

1) Loss on Drying:	2.17 %
2) Total Ash:	5.52 %
3) Acid Insoluble Ash:	0.16 %
4) Water Soluble Extractive:	0.71 %
5) Alcohol Soluble Extractive:	0.33 %
6) pH (10%)	7.46
7) Thin Layer Chromatography:-	
Extraction -	Methanol Extract.
Adsorbent Used:	Silica gel $G_{60}F_{254}$.
Mobile Phase –	Toluene:Ethyl Acetate:Glacial Acetic Acid:Formic Acid (2:4.5:2:0.5)
Detection-	
UV-254 nm –	No separation was observed.
UV – 365 nm-	-"- -"-
Iodine-	-"- -"-
Anisaldehydr Sulphuric acid: Reagent.	-"- -"-

Preliminary Phytochemical tests (Water extract).

1) Alkaloid	Negative.
2) Flavonoid:	Negative.
3) Steroids:	Negative.
4) Tannin:	Negative.
5) Terpenoid:	Negative
6) Saponin:	Negative
7) Sugars:	Negative.

For I.D.R.A.& L. Pune.

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E-mail : idralpune@gmail.com, Website - www.idra.in

Ref. No. _____ Date 13-04-15.

Report No. 333.

CERTIFICATE OF ANALYSIS
CONFIDENTIAL

Name of the Party.	Dr Nilambari Patil, Pune.
Your Ref.No.	Your letter dated 18-3-2015.
Type of the Sample.	Sample-C.
Date of receipt.	18-3-2015.
Quantity Received.	1 X 50gm. Sample Drawn by Party.

Description: Brown coloured free flowing coarse Powder.

Physico Chemical test:-

1) Loss on Drying:	10.53 %
2) Total Ash:	2.08 %
3) Acid Insoluble Ash:	0.15 %
4) Water Soluble Extractive:	16.23 %
5) Alcohol Soluble Extractive:	14.06 %
6) pH (10%)	2.80
7) Thin Layer Chromatography:-	
Extraction -	Methanol Extract.
Adsorbent Used:	Silica gel $G_{60}F_{254}$.
Mobile Phase –	Toluene:Ethyl Acetate:Glacial Acetic Acid:Formic Acid (2:4.5:2:0.5)
Detection-	
UV-254 nm –	Four spots. Rf-0.23,0.40,0.58,0.81(All dark blue)
UV – 365 nm-	Two spots. Rf- 0.35, 0.81.(All green)).
Iodine-	Six spots. Rf-0.06,0.21,0.33,0.41,(Yelllow).56,0.81 (Blue).
Anisaldehyde Sulphuric acid: Reagent	4 spots. 0.11,(Green)0.41,0.81(Brown),0.95,(blue)

Preliminary Phytochemical tests (Water extract).

1) Alkaloid	Negative.
2) Steroids:	Negative.
3) Flavonoid:	Negative.
4) Tannin:	Negative.
5) Terpenoid:	Negative
6) Saponin:	Negative
7) Sugars:	Positive.

For I.D.R.A.& L. Pune.

Accurate Analytical Laboratory
House for Chemical, Microbiological Analysis
Prof : Mrs. S. M. Nadkarni M.Sc (Lond.)
Phone : +91-20-25671998 TeleFax : +91-20-25652744
E mail : accurate.analyticallab@gmail.com
: shalini_nadkarni2006@yahoo.co.in
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967 / 3 / 1, Bhagyadeep,
Near Ratna Memorial Hospital,
Senapati Bapat Road,
Pune - 411016.

TEST REPORT

REPORT No : ATM / L - 778 2.3.2015

To,
Dr. Nilambari M Patil,
PHD (Scholar),
T.M.V., Pune

Your Ref No : Your Letter Dated :- 21.2.2015 Received On :- 21.2.2015.

Samples : Three solid samples marked as,

1) Sample - A For :- Vitamin C , Total Organic Carbon .
2) Sample - B For :- Vitamin C, Total Organic Carbon .
3) Sample - C For :- Vitamin C.

Test Conducted : To determine Vitamin C, Total Organic Carbon .

TEST FINDINGS :

Sr.No.	Samples	Unit	Vitamin C	Total Organic Carbon
1.	Sample - A	%	0 . 0329	42 . 712
2.	Sample - B	%	0 . 0153	3 . 2367
3.	Sample - C	%	3 . 7742	---

For Accurate Analytical Laboratory

M.T.G./ M.M.

Mrs. S.M. Nadkarni

Disclaimer:

- Sample submitted by the Customer in their own container.
- Above analysis result is valid only for the specific sample as stated above, submitted for testing, without any bias to its source.
- We claim no responsibility for changes made in the report after dispatch e.g use of whitener or eraser.
- Above Test Report cannot be produced as legal evidence without our prior written permission.
- Sample stored for one month and Test Report for one year from the date received.
- Duplicate copies of Report or Invoice will be charged extra.

Sophisticated Analytical Instrument Facility (RSIC)

Indian Institute of Technology Powai, Mumbai 400076

Phone : (022)2576 7691 / 2 /3

Gram :Technology, Bombay 400 076
Fax : 091-22-2572 3314
091-22-2572 3480

IIT Bombay

Ref: -ICP AES-
141 Date:
14/12/2015

Analytical report of the samples submitted by **Dr. Nilambari M Patil, Tilak Maharashtra Vidyapeeth, Pune,** using Inductively Coupled Plasma Atomic Emission Spectroscopy.

Sample	P	S	Ti	Hg	Zr	Ce
	%	%	%	%	%	%
SAMPLE-A	0.119	0.083	0.0042	0.0034	ND	ND
SAMPLE-B	0.144	0.068	ND	0.012	ND	ND
SAMPLE-C	0.058	0.023	0.00004	ND	ND	ND

Sample	Al	B	Ba	Ca	Cr
	%	%	%	%	%
SAMPLE-A	0.025	0.010	0.00079	0.365	0.00036
SAMPLE-B	0.021	0.006	0.00083	0.435	ND
SAMPLE-C	0.007	0.001	0.00049	0.306	ND

Sample	Cu	Fe	K	Pb	Sr	Zn
	%	%	%	%	%	%
SAMPLE-A	0.0016	0.013	1.03	0.001	0.0011	0.0029
SAMPLE-B	0.0018	0.017	1.764	0.001	0.0014	0.0037
SAMPLE-C	0.0009	0.0019	0.658	0.0007	0.00079	0.0035

Sample	Li	Mg	Mn	Na	Ni
	%	%	%	%	%
SAMPLE-A	ND	0.098	0.00096	0.018	0.00031
SAMPLE-B	ND	0.104	0.0011	0.02	0.00073
SAMPLE-C	ND	0.061	0.0006	0.016	ND

ND means less than 0.01 ppm

Note: Content of this report is meant for your information only and should not be quoted as certificate to third party for advertisement, evidence or litigation.

Regd. Office: S. No. 36/1/1, M.N. 199, Vadgaon Khurd, Pune 411 041, Maharashtra, India.
● **Tel.:** 020 - 2439 2933 / 2439 4803 ● **E-mail:** ntcpune@gmail.com ● **Web:** ntc-apt.com

APT RESEARCH FOUNDATION

Date: 22/June /2015

C E R T I F I C A T E

This is to certify that Dr. Nilambari M Patil, Tilak Maharashtra Vidyapeeth, Ayurveda Faculty, Pune, under the guidance of Dr. Vilas A Dole, Tilak Maharashtra Vidyapeeth, Ayurveda Faculty, Pune,has satisfactorily conducted her experiments for the degree of Ph.D. (Pharmaceutical Sciences) in this Institute as per the approved protocol through Research Project No. 45/1415 entitled "ACUTE ORAL TOXICITY (OECD 423), SUB ACUTE ORAL TOXICITY (OECD 407) AND ANTI ULCER ACTIVITY OF AAMALAKI MASHI." as a part of the thesis entitled "COMPARATIVE PHYSICO-CHEMICAL CHARACTERIZATION AND *IN VIVO* ANTI -ULCER ACTIVITY OF AAMALAKI MASHI."

For APT Research Foundation,

APT RESEARCH FOUNDATION PUNE

Dr. K. G. Apte

Study Director & Head,

APT Research Foundation, Pune.