

1. INTRODUCTION

Siddha system is one among the Indian systems of Medicine which has been practiced in Tamil speaking part of world. This system was founded by ‘*Siddhars*’ who are known for their protean abilities. Siddhars, were the incorporeals who explored and explained the reality of nature and its relationship by their yogic sentience and experimental findings. They preached the concept of spiritualism for self empowerment and then from that, different practices were evolved to be known as the “*Siddha System*” (1).

Whatever present in the Megacosm is present in the Microcosm
Whatever present in the Microcosm is present in the Megacosm
Megacosm and Microcosm are one
When you look in right understanding

- *Sattamuni*

The human being’s body is dependent on nature’s primary and its supportive system. The Man is a miniature of the universe in which he lives. Any change in the universe due to natural or unnatural causes will create changes in human systems. For example the natural disorders like cyclone, heavy rain, mist and scorching sun or man created impurities of air and water will create changes both in the atmosphere and in the human body. Hence the change in the elementary conditions of external world has its corresponding change in the human organs.

Fever is a rise of body temperature that may exceed the circadian variation. A normal body temperature is maintained habitually, inspite environmental variations because the heat production from the metabolic activity happening in liver and muscle with heat exacerbation is regulated by thermoregulatory centre in hypothalamus (2). As per studies conducted in healthy subjects with 18 to 40 yrs of age, the mean oral temperature is $36.8 \pm 0.4^{\circ}\text{C}$ (3).

As per *Siddha* literature (4) *Suram* which can be correlated to fever.. Ancient *Siddha* literatures numbered the diseases as 4448. Among them *Suram* is one of the disease. According to the concept of *Siddha*, the taste of food which consumes in improper proportional plays a key role for diseases. Sage *Theran* denotes Pyrexia (*Suram*) is a disease, which is caused by the accumulation of *Seetham* in the GI tract which makes

increase of body temperature (5). Pyrexia (*Suram*) can be defined as increased body temperature above its normal range, burning sensation in eyes, pain in the body, nausea and vomiting (6). Several etiological factors are described for causes of Pyrexia (*Suram*) in *Siddha* literature. In ancient time, by the correlation of symptoms what the patient narrated led to the types of Pyrexia (*Suram*). Further by means of Tridhosas, associated manifestations and various etiological factors, it can be classified into two major types viz *Thanvazhi* and *Puravazhi Suram* (5).

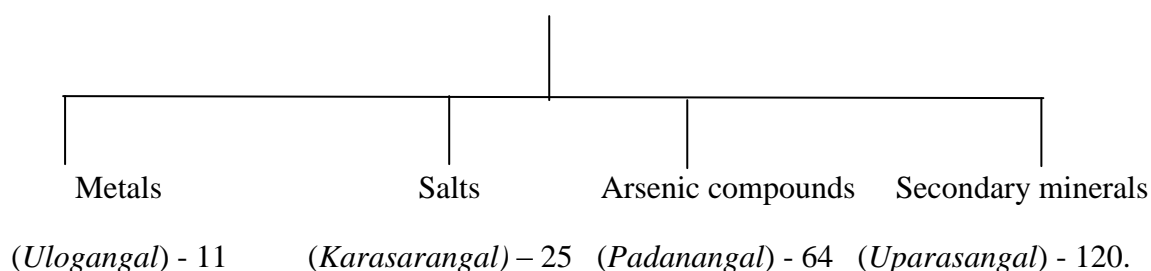
Much therapeutic management indicated for fever according to their type. Suleyman et al (2007) studied the indigenous treatment of fever using non-steroidal fabricated anti pyretics has been associated with gastro- intestinal toxicity, nephro toxicity, hepato-toxicity central nervous system and dermatological toxicity (7). In western medicine, cause of fever and the diagnosis of the diseases are assessed using various tests, then treated appropriately by using antibiotics, antipyretics or external cooling methods (8). Before a confirmed diagnosis even when the infection is suspected antibiotics are generally not recommended. Preventive administration of antibiotics can develop a risk of clinical misjudgment and cause serious side effects. The role of *Siddha* drug in the management of *Suram* is not only in normalizing the elevated body temperature but also in eradicating the etiological factor of that particular type of *Suram*.

Universe is made up of by five basic elements and the objects in its were classified into two, namely movable and motionless objects. The above view has been declared by a rational *Tamilian* as follows:

“Classifying all movable and immovable things
Within the classification of five material elements....”

The Earth whirls with all these objects. The Sky provides shelter to the objects while the Water, the Fire and the Air provide protection. Both mobile and motionless objects may be classified under three major categories such as Mineral, Herbal and Animal Kingdom (9).

Minerals – 220



Among the Metals, *Lingam* (Cinnabar) is a one of the important metal which has been used as an ingredient in the preparation of various *Siddha* medicines. Treatment in *Siddha* system divided into two types such as Internal and External medicine. Further they are divided into 32 types in each. Chendhuram is one among the 32 forms of internal medicine pictured in *Siddha Materia medica*. Chendhuram means sulphide form of toxic salts reduced into red coloured powders, by the process of either drying or burning them or exposing to the sunlight of keeping them in specialized tubes by adding decoctions, *ceyneed*, and acid etc - Calcined red oxides / sulphides (10).

Linga chendhuram is a single metallic drug preparation useful in *Siddha* system of medicine. It has been widely used in therapeutic management of arthritis, delirious fevers, anaemia etc (11). It is made from cinnabar (*Lingam*), the chief ore of mercury. Generally there are two types of *Linga chendhuram* viz *Linga chendhuram* number 1 and *Linga chendhuram* number 2 based on the preparation methods. Both are very often useful in practice. *Linga chendhuram* number 1 is prepared by titration (*Churuk*)'process (12) and *Linga chendhuram* number 2 is prepared by burning in cow-dung cakes (*Pudam* process) (13). The author selected for *Linga chendhuram* 1 in this study which consists of *lingam* and *Citrullus colocynthis* (*Atruthorumatti*). The preparation of trial drug *Linga chendhuram* is as follows. 35g of purified *Lingam* was placed over the mud pan (*Agal*) and started to heat at low flare using LPG stove. 1.3 lit of *Citrullus colocynthis* juice of whole plant (*Atruthorumatti*) was added drop by drop over the *Lingam* simultaneously while heating. Later on, *Lingam* was taken to *kalvam* and ground into very fine powder until the disappearance of its luster. (9). The finished test drug was kept in an air tight sterile glass vessel in a dark place.

Robert sapper et al (2004) in JAMA reported that Ayurveda and *Siddha* drugs are to be banned because of the presence of heavy metals in these drugs which they got through unreliable net source. After the effort from AYUSH, they accepted that these drugs are safe to consume while prepared traditionally as per literature. The modern scientists need the preclinical data of Indian system of medicine. It is a known fact that the *Siddha* medicines are safe and powerful for treating diseases, but for worldwide acceptance, *Siddha* system of medicine has to be explored only by scientific manner by conducting experiment on animal model and clinical trial. The present study report the qualitative and quantitative analysis, safety and efficacy studies in animal model and efficacy in clinical trial.

In the aspect of *Suram* (pyrexia) management scientific there are no data is available of the siddha drug LC in detail from clinical trials to justify the scientific safety use of *Siddha* treatment regimen of *Suram* (pyrexia) management and LC which is extensively used as a antipyretic in siddha system of medicine is yet to be standardized scientifically. Hence keeping these drawbacks, Standardization of *Linga chendhuram* preparation as per Pharmacopoeias Laboratory for Indian Medicine was performed. Toxicity studies were carried out as per OECD guidelines. Pharmacology studies such as antipyretic activity, analgesic activity and anti-inflammatory activity were carried out as per available standard guideline. Clinical study was conducted to evaluate the efficacy of *Linga chendhuram* among the pyrexia patients

2. AIM AND OBJECTIVES

AIM

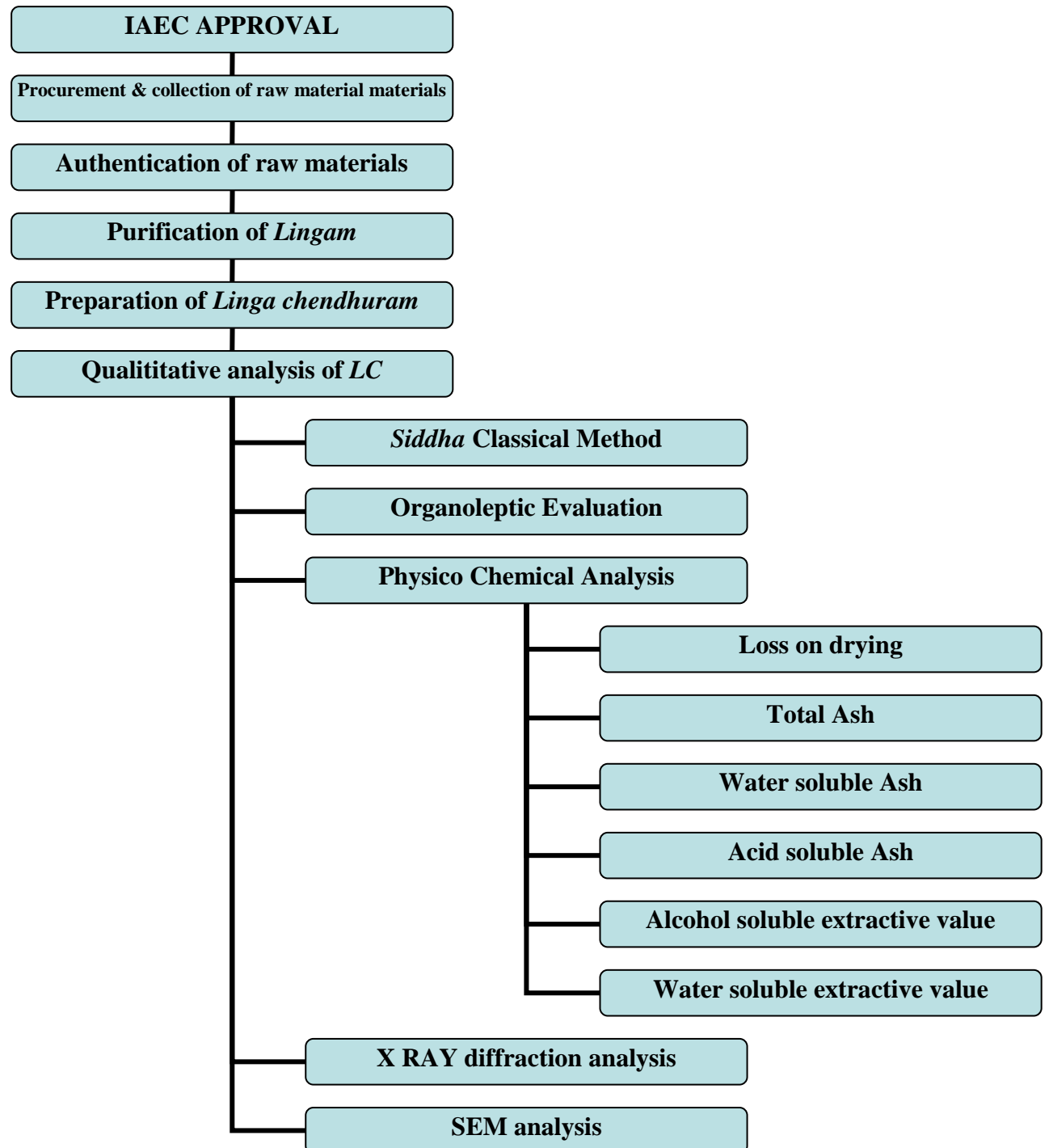
- To validate the safety and efficacy of a Siddha Sastric Formulation *Linga chendhuram* at its human intended therapeutic dosage in the management of *Suram* (Pyrexia)

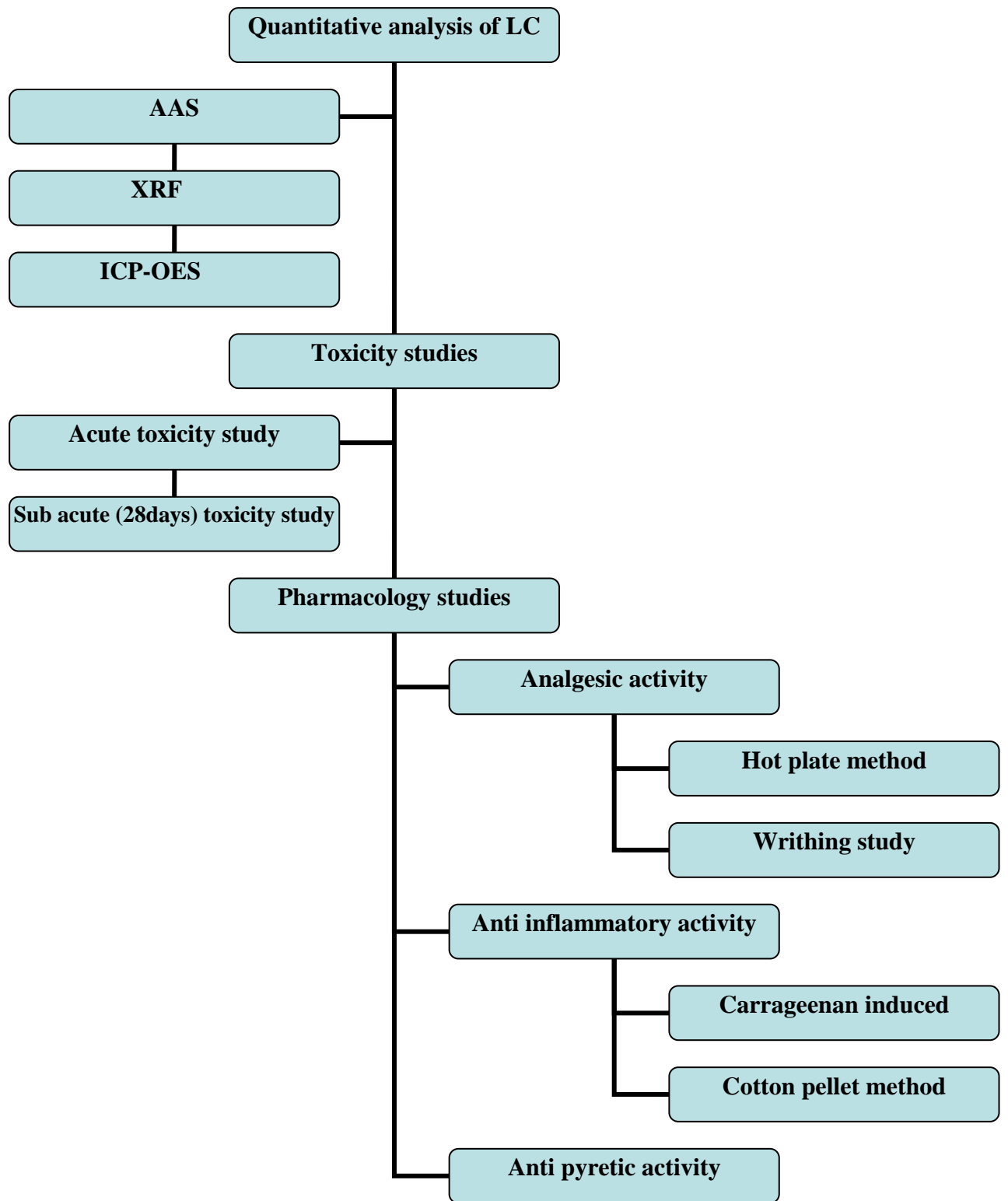
OBJECTIVES:

- To analyze the Physico chemical parameters of *Linga chendhuram*
- To establish the safety profile of *Linga chendhuram* in Rat model by doing Acute and 28-day Repeated oral toxicity studies following OECD guidelines
- To validate the efficacy of *Linga chendhuram* against pyrexia in Rat model by screening it's Analgesic, Anti inflammatory and Anti pyretic activity
- To validate the safety and efficacy of *Linga chendhuram* for the management of Pyrexia (*Suram*) by doing open labelled non randomized clinical trial

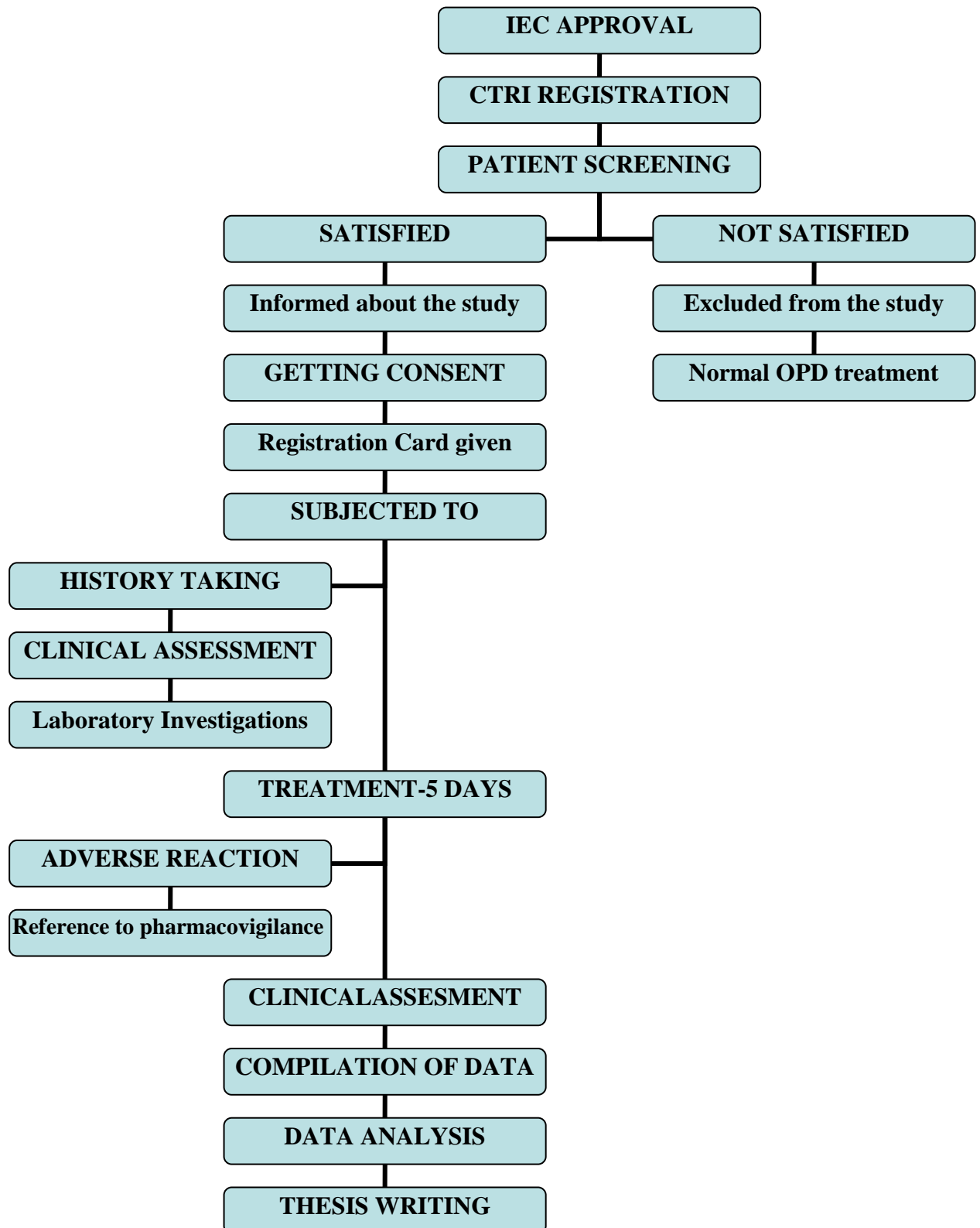
3. SCOPE AND PLAN OF WORK

Methodology of preclinical studies





Methodology of Clinical studies



4.1. CINNABAR (MERCURY II SULPHATE) – MODERN ASPECT

- Division - Metallic substance - Sulfide mineral
- Chemical Formula - Mercury II sulfide, HgS
- Colour - Brick-red, towards brownish red and lead-gray (W 1)
- Crystal habit - Rhombohedral to tabular; granular to massive and as incrustations (w1)
- Specific gravity - 8.176
- Cinnabar and cinnabarite, likely evolving from the Greek kinnabari, represents the common bright scarlet to brick-red form of mercury (II) sulfide, formula HgS that is the most common source ore for refining elemental mercury, and is the historic source for the brilliant red or scarlet pigment termed vermilion and associated red mercury pigments.(3)
- Cinnabar generally occurs as a vein-filling mineral associated with recent volcanic activity and alkaline hot springs. The mineral resembles quartz in symmetry and in its exhibiting birefringence; cinnabar has a mean refractive index of ~3.2, hardness between 2 and 2.5.



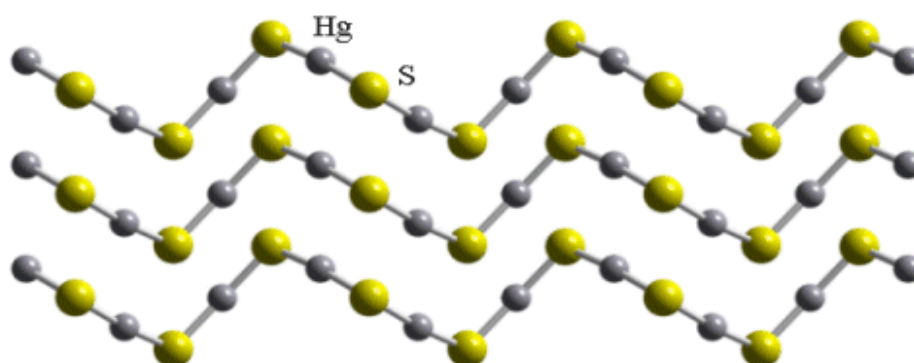
Plate 4.1.1: Raw *Lingam*

4.1.1. STRUCTURE:

- Structurally, cinnabar belongs to the trigonal crystal system.
- It occurs as thick tabular or slender prismatic crystals or as granular to massive incrustations. Crystal twinning occurs as simple contact twins.

- Mercury (II) sulfide, HgS, imitate the cinnabar structure described, and one additional structure, i.e. it is dimorphous. (14)
- Cinnabar is the more stable form, and is a structure similar to that of HgO: each Hg center has two short Hg-S bonds and four longer Hg•••S contacts.
- In addition, HgS is found in a black, non-cinnabar polymorph (metacinnabar) that has the zincblende structure

Crystal structure of the Cinnabar form (Red) of Mercury (II) Sulfide



4.1.2. ABSORPTION:

- Through the Respiratory tract the mercurial vapour is respired from the atmosphere. After ingestion the metal is absorbed from the Gastro intestinal tract.
- The absorption and solubilization is decreased markedly because the metallic mercury is concealed within the lumen of gastro intestinal tract.
- The uptake of elemental form of mercury dermally is rather low when compared with the inhalation uptake. (15).

4.1.3. DISTRIBUTION AND METABOLISM:

- The distribution of mercurial elements depends upon the chemical form, exposure pathway and dose.
- Elemental Mercury can be metabolized in the mammals. The absorbed mercurial vapour is distributed in the body within 24 hrs after a single exposure.
- The appearance in the blood is comparably rapid (about 2% of the dose/ liter).
- The Erythrocytes uptake get completed within 10 minutes, whereas the plasma concentration is reached after 5 - 10 hrs, and the distribution into the brain (after

short – term exposure, about 75%) is very slower and reached to maximum level only after 2 to 3 days.

- Equal amount of mercury are transported by the blood plasma and RBCs during the uptake of Hg II salts.
- After single dose, the mercury preferentially accumulates more in the renal cortex of kidney. (15).
- The blood barrier is not that much permeable for organic Hg II.

4.1.4. EXCRETION:

- Mercury vapor inhaled after exposure may get excreted mainly through renal and fecal excretion as Hg^{2+} .
- Through the exhalation also the mercurial vapour get excreted.
- Excretion through exhalation plays predominant than renal in a short term exposure of mercurial vapour. The biological half life of mercury is determined by kidney.

4.1.5. TOXICOLOGICAL ASPECT

Acute poisoning:

- The soluble salts of mercury also inactivate sulfhydryl enzymes and thus interfere with cellular metabolism and functions.
- The symptoms are mostly due to corrosive sublime and commence immediately after swallowing the poison and are rarely delayed beyond half an hour.
- The symptoms are acrid, metallic taste and feeling of constriction or choking sensation in the throat, hoarse voice and difficulty in breathing.
- The mouth, tongue, and feces become corroded, swollen and coated with greyish white coating. Hot burning pain is left in the mouth, extending down to the stomach and abdomen, followed by nausea, retching and vomiting.
- The vomiting contains greyish slimy mucoid material with blood stained stools and tenesmus.
- The urine is suppressed or scanty; containing blood and albumin, necrosis of renal tubules and damage to glomeruli may follow within 2 days or 3 days if the patient survives.

- The pulse become quick, small, and irregular, and circulatory collapse soon supervenes. In some cases spasms, convulsions and unconsciousness are observed before uremia cause death. Gangrenous colitis may be observed if the patient has survived 6 or more days.
- Metallic taste, salivation, gingivitis and loosening of teeth with foetid breath are usually common when mercurial vapors are inhaled, strong concentrations cause lethargy, slurring of speech, diarrhoea, pneumonitis, cough, cyanosis and anuria (15).

Chronic poisoning:

- This form of poisoning occurs among those who are exposed to the vapors of mercury dust in factories where mercury and its salts are largely used. It also occurs among those who have taken internally for a prolonged period excessive dose of mercury compounds or used the mercurial ointment in the form of an external application.
- Symptoms are nausea, digestive disturbances, colicky pain, vomiting and diarrhoea.
- Salivation is a constant symptom, which is accompanied by foul breath, swollen and painful salivary glands and inflamed and ulcerated gums, which occasionally present a brownish blue line at their junction with the teeth, later, the teeth may become loose and rarely necrosis of jaw develops.
- Evidence of nephritis may be seen. Skin eruptions of erythematous, eczematous or popular type with some thickening of skin of the hands and feet may be noticed (15).

4.2. CINNABAR - SIDDHA ASPECT

JAATHI LINGAM (RED SULPHIDE OF MERCURY)

(NATURAL CINNABAR (or) LINGAM (or) VIRMILION)

- Red sulphide of mercury is also known as Inkuligam, raasam, kadai vanni, karpam, kalikka, kaanjanam, kaaranam, sandagam, samarasam, saaniyam, chendooram, maniraagam, milecham, vani and vanni. (9)
- Nowadays, the red sulphide of mercury used by us, is called as jathi linga paadana, grouped under ‘vaippu paadanam’

4.2.1. Method of preparation

Purified Mercury	280 gm.
Sulphur	70 gm.
Pottasium nitrate	70 gm.

Mercury is thoroughly mixed and triturated with sulphur. Pottasium nitrate is then added; placed in a conical flask and burnt for 18 hours. After cooling, the red sulphide of mercury is collected out. It is hard when it is put into fire it becomes smoke; not soluble in water, it has no smell and taste and has hot potency. It has properties of a tonic.

This preparation is effective in the treatment of diarrhoea, tuberculosis, scabies, unknown insect bites, syphilis, leprosy, eczema, skin diseases, throbbing pain (soolai) and vatha diseases.

It has the properties of curing the diseases caused by the earth element and cures the diseases caused by the water element.

4.2.2. Method of Purification:

- Algangium bark (Alangium savlifolium) – 1400 gm is powdered and added with vinegar 5.2 litres and placed in dew in the night.
- The next day it is rubbed and kindled well. 35gm. Of cinnabar is tied well in a cloth and put into the above liquid.
- The pot is covered with another pot concealed with cloth over which mud get gummed, after which it is dried and exposed in dew for one day.
- It is heated with low intensity fire (flame) until the liquid is dehydrated for 24 hours. Then the cinnabar is taken out and cleaned well.
- This procedure is repeated using the vinegar soaked individually with the whole plant of Vitis lanata (puli karunai) and Indian sarasaparilla root as stressed in the following Tamil verses:

Another Method of Purification:

- Lemon juice, cow's milk and the Indian acalypha juice are mixed in equal proportion and allowed to fuse cinnabar so as to get it in a consolidated potency state.

- When the crude form of red sulphide of mercury is soaked for one day in mother's milk and lemon juice respectively, it becomes purified.

Dosage: For intake 650 mg and for fumigation 2.1 gm

4.2.3. Parpam:

Purified Red Sulphide of mercury (35 gm) is taken and triturated with the juices as mentioned in the following table. It is then dried and put to puda to get the parpam.

Table 3.2.1.: Linga parpam as per Sage Theran

Name of the plant juice	Quantity required in grams	No. of days required for grinding	No. of days required for drying the cakes	No. of days required for drying kavasam	No. of dung cakes required for puda
1. Whole plant juice of Indian laburnum (Classia fistula – sarakkonrai)	140	4	3	1	32
2. Fruit juice of Nuxvomica (Strychnos nuxvomica – naattu etty)	140	4	3	1	30
3. Whole plant juice of Alexandrian Laurel (Calophyllum ino phylum – punnai)	140	4	3	1	32
4. Whole plant juice of Nut grass (Cyprus rotundus – vaatkorai)	140	4	3	1	30
5. Whole plant juice of Holy mountain ebony (Bauhinia tomentosa – thiruvaaththi)	140	4	3	1	32
6. Whole plant juice of mango tree (Mangifera indica)	140	4	3	1	30
7. Whole plant juice of palmyra tree (Borassus flabelifer)	140	4	3	1	32
8. Whole plant juice of Hairy stalk figged banyan (Ficus dalhousiae – kallal)	140	4	3	1	30

Dose: 1/8 size of a pigeon pea (*Cajanus cajan*).

Suitable land for taking the *parpam*: Kurinji (hilly region) and Neithal (seashore).

Unsuitable lands: Marutham (pastoral region), Mullai (land of forests) and Paalai(waste lands) are not suitable.

Curable diseases and Adjuvants:

Asthma, swelling, dropsy, fevers, ulcers, venereal disease, perversion, bearing down pain, rheumatism, itching and delirium are cured by the following adjuvant respectively.

1. Hog weed (*Bauhanian procumbens* – Mookirattai)
2. Boerhaavia diffusa (Irattai piramattai)
3. *Nymphaea alba* (Aambal)
4. *Nymphaea edulis* (Sevvalli)
5. Chebulc myrobalan
6. Beleric myrobalan
7. *Ficus dalhousiae* – (Kallal)
8. *Ficus microcarpa* (Kuriviyaal)
9. Curd
10. Butter Milk
11. Cow's milk
12. Breast milk
13. Butter
14. Sugar
15. Sugar candy
16. Palmyra jiggery
17. Jaggery
18. Sugarcane juice
19. Ghee
20. Coconut oil
21. Tender coconut
22. Rose water
23. Basil (*Ocimum sanctum*)
24. Rosary nut (*Elaeocarpus ganitus* – Uruthira sadai)
25. Gooseberry (*Emblica officinalis*)
26. Indian Phyllanthus (*Phyllanthus amarus*)

27. Sessil Plant
28. Trailing eclipta
29. Thorney Shrub (Viral mulli)
30. Hygrophila auriculata (Neer mulli)
31. Thumbbe (Leucas aspera)
32. Chamomile flower (Chrysanthemum indicum – chamanthi)
33. Aquatic root (Aponogelon monostachyon – kotti)
34. White Indian water lily (neithal)
35. Hot water
36. Water

4.2.4. Linga chendhuram

Purified red sulphide of mercury is consolidated with the juice of garlic (*Allium sativum*) (1.3 litre) and root bark juice of *Cleome viscosa* (1.3 litre) individually washed and powdered.

Dose : 65 mg.

Adjuvant : Honey.

Another Method:

Purified red sulphide of mercury (35 gm) is consolidated with the whole plant of bitter apple (*Citrullus colocynthis*) (1.3 litre). It is then washed and the chendooram is collected. The chendooram is useful in the treatment of fever associated with chill, diseases of vata and kapha and venereal disease (gonorrhoea).

Dose: 65 mg

Adjuvant: Honey

4.2.5. Toxic symptoms of Lingam

Loss of taste, difficulty in eating and drinking water. Ulcers in the buccal floor, uvula (base of the mouth), inner portion of the tongue, larynx and large intestine, foul odour from the mouth, discharge of viscous, whitish saliva, difficult to speak and burning sensation are the toxic features of red sulphide of mercury.

Antidote:

Nutmeg (*Myristica fragrans*), cubeb pepper (*Piper cubeba*), root bar of red cotton tree (*Gossypium arboretum*) and regular candy each 4.2 gm are made into a decoction and administered twice a day for 48 days.

4.3. CITRULLUS COLOCYNTHIS (Aatruthumatti)

Botanical Name	- <i>Citrullus colocynthis</i>
Family	- Cucurbitaceae
Regional Name	- Colocynth, Indian wild gourd, Bitter apple, Bitter cucumber
Tamil Name	- Paedikari Aatruthumatti, Pey thumatti, Pey cumatti
Sans & Can	- Indravaruni, Vishala, Chitrapala
Telugu	- Eti-puchcha
Hindi	- Indrayan (16)



Plate 4.3.1: *Citrullus colocynthis* collected from field of Kancheepuram district for preparation of *Linga chendharam*

4.3.1. Habit & habitat:

A scabrid perennial with prostrate or climbing angular stems and bifid tendrils. Found in arid and sandy lands.

4.3.2. Botanical description:

Leaf - The angular leaves are located alternately on petioles. Leaves are 5 to 10 cm in length. It contains 3 to 7 lobes. The leaves have hairs with open sinuses.

Fruit - The outer portion is green in colour with yellow strips. The fruits have numerous seeds with soft white pulp.

Flowers - Yellow in colour. Monoecious, each flower comprised of yellow campanulate.

Seed - Size-6mm, presentation-partial presentation, colour-yellowish orange to dark brown

Root - Roots usually has a tendency to climb over other herbs or shrubs by their axillary Tendrils- Roots sends long. Rough vine like stems (16).

4.3.3. Phytochemical constituents:

Pulp contains - Colocythin-a bitter principle, a glucoside also Colocythetin, Resin, Colocyntherin, pectin, gum and ash., a anticancer glycoside, α elaterin, 2-D glucopuranoside.

Seeds - 7% of fixed oil, 6% of albuminoids and 3% of ash, α spinasterol.

Leaves & Flowers - Quercetin and kampferol

4.3.4. Phrase about aatruthumatti in *Siddha* text:

“Where is joint stiffness, where is drowsy dullness
Where is the wind humor that is full of harmfulness
Where is menorrhagia and where is blockade of menstruation
When there is *Citrullus colocynthis* say with admiration” (17)

4.3.5. Ethnobotanical Studies:

Fruits - Are used as a purgative, used on boils, stomach ache, Amenorrhoea, Malaria, Jaundice, urinary tract disease, as an antidote in snake poison and calcaneal spur.

Seeds - Used to induce abortion

Leaves- Used to cure stomachache, migraine.

Root - To treat jaundice, Urinary disease, Rheumatism, Amenorrhea, Epilepsy, Ascites and Piles.

Seed oil - Used to cure cancer of various types like sarcoma, lymphosarcoma and carcinoma.

4.3.6. General pharmacology:

- The active principle coloside a isolated from the pulp was screened for its action on smooth muscles and cardiovascular system.
- It demonstrated uterine depressant action on isolated rat uterus.
- The compound exhibited antihistaminic and anticholinergic activities when investigation done with intestine of rabbit and ileum of guinea.
- Coloside A showed negative chronotropic and negative ionotropic effects on isolated rabbit and frog heart preparations. (18)

4.3.7. Chemical contents of Citrullus colocynthis:

Seed

- A Phytosterol- a hydrocarbon, Saponin, glucose.
- Ripe seeds were containing 4.1 percent nitrogen and 25.62 percent protein and free amino acids valine, alanine and cysteine.
- The fatty acids in seed fat were established as palmitic, stearic, arachidonic, oleic, linoleic acids.
- The seed showed the presence of steroids, terpenoids, alkaloids, tannins, saponins and absence of flavonoids, phenolics.
- Saponification of oil yielded linoleic acid, oleic, myristic, palmitic and stearic acids. A phytosterol were isolated from the unsaponifiable fraction (18).

Fruit

- Three glucosides were found to be isolated from the fruits, of which one was identified as α -elaterin glucoside.
- The ether fraction shows Cucurbitacin E and I, the chloroform fraction shows Cucurbitacin E-2 glucoside.

- The fruit coat contained Hentriacontane, while the fruit pulp yielded elateridine hexanocucurbitacin I and 16-O acetylhexanorcucurbitacin I.
- The free aminoacida reported from the pulp were valine, alanine, cysteine, and an unidentified aminoacid (18).

Root

- Saponins and glycosides were present.
- In another study presence of alkaloids, glycosides, saponins, sterols and absence of flavonoids and terpenoids were reported from the root.
- Isolation of α -elaterin and hentriacontane were reported. Two aliphatic compounds namely 1, 11-undecanediol monoacetate and nonyl hexadecanoate were isolated from the root along with stearic acid (18).

Flowers

- Flavonoids quercetin in free form and kampferol in constrained form were isolated.
- Male flower - The amino acids namely alanine, arginine, aspartic acid, glutamic acid, glycine, serine, proline, valine, lysine were present in trace amounts.
- Female flowers - The amino acids namely alanine, arginine, asparagine, aspartic acid, glutamic acid, ornithine, serine, valine were reported while leucines, lysine, phenylalanine were present in traces (18).

Leaves

- Cucurbitacin B and E.
- Flavonoid quercetin in free form and kampferol in bound form were isolated (18).

4.3.8. Minerals in Citrullus Colocynthis:

- The various elements like sodium, magnesium, aluminium, calcium, chromium, manganese, iron, cobalt, nickel, coper, zinc, silver and serenium were reported (19).

4.4. FEVER - Modern aspect

- Body temperature is regulated by nervous feedback mechanism through temperature regulating centres situated in the hypothalamus.

- Classification of temperature (20):
 - Normal temperature – 97.7 to 99.5 °F
 - Hypothermia – below 95°F
 - Hyperthermia – 99.5 to 100.9 °F
 - Hyperpyrexia – 104 to 106.7 °F

Human body temperature depends on following conditions

- Age
- Sex
- Health status
- Reproductive status
- Time of the day
- Emotional activities

The fever may caused by the following reasons,

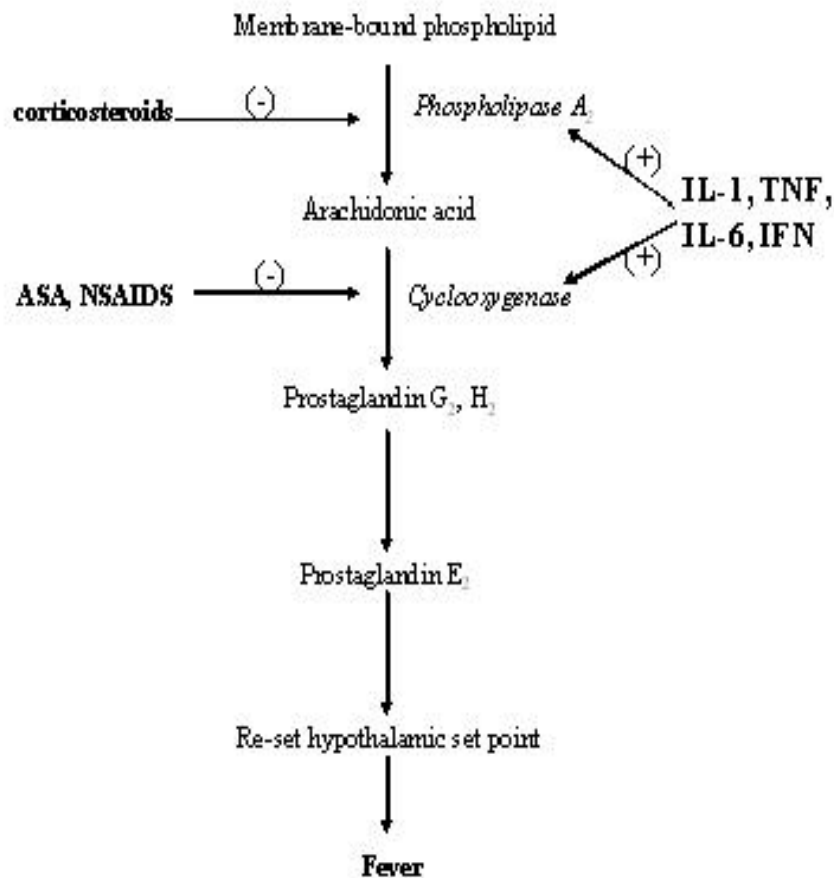
- Pyrogens
- Immunological reactions
- Hormones
- Inability to lose heat
- Drugs and
- Malignancy

4.4.1. PATHOGENESIS OF FEVER

PYROGENIC CYTOKINES

- Cytokines are proteins that control immune, inflammatory, and hematopoietic processes.
- Fever causing Cytokines are known as Pyrogenic Cytokines. The era of cytokine biology began with laboratory investigations into fever induction by products of activated leukocytes in the 1940s. These fever-inducing molecules were called as endogenous pyrogens. The main pyrogenic cytokines may include IL-1, IL-6, ciliary neurotropic factor, TNF and interferons.
- Each and every cytokines are encoded by a distinct gene, and each pyrogenic cytokine has been shown to cause fever in laboratory animals and in humans. The pyrogenic cytokines IL-1, IL-6, and TNF produce fever at low doses (10 to 100 ng/kg) when it is injected into humans.

- The production and discharge of endogenous pyrogenic cytokines are promoted by a wide range of exogenous pyrogens, most of which have observable bacterial or fungal origin. Viruses also promote pyrogenic cytokines by infecting cells. However, in the absence of microbial infection, inflammation, trauma, tissue necrosis, or antigen antibody complexes can also induce the production of IL-1, TNF, IL-6, which-individually or in combination - trigger the hypothalamus to increase the set point to febrile levels.
- Monocytes, neutrophils, and lymphocytes are the primary cellular sources of pyrogenic cytokines. Many other types of cells can also produce these molecules if activated (3).



- **Quartan fever:** Fever with a periodicity of 72-hour
- **Remittent fever:** There is fluctuation of body temperature more than 1 °C in 24 hours and remains above normal level for whole day.
- **Ebstein -cardarelli fever:** A fever associated with Hodgkin's lymphoma.
- **Neutropenic fever** - Fever due to the lack of neutrophils.
- **Febricula:** Low-grade fever with unknown cause without exhibiting any symptoms

4.4.5. Accompanying features:

- Headache - Headache and photophobia, although features of meningitis may be associated with other infections.
- Delirium - Mental confusion during fever is common in young children as well as elderly.
- Muscle pain - Myalgia may accompany with viral infections, such as, influenza, and with septicemia including meningococcal sepsis.
- Shock - Shock may accompany severe infections and sepsis.

4.5. SURAM (FEVER) - SIDDHA ASPECT

In Siddha system of medicine fever is considered as a disease which causes body heat to raise above normal level. Fever is called as vemmai, veppu noi, kaichal, etc. in Tamil (21).

4.5.1. Causes of Fever:

It is considered that fever is caused by the following factors; constipation, excessive sexual activity, toxicity, sleeplessness, heavy running, intake of fast foods, excessive anger, walking in the morning sun and hot sun, tolerating excessive anger, excessive eating, carrying excessive weight, excessive shouting, controlling fourteen natural urges of the body, indulging in sexual activity on the day of taking oil bath, eating full stomach after heavy hunger, drinking chilled water, malaise, etc.

4.5.2. Signs and symptoms of fever:

Lack of interest in any food, heaviness of body, change of body color, hyperesthesia, dislike to any substance, giddiness, delirium, loss of taste in tongue,

shivering of body, loss of sleep at night, pain in the muscles of the thigh and leg, pain all over the body, dislike to neighbours, watering from eyes, dryness of tongue, lack of interest, dislike to sweet, sour and bitter tasty foods, dizziness, excessive sweating, changing pattern of like and dislike to musical sounds, hot and cold water, shadow, etc., enigmatical activity towards wife and children. In fever, a few or many of the symptoms mentioned above will appear (21).

4.5.3. Types of fevers:

It is considered that there are sixty four types of fevers. Of these twenty are included in *Vatha*, twenty four in *Pitha* and twenty in *kapha*. Of these, eight types of fever under *vatha*, ten under *pitha* and nine under *kapha* are considered incurable.

It is considered that of these sixty four types of fevers fifty two are of self origin and the twelve are originated from secondary sources.

Vatha fever (Valisuram):

In this type of fever the following features may be seen:

Black discoloration of body, puffiness of face due to edema, body pain, loss of appetite, heaviness of head, etc. Sweating after the fever was reduced, shivering of body, distension of abdomen with dyspnoea, goose flesh, chillness of body, tremors of hand and leg, joint pain, shining of face, constipation, oliguria, pain in legs, body becomes ashen coloured and pale, chest pain, dryness of skin and formation of wrinkles, excessive thirst, anxiety, vomiting, insomnia, excessive tiredness, delirium, severe cough, watering of eyes, headache, excessive bowel sounds, excessive laziness, yawning and hiccup. As the incubation period of the fever is about ten days, in addition to the above mentioned features the following signs and symptoms may also appear.

Vathapitha fever (Valiazhal suram): In Vathapitha fever the following signs and symptoms may manifest. Yellow discoloration of tongue and conjunctiva, nausea, continuous fever, goose-flesh, chillness of body, cold fever, blabbering, hard breathing, frequent sneezing and confusion of mind. After the fever subsides, there will be diarrhoea and dysentery.

Vathakapha fever (Valiyya suram):

In this type of fever the following signs and symptoms may appear. Vomiting, dyspnoea, edema of legs and arms, cough, asthma, intractable fever, tremors, throat pain, pale face, hiccup, gooseflesh, dryness of mouth, joint pain, excessive sleep, chillness, white coloration of stool and urine, excessive salivation, desire for sunheat and hot climate, paleness of tongue, excessive thirst, mental depression, delirium, excessive sweating etc.

Pitha fever (Azhah suram):

This is severe form of fever which produces the following clinical features. The patient will develop abnormal behaviors. He will develop fearful look, thought distortion, reddish appearance of the body, delirium, reddening of eye, red colored urine, asthma, excessive saliva, diarrhoea, tastelessness, excessive thirst, continuous fever, etc. in addition, the patient will try to run out of the bed.

Pithavatha fever (Azhahvali suram):

This fever is due to vatha and pitha humour with the following clinical features may be seen: Hyperpyrexia, yellow discoloration of the body, vomiting due to indigestion, dryness of neck, stomach and head, chillness of body, decrease in body shining, anorexia sweating, liking to sunheat, intermittent fever, biting of teeth during sleep, heart burning, restlessness in bed, partial sleep, eye disease, etc.

PithaKapha fever (AzhahIya Suram):

In this type of fever, the following signs and symptoms may be seen. Mental depression with impairment of learning, intractable cough and fever, insomnia, yellow coloration of stool and urine, insomnia, edema of face and leg, hiccup, palpitation, headache and pain in back of neck, dyspnoea, excessive sweating etc.

Kapha fever (Iya suram):

In this type of fever, the patient will appear as if he is frightened. He may hear some thundering noise. The body will develop a pale appearance. There may be dyspnoea, swelling of the body, cough and tiredness, blabbering with incoherent speech, thirst, phlegm, and intractable fever, the temperature over the head may be raised: there

may be delirium sweating and ringing of the ears; later deafness may also develop..In addition to the above features there may be hiccup, insomnia, discharge from the eye and diarrhea etc.,

Kaphavatha fever (Iyavali suram):

In this type of fever there will be shivering of head, and the body may become stiff besides continuous cough and chillness of hand and foot. The patient may develop loss of taste and breathlessness and mucous sputum. The patient may also feel tired and have liking to sweet and sour foods.

Kaphapitha fever (Iyaazhal suram)

In this type there will be high fever and sweating after the fever was subsided. The tongue will dry and will have bitter taste. There may be also sore throat. A few days after the fever was subsided the hair on the head will turn grey. Other features of this fever include the following: the body will become chill after fever subsides; the chin will appear as if it is hanging; there may be polydipsia and vomiting with plenty of mucous; there may be continuous fever and the blood will increase in volume; as a result. There may be bleeding from the nose, tongue and mouth. There may be discharge from the nose, nausea and severe cough. The patient will often try to run out of the bed; he may develop excessive sexual desire towards women.

Triple dosha diseases (Muppini suram):

Other names: Kalappu suram, thondha suram and sannipatha suram. In addition to the above features the patient will also develop the signs and symptoms of triple dosha diseases (Muppini). The other features are delirium, hallucination, dryness of tongue, polydipsia, hyperpyrexia with chill and rigor, during high fever the patient may throw away all clothes he is wearing; after the fever is subsided the patient will develop giddiness, itching sensation over the head and severe joint pain.

Indigestion fever (Mandha suram):

In this type of fever there may be distension of abdomen. There may be indigestion, pain in chest and thigh, sour belching, vomiting, uncontrolled thirst, hard breathing and hiccup besides having chillness during the fever.

Fever with rigor (*Nalir suram*):

In this type of fever there may be goose flesh, yawning and vomiting prior to the starting of the fever. Thereafter there will be fever with chills and rigor; in addition other features such as watering of eyes. Abdominal pain, diarrhoea, bilious vomiting, giddiness, delirium, etc. may also occur. The fever then may subside. There may be recurrence of fever on alternate days along with above mentioned signs and symptoms. Sometimes the recurrence of the fever may be once in 3 days or 4 days or even 9 days.

Cruel fever (*Venjuram*):

In this fever, there will be uncontrolled headache, reddening of eyes, excessive salivation, goose flesh and very high fever. After the fever is subsided there will be sweating and extreme tiredness. Pustular lesions may appear in the nostrils.

Body fever (*Megasuram*):

In this type of fever the whole body will appear reddish in color. The fever will be associated with vomiting, diarrhea, itching, giddiness, dizziness and burning pain in the body.

Internal fever (*Utsuram*)

This type of fever often occurs in people who are having lowered immunity. The patient will have a sensation of heat over the body; he will be dull and depressed; there may be pain in the limbs. Anorexia, nausea, headache, loss of weight, etc. are other features of the disease.

External fever (*Veli suram*):

In this type of fever will be nausea, indigestion, excessive thirst and bitter taste. The body will appear as if always hot. Another special feature of this disease is that the patient may dislike to lie in the bed and will be interested in carrying out his routine duties.

Intermittent fever (*Vittuvarum suram*):

In this type of fever, the fever will appear in morning and will subside after sometime. The fever will again reappear. The fever will occur daily. the body will appear

black in colour and there will be loss of weight. There may be atrophy of limbs and distension of the abdomen.

Continuous fever (*Vidaa suram*):

There will be redness of the eye, dryness of tongue. Excessive thirst and pain in the limbs just before the onset of the fever. The fever will gradually increase in intensity and will persist for few days. Patient will also develop loss of body strength.

Fever affecting blood tissues (*Raththa thadhu suram - Saaral suram*):

In this type of fever, the patient will develop perturbation of mind, uncontrollable sweating, excessive thirst, body pain and frequent fainting. In addition, the tongue will appear yellow in color with lubrication, development of phlegm in the chest; insomnia and constipation are other features of this disease. Other unusual features are loss of taste, hiccup, vomiting, etc.

Fever due to altered condition of blood (*Rakha thadhukatha suram - Sengarai suram*):

In this type of fever there will be high fever. The whole body will appear edematous and reddish in colour during the fever; excessive thirst and sweating, general body pain, restriction of the movements of the joints, etc. are other features of the disease. In addition, there may be headache, perturbation of mind, chillness of limbs, giddiness and yellow colouration of tongue with lubrication. Patient may also develop diarrhea.

Flesh fever (*Oon suram*):

In this type of fever, there will be high fever with breathlessness, sweating, pain throughout the body, dryness of lips, goose flesh, and perturbation of mind and frequent watering of eyes. In addition there may be dryness of the body, involuntary movements of the limbs, shining of the skin, yawning, hiccup and weakness of body.

Fat Fever (*Kozhuppu suram*):

In this type of fever the patient will develop anorexia and indigestion before the onset of fever. There may be pin prick sensation on the head with giddiness.

Bone fever (*Enbu suram*)

In this type of fever, there will be excessive thirst, even though the skin appears cold, there will be high fever, if the fever continues for many days, patient will develop loss of weight, paleness, pain in the lower abdomen, indigestion and diarrhoea.

Sperm fever (*Vindhu suram*):

In this type of fever, there will be pain in the so called 14 vital sites of the body (vertex, eyebrow, lips, neck, chest, xiphisternum, umbilicus, knee, ankle, hip, big toe of the feet, little finger, skin and scrotum). There will be headache with head movements, tooth pain, fever, loss of taste and appetite, indigestion, hiccup, vomiting, frequent diarrhoea.

There will be feeling of mouth tasting sweet. There may be also continuous cough, hyperpnea, insomnia, paleness and oedema of body, decreased haematopoiesis, anxiety, giddiness and chillness of limbs. In men, the size of penis may also shrink. When the fever progresses, there may be delirium.

Sorrow fever (*Thunba Suram*):

This fever occurs due to unexpected onset of sad events. There will be giddiness, mental disturbances, redness of eye and high fever. In addition, there may be vomiting, tremors of limbs and incoherent talk, singing and dancing.

Periodical fever (*Murai suram*):

In this type of fever, there will be chillness and rigor, burning sensation of eyes with fever. When the fever subsides, there will be severe headache and sweating. The fever may occur once in two or three or four days. Sometimes the fever may come once in a month or once in a year. There will be joint pains, goose flesh, chest pain and frequent yawning.

Giddiness fever (*Mayakka suram*):

In this type of fever, there may be giddiness, vertigo, vomiting and mental retardation during the fever.

Delirium fever (*Pithatral suram*):

There may be goose flesh, redness of eyes, pain in the joints and limbs before the onset of the fever. When the fever reaches its peak, the patient will develop delirium and try to run out of the bed.

Worm fever (*Puzhu suram*):

This type of fever usually occurs in children due to worm infestation. There may be fever with convulsions and hyperpnoea.

Yellow fever (*Manjal suram*):

The fever will be continuous with yellow coloration of the body, intractable vomiting, decreased hematopoiesis, bilious vomiting and generalized anasarca.

Intense fever (*Theevira suram*):

This fever is also called as skin fever because it is of skin in origin. This fever also affects the bone and causes hyperpyrexia, the body will become pale and the patient will appear emaciated. There will be incoherent talk and excessive salivation and body pain. There may be frequent attack of giddiness.

Chronic fever (*Pazhanjuram*):

This is also known as Maaral fever. The fever may come on alternate days or once in two days. Sometimes the fever may occur daily. It is considered that the signs and symptoms occur as a result of combination of Tridoshas.

Thirst fever (*Thaaga suram*):

In this type of fever, there will be hyperpyrexia, excessive thirst, burning and sensation in abdomen and in limbs. The patient may develop incoherent talk, bite the teeth and try to move out of bed. There may be dryness of tongue and hyperpnoea.

***Migu kazhichal suram- Adhisaara suram- Pitha adhisaara suram* (Excessive diarrhoeal fever):**

There may be indigestion, dryness of tongue and chillness of limbs before the onset of fever. There will be frequent diarrhoea with large volume stool. The patient may

be anxious and unable to move the organs. There may be blood in the stools, and severe pain all over the body.

Fever from secondary sources;

Fever due to strenuous work: (*Modhu suram – abikatha suram*)

This fever results from excessive walking, riding on horse or on elephant and carrying excessive load against one's capacity. The fever may also occur on exposure to cold *Vatha* in the dew. In this fever, the whole body will be painful and there will be excessive sweating, flatulence. The patient will develop liking to cold food.

Naaveru suram - Saaba suram:

This fever results when a person gets scolding and hard words from elders. In this fever, the patient will be mentally depressed with fever, headache, vomiting and emaciation. He may also exhibit incoherent talk and feel guilty for his act.

Eaval suram:

This type of fever results from witchcraft and magic. In this fever, there will be boils throughout the body with uncontrolled fever. Since this fever is associated with mind, there will be mental depression, anxiety and delirium.

Pootha suram:

In this fever, there will be convulsion, diarrhoea, prominent sight, blackening of the body, desire to eat excess, liking to any object, incoherent talk goose flesh and deep sleep. The other features of the fever are; talking angrily with loud voice, throbbing pain in the head, frequent attack of giddiness, heaviness of body, excessive thirst, fire like body heat, sweating, redness of eye, hyperpnoea etc.

In addition to the above features, the patient may cry or laugh without any reason. There may be burning sensation all over body, yawning, hiccup and mental disturbances. It is considered in this fever, that the Tridoshas combine together and aggravate the triple diseases; as a result there will be sweating and chillness of whole body which may be fatal. The following is considered as treatment for this disease. Tender coconut water is boiled till it gets concentrated. It is then triturated with *Parpaadagam* and dried ginger. This preparation is taken orally

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Fever due to drug response (*Marundhu vega suram*):

This type of fever results from giving improper doses of medicines prepared from mercury, sulphur, yellow arsenic trisulphide, Indian aconite and *Seangkottai* (Making

nut). Ulcers in the mouth, swelling of the body with fever. There may be also cough, sneezing, diarrhoea, vomiting and paleness of the body.

Toxic fever (*Nachu suram - Vida suram*)

This fever results from bite of small worms of poisonous in nature. There will be swelling, itching and burning sensation of the site of bite. There will be oedema in face or the whole body with fever. The other features of the fever are; hiccup, indigestion, green colored vomits and giddiness.

In addition to the above features, the limbs will be hot. There may be dryness of tongue and intermittent high fever. The patient will have fear of death.

Angry fever (*Cinasuram*):

This fever results when a person develops uncontrolled anger and talks without knowing the consequences. There may be throbbing pain in the head, blurring of vision, giddiness and high fever.

Fear fever (*Paya suram*):

In this fever, the patient may develop sudden fear. There will be tremors, sweating, heaviness of head and fever. In addition, there will be burning pain in abdomen, redness of eye, vertigo, goose flesh, hyperpnoea, gnashing the teeth, palpitation and a sensation of empty of stomach.

Fever due to sad events (*Varutha suram*):

This fever occurs as a result of grief due to sad events. The patient will develop mental retardation and delirium. He may dance, sing or cry.

Sexually induced fever (*Ichai suram – Kama suram*.)

This type of fever occurs as a result of excessive sexual urge. In this type of fever, the patient will lose all his good characters. There will be burning sensation of the body and eye, giddiness, dryness of tongue, thirst, palpitation and high fever.

Fever due to God's angry (*Deiva suram*):

This type of fever results when a person steals and eats the worship food items before offering to the God. After eating, the person usually realizes his fault and the

severity of fever will depend upon the intensity of his guilt feeling. There may be also headache, giddiness, vomiting and vertigo. The patient may also become mad.

Vomiting fever (*Vaanthi suram*):

In this fever, there will be frequent vomiting, sweating, diarrhoea, excessive bowel sounds, inability to hear, dryness of tongue, loss of appetite, pain in the lower part of the mouth. etc.

Salivary fever (*Ooruvaai neer suram*):

In this fever, there will be excessive salivation in the mouth, vomiting and diarrhoea. There may be sour taste in the mouth and the body may become very weak; the eyes will be blackened; there will be chillness in the limbs. Patient may develop polydipsia but if he drinks water they may vomit.

Morning fever, day fever, evening fever, night fever and early morning fever:

The clinical features of these fevers will be more or less similar other fevers. it is considered that these fevers are named according to the time of their occurrence.

Rigor fever (*Nadukkal suram*):

This fever has been named in view of the rigor occurring during the fever.

Belching, yawning and hiccup fever:

As these types of fever develop as a result of malfunctioning of *Vatha*, they come under the *Vatha* fever.

Sweating fever, insomnia fever and Pitha fever:

As these fevers arise as a result of bilious malfunctioning, they are included under the pitha fever.

Cough fever, abscess fever, multiple abscess fever, pus fever, fever due to sexual activity, artificial fever:

As these fevers originate from phlegm, they are included under the *Kapha* fever.

Anal suram:

This fever is due to skin disorder and it is included under the head ‘Intense fever’. It is considered that the anal fever results from variations in the six tasty foods eaten or from bad conduct or by combination of one or two factors of the triple dosha disease. The patient will develop indigestion to energy yielding foods. He may also develop obstruction in the gastrointestinal tract. The whole body will become hot. Since in this fever the gastrointestinal tract is affected, this ultimately produces defective functioning of blood, lymph, muscular and urinary system of the body (21).

4.5.4. Pulse:

- If the pulse related to *Vatha* and the pulse related to phlegm accompanies each other, it is suggestive of fever disease.
- If the pulse related to fire is bounding in nature, it is suggestive of fever disease especially fever of bony origin.
- If the good character *Pitha* is associated with *Sethumam*, it is suggestive of *Aththi* fever.
- If the pulse is related to fire and pulse is related to phlegm accompany each other, it will denote bone fever.
- If the *Vatha* pulse is associated with the special *Pitha*, it denotes intermittent fever.
- If the pulse is associated with fire and the pulse associated with *Vatha* accompany each other, it also denotes intermittent fever.
- If the pulse associated with *Vatha*, beats like running of a ball, the fever is likely to be due to disorders of the intestine. This type of fever will be continuous. The patient will develop loss of strength of body and impairment of intelligence.
- If the pulse associated with *Vatha* is of shagging in nature and associated with bradycardia, the fever is likely to be bone fever which is one of the fever coming in the evening.
- If the pulse associated with fire is straight and low volume, this denotes bone fever caused by dehydration of the body.
- If the pulse is associated with *Vatha* is bounding in nature, it will denote serious diseases. During this period,

- If the pulse associated with fire is also bounding in nature, it will denote very severe fever (21).

4.5.5. Treatment of fever:

As it is considered that fever arises mainly from malfunctioning of triple doshas, the following modes of therapy is advocated with the purpose of restoration of the functions of triple doshas.

- By subjecting the patient to starvation or inducing vomiting or diarrhea.
- When the above measures fail, administration of decoction, powder, tablets, wax Mezhugu, Parpamand chendharam.
- For treatment of adjuvant diseases, topical medications to the eyes and nose are given
hot fermentation.

Starvation:

The number of days of starvation suggested for various diseases is as follows:

Vatha fever - 7 days

Pitha fever – 6 days

Kapha fever – 9 days

Mukkoottu fever – 10 days

It is also considered by some physicians that severe starvation should not be allowed. They advise that in Vatha fever, three days starvation alone is sufficient. In pitha fever, decoction and small quantity of food may be allowed. In kapha fever, drug and rice gruel may be given.

Method of three days starvation:

The patient is subjected to starvation on the first day of starting of fever. On second day, patient should brush his teeth, clean the tongue, and gargle the mouth with hot water. Face is washed with cold water. The head and body of the patient is covered with cloth and the patient is advised lie over his left arm.

The procedure is repeated on third day. On fourth day, patient is directed to drink water which was cooled after boiling; patient should chew clearing nut and eat the same; usually the fever will be controlled.

However the famous ancient sage Theran different opinion with regard to starvation of patients. As per his view, most of the people living in the south take diet which are easily digestible and give strength to the body. People in the south are not having much strength and in such people, starvation for seven to ten days during fever will do more harm than good.

Now it is generally believed that instead of putting the patient under severe starvation. Allowing the patient to take small quantities of easily digestible foods will help in the speedy recovery of the patient.

In *Vatha* fever, there will be usually constipation. To relieve the same, sanjeevi maathirai, kodosoori maathirai, attapyravam, thazhampoo maathirai, ilavankathi, agathiar kuzhumbu, kowsigar kuzhumbu are administered.

In *Pitha* fever, diarrhea itself is one of the features and hence there is no need to give laxatives; in case there is constipation, the following may be given; liquorice, oldeniandia corymbosa, European chamomile anthemis (*Seemai samanthi*) and cassia senna (*Nilavaagai*) are taken (each 8.75g). They are boiled with half liter of water. The filtered water is taken internally; this will produce diarrhea once or twice.

In *Kapha* fever, even though there will be usually constipation, sometimes there may be frequent passing of small quantities of motion. For relieving constipation in this type of fever, Agathiyar kuzhambu and kowsigar kuzhambu may be administered. When the patient is very weak because of the fever, instead of giving laxative orally, this may be applied topically over the skin or teeth or inhale by nose (21).

Vomiting:

It is not desirable to give emetics in *Vatha* and *Pitha* fever. In *Kapha* fever, there will be mucus secretion in the lungs and in this fever judicious use of emetics is justifiable. It is preferable to use drugs which have emetic as well as laxative properties. For example, when sanjeevi maathirai is given in the juice of daemia extensa (*Thamani*), it produces vomiting as well as diarrhea. Similarly, when the venkaattan mathirai and Ox gall powder are given together, vomiting and diarrhea are produced.

Hot fomentation:

Fever which is originated from toxins will produce headache and sensation of heaviness of body because of the mucus secretions. Hence, hot fomentation will be useful in this fever.

Hot fomentation relieves severe heaviness of head caused by mucus secretion, uncontrolled fever and hyperpyrexia.

The hot fomentation will be useful in cold fever and fever resulting from *Kapha*. Because of the hot fomentation, there will be sweating and the bad fluids of the body are removed.

Method of hot fomentation:

Four fistful of leaves of *Vitex negundo* (Five leaved chaste) and turmeric (each 8.75g) are powdered together and boiled with water and filtered. Again, it is boiled after putting the pieces of red hot brickstone one by one. The patient is instructed to inhale the vapour evolved.

Eye medicine:

Usually medicines are applied in the eyes for delirium diseases resulting from malfunctioning of *Kapha* and triple dosha disease.

Cinnamon is kept soaked in mother's milk. It is then placed in a cloth and the cloth is squeezed and few drops which come out are instilled into the eye.

Preparation of eye medicines:

Kalikkam (method 1)

Equal quantities of dried ginger, pepper, piper longum and root of *leucas aspera* (*Thumbai*) are taken and triturated with mother's milk and then with honey. They are rooled inbo balls, dried and kept stored. The ball is rubbed in the juice of *ocimum sanctum* and applied in the eyes.

Another method:

Chinnabar, yellow sulphuret of arsenic and *trum foliclosum* (Gold thread thalic) are trituratd with lemon juice, dried and applied to the eyes.

Nasal medicines:

The cloth is soaked in the juice of *leucas aspera* (*Thumbai*) is squeezed and the drop is instilled in to the nose.

Nasal Thread:

The burnt smoke of piper longum, turmeric, bishops weed and pepper are inhaled through both nostrils. They may be powdered together and sprinkled in a cloth. The cloth is rolled into thread. The thread is soaked in neem oil and burnt. When inhaled, the patient will develop watering from the nose; this results in control of fever, heaviness of head and swelling of body.

Patru (poultice):

The juice of daemia extensa leaf, white garlic, seed of bamboo, bamboo salt, and potassium nitrate are triturated with water and boiled. It is then applied over the skin as poultice; fever and body pain will be relieved by this.

Another method:

Pottasium nitrate, jambul sed and the root of croton oil seed are powdered and mixed in ghee of Alexandrian laurel and applied as poultice for the control of triple dosha disease and throbbing pain of upper and lower limbs.

Clearing nut is powdered and dissolved in neem oil and applied as poultice for control of chest pain occurring in fever disease, cramp, vatha disease and splenomegaly.

Bed:

As the patient who is chronically ill should lie in the bed for many days, the mattress should not be hard and irritant to the skin. Hence the mattress should be made of bedsores. For those who cannot afford to silk cotton mattress, the mat made of leaves of phoenix dactylifera tree may be the cheaper alternative.

Handmade fan:

After the intensity o fever is over, patient will be usually exhausted and sweat. Hence the patient should be exposed to purified air by using handmade fan. For those feathers is used; for patients suffer from triple dosha disease, fan made of palm leaves is preferable; for those who suffer from sensation of burning like fire; fan made of root of cuscus grass is preferable.

Properties of fan made of palm leaves:

The vatha produced by this type of fan can control kapha disease. it will also restore the loss of taste of the tongue.

Properties of fan made of root of cuscus grass:

This type of fan can control the burning pain of the fever, thirst and sexual desire. In short; the fans will be useful in various fevers as follows:

Table 3.5.1: Fan made of palm leaves for fever

S.no	Fever	Fan made of
1	<i>Vatha</i> fever (Vali suram)	Peacock feather and palm leaves
2	Azhal fever	Root of cuscus grass and pain leaves
3	Kapha fever	Palm leaves
4	Alanalayam, iyavali	Palm leaves
5	Delirium (Sanni)	Peacock feather

Blanket:

Blanket is useful in cold fever and fever associated with *kapha*. Due to chill weather, patients who are emaciated due to fever will further suffer; woolen clothes are preferable for these patients.

Medicines:

1. Decoction for vatha fever (vali sura kudineer)
2. Decoction for vatha pitha fever (vali azhalsura decoction)
3. Vathakapha fever (vali iya suram)
4. Fever due to deranged vatha humour; (vali mukkutra suram)
5. Neem decotin (vaambu kudineer)
6. Pavonia zeylancia decotin (citramutti kudineer)
7. Dried ginger decotion (chukka kudineer)
8. Decoction for pitha fever (azhalsura kudineer)
9. Decoction prepared with mother's milk (thaaipaal)
10. Decotion for vomiting fever (saththi suram)
11. Decoction for kapha fever (iya sura decoction)
12. Decoction for kaphavatha fever (iyavali sura kudineer)

13. Decoction for kapha pitha fevers (iya azhal sura kudineer)
14. Decoction for cold fever (Kulir sura kudineer): *Paalaitandu* kudineer:
15. Toddy of country fig (*Aththi kallu*)
16. Decoction of bitter snake gourd (*Paeipudal* kudineer)
17. Varieties of medicinal paste (*Karkam*):
 1. The following ingredients are taken in equal quantities: tender leaf of calotropis gigantea, white garlic, raw sweat flag, pepper, dried ginger and raw drumstick flower. They are triturated and rolled into balls to the size of Alexandrian laurel (*Punnai*). They are taken internally along with hot water.
 2. Root of acalypha indica (*Kuppaimeni ver*) (17.5g) white garlic (175 g) and pepper (52.5g) are triturated and taken internally the quantity being equal to the size of Alexandrian laurel (*Punnai*), this preparation is taken twice a day along with hot water.

18. Internal medicines:

Maththirai

Vasanthakusumagara maathirai,
 Bramanandapayiravam,
 Vishnu chakkaram,
 Vaathaatchan and Emathandakuligai.

Parpam,

Parpa of yellow sulphuret of arsenic, mica parpa, red coral parpa, pearl parpa, silver parpa, lead parpa, conch parpa and parpa of feather of large hootingowl.

Chendhuram

Sulphur, mica, red coral, cinnabar, red orpiment, silver, gowri shells, camphor and perchloride of mercury.

Karuppu

kasthuri karuppu, thaalaga karuppu, korosanai karuppu; sivanaar amirtham and gowri chinthamani (21).

4.6. Biological activities for the ingredients of *Linga chendhuram*

- Maximum tolerated dose for Cinnabar in mice is estimated up to 24 g/kg.
- Jing-Zhen Shia et al (2011) studied the nephrotoxicity of Cinnabar which shows the toxic potential of Cinnabar is much less than Mercuric chloride and Methyl mercury after chronic oral administration for 60 days in adult Sprague-Dawley rats at the dose of 0.2g/kg body weight. Histopathology shows severe kidney injury in methyl mercury treated group. Moderate renal injury in mercuric chloride treated group but only mild or no injury in Cinnabar treated group. Kidney sensitive markers Kim-1, MMP7 and N-cadherin are not altered in Cinnabar treated rats but altered in Mercuric chloride and Methyl mercury treated rats (22).
- It is assumed that conversion of cinnabar into methyl mercury form in intestine produce toxicity. Xinrui Zhou et al (2011) reported that on ingestion of Cinnabar, it is biotransformed as non-toxic form of mercuric polysulphides by the intestinal bacteria rather converted into highly toxic methyl mercury under gastrointestinal environment. (23).
- Yuan-Fu Lu et al (2011) studied the toxicity of Gong – Niu –Huang Wan (AGNH), a 10% Cinnabar containing Chinese traditional medicine used for brain disorder in mice compared with the toxicity of Mercuric chloride and Methyl mercury. Mice were given orally cinnabar containing AGNH for 44 days at the dose of 300 mg/kg. The liver toxicity study showed that more liver damage in Mercuric chloride and Methyl mercury treated mice than Cinnabar AGNH group. Expression of hepatic cytochromes P450 genes such as cyp1a1, Cyp1a1 and Cyp4a10 was increased only after MeHg and HgCl₂. (24).
- Feng Zhanga et al (2012) reported the protective effect of Cinnabar contained Chinese traditional medicine – Wan Sheng Hua Feng Dan against Lipo polysaccharide induced dopaminergic neurotoxicity in Wistar rats (25)
- Feng Zhanga et al (2012) studied the ototoxicity effects of Cinnabar on the auditory brainstem response system during 2-10 weeks administration at oral dosage of 10 mg/kg/day in mice. Male mice were more sensitive to Cinnabar in producing hearing impairment correlated with the biochemical alteration in plasma and brainstem. These finding provide important information that the

clinical dosage of Cinnabar (10 mg/kg/day) still exhibited ototoxicity after continuously long term exposure (26).

- Chun-Fa Huang et al (2007) elucidate the effects of Cinnabar on the time course of changes in locomotor activities; pentobarbital induced sleeping time, motor equilibrium performance and neuro biochemical activities in mice treating with cinnabar at the clinical dose of 10 mg/kg/day for different duration 3, 6 and 11 weeks. The results showed that Cinnabar was significantly absorbed by GT tract and transported to brain tissues. Moreover, frequency of jump and stereotype – 1 episode were progressively decreased on 3 week oral administration in male and female mice (J7). The signaling of reactive oxygen species, nitric oxide, sodium /potassium ion – ATPase and mercury accumulation in brain are the factors causes the neuro toxicology effects of Cinnabar (27).
- Qi wang et al (2007) studied that the anxiolytic effect of Cinnabar on chronic administration at effective doses in mice. The study shows the changes in the level of monoamine neurotransmitters and their metabolites monoamine oxidase activity. The anxiolytic effect of cinnabar is due to the reduced level of serotonin but it didn't involve in alteration of serotonin metabolic pathway (28).
- Qin wu et al (2011) studied the potential toxicity of cinnabar compared with mercury and arsenic on the cells - brain and liver treated with chemical for 48 h and cytotoxicity was determined by the MTS assay. The study result reported that cinnabar is less toxic when compared with mercurial and arsenical compound (29).
- Jing-Zhen Shia, Feng Kanga, Qin Wub, Yuan-Fu Lub, Jie Liub, Y. James Kangd, et al. Nephrotoxicity of mercuric chloride, methylmercury and cinnabar-containing Zhu-Sha-An-Shen-Wan in rats. *Toxicology Letters*. 2011; 200:194-200 (30).
- S. K. Atole et al (2009) reported in their study that *Cirullus* his is safe for use as on antidiabetic dose of 50 mg & 100mg/kg b.w (31)
- Farzaneh & Mohammed (2006) studied the toxic effect of alcoholic extract of *Cirullus Colocynthis* on rat at 50, 100, 200 and 400 mg/kg body wt. The study showed that the toxic effect was nil at 50 & 100 mg/kg body wt less intense at 200 and 400 mg/kg/body wt (32).

- Farzaneh & Mohammed (2006), *Citrullus Colocynthis* seed caused some morphological change in liver cells including karryhexis, Chromatolysis & granulation of cytoplasm with doses of 200, 400 mg/kg can have hepatotoxic effect (Toxic effect of liver cells) which may induce hepatocyte necrosis & liver fibrosis (32).
- Diwan et al., (2000) reported that the saponin extract of *Citrullus colocynthis* shows mortality and histopathologic changes in Mice (33).
- Pratap Chand Mali et al ., (2008) concluded in their study that 50% ethanol extract of *Citrullus Colocynthis* root produced anti infertility activities in male albino rats at doses of 50, 100 and 200 mg/kg b.et /day for a time period of 60 days. It revealed the arrest of spermatogenesis at the stage of round spermatid, degenerative changes in the seminiferous epithelium, cell lysis and the lumen was filled with esinophilic substance ---- change in the leydig cell nuclear diameter were also decreased. Thin it produced anti infertility activities in male albino rats (34).
- Grossmans et al., (2007) study showed the effects of Cucurbitain glucosides extracted from *Citrullus Colocynthis* leaves, exhibit pleiotropic effect on human breast cancer cells causing both cell cycle arrest and apoptosis (35).
- Qazan WSH et al., (2007) showed in their study that exposure of female rats to *Citrullus Colocynthis* for long term causes adverse effects on fertility and the reproductive system (36).
- Dazadka tt et al.,(2007) studied the effect of *Citrullus Colocynthis* (70% ETOH) extract on lipid profile of liver and heart muscles showed serum Cholestrol levels, phospholipids and triglycerides were reduced it indicates that *Citrullus Colocynthis* possess active hypolipidaemic constituents (37).
- Soufane S et al., (2013) conducted the acute toxicity study on *Citrullus Colocynthis* fruit methonal extract in albino rats. The study showed that intake of ripe *Citrullus Colocynthis* fruit presented some adverse effects on the function of the liver, kidney and bone marrow in rats. The lethal dose of the extract was 45 mg/kg. The plasma ALT, AST, urea and creatinine levels were significantly affected an indication that the extract is hepato-nephro toxic. The results obtained for hematological parameters reflect that methanol extract with a dose of 131

mg/kg did not affect quantitatively. But disrupted qualitatively some functions of the bone marrow (38).

- Hajar Shafari et al., (2012) studied the toxic effects of *Cirullus Colocynthis*. Study showed that no animal were survived when treated with 200 mg/kg/day of pulp extract. Animal treated with 100 mg/kg/day of pulp extract displayed severe lesions in small intestine, kidney and liver. Animal treated with 100 or 200 mg/kg/day of seed extract shows only minor intestinal toxic effect. In contrast to the seed extract, Pulp extract of *Cirullus Colocynthis* can be fatal for rabbits. Seed extract may be the preferable way for the therapeutic use. (39).
- Prasanth Reddy et al (2012) studied the antipyretic activity of ethanolic extract of *Cirullus Colocynthis* at doses of 200 mg/kg and 400 mg/kg via oral showed significance compared to that of control group (40).
- Marzouk B et al (2010) studied the aqueous extracts from root, stems, fruit and seeds of *Cirullus Colocynthis*, which shows analgesic and anti-inflammatory activities at variable doses without inducing toxicity (41).
- Sandhya V et al (2013) examined the antimicrobial activity of *Cirullus Colocynthis* plant extract against bacterial stains like *E.cloi*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella shiogella* and *Candida albicans*. Anti microbial activity was compared with standard antibiotic cephalexin. It proved that antimicrobial activity of fruit extract was higher than that of antibiotic cephalaxon against studied test organisms (42).
- Gupta M et al (2008) To evaluate the antipyretic effect of aqueous extract of 10 herbal drugs, a study carried out as a clinical trial on 60 patients using Aspirin (60 mg/kg b.wt per day) as a standard drug for comparison were done. This study results is significant reduction in body temperature and also prostaglandin when compared to that of aspirin (43).
- Sun-ju-lee et al (2011) designed a study to investigate the effect of herbal medicine extracts for a treatment of fever. The body temperature was dropped over in the herbal extract medicine only group along with the antibiotic/antipyretic group (44).
- Akindele AJ et al (2007), the antipyretic effect of aqueous leaf extract of *Byrsocarpus coccineus schum* and thon was investigated A. J. Akindele and O. O. Adegemi in rats and rabbits by using yeast, amphetamine and lippolysaccharide

induced pyrexia models. The study results in effective antipyretic activity by inhibiting the activity of prostaglandins production in hypothalamus largely (45).

- Gopianand K et al (2010), to elucidate the antipyretic and anti-inflammatory extract of cassia fistula. The ethanolic leaf extract were administered to experimental rat. To carry out anti-inflammatory activity carrageenan induced rat paw oedema and cotton pellet granuloma models. Which the antipyretic effect was evaluated using against TAB vaccine induced pyrexia. Dose variety ranges from 50,100,250,500 and 750 mg/kg b.wt. The study results are exhibiting the potential benefit of cassia fistula in existing conditions associated with fever and inflammation (46)

5. MATERIALS AND METHODS

Table 5.1: Study names and study places

S. No	Study name	Done at
1	Procurement raw drug	M/s Gopal Aasan Country drug store, Nagercoil, Tamilnadu, India
2	Collection of raw materials	The field of Kancheepuram district, Tamil nadu, India
3	Purification and detoxification of Lingam	NIS, Chennai, India
4	Preparation of Linga chendharam	NIS, Chennai, India
5	Physico chemical analysis	RRIUM, Chennai, India
6	Atomic Absorption Spectroscopic study	RRIUM, Chennai, India
7	X ray diffraction analysis	Madras University, Chennai, India
8	High Resolution Scanning Electron Microscope (HR-SEM) analysis.	SAIF, IIT Madras, Chennai, India
9	Wavelength dispersive X-ray fluorescence spectroscopic study	SAIF, IIT Madras, Chennai, India
10	Inductively Coupled Plasma – Optical Emission spectroscopic study	SAIF, IIT Madras, Chennai, India
11	Toxicity studies	NIS, Chennai, India
12	Pharmacology studies	KMCH college of Pharmacy, Coimbatore, India
13	Clinical study	NIS, Chennai, India

5.1. PREPARATION OF TEST DRUG LINGA CHENDHURAM

5.1.1. Procurement and collection of raw materials

The raw material Lingam (Cinnabar) was purchased from M/s Gopal Aasan Country drug store, Nagercoil, Tamilnadu, India. The herbs such as Atruthumatti (Entire part of *Citrullus colocynthis*) and Kuppaimeni ilai (Leaves of *Achalypa indica*) were collected from Kancheepuram district, Tamil Nadu, India. Cow's milk and Lemon fruit were purchased from the local market at Chennai, Tamil Nadu, India.

5.1.2. Authentication of Raw materials:

Lingam was authenticated by Dr. M. Suresh Gandhi, Geology department, Madras University, Chennai, Tamil Nadu, India after studying its physicochemical properties. The herbs were authenticated by the Dr. D. Aravindhan, Associate professor of Medicinal Botany, NIS, Chennai, Tamil Nadu, India.



Plate 5.1.1: The raw Lingam purchased from country drug store to be subjected for purification processes

5.1.3. Apparatus used

Agal (Mud plate), Hot plate, Glass jar, Kalvam (Black stone mortar) and LPG stove.

5.1.4. Purification and detoxification of Lingam

Numerous purification processes were cited in Siddha literature and in traditional practice which were having complicated and laborious methods. In this study, we selected Surukku process for the purification of Lingam

5.1.5. Purification and detoxification of Lingam by Surukku process

This is the common routine method employed for the purification of Lingam which is cited in the text of Gunapadam (9).

Materials

- A single piece of Lingam (Cinnabar, Red Sulphide of Mercury –Natural) – 57.83g
- Juice of Leaves of *Acalypha indica* – 150 mL
- Lemon juice – 150 mL
- Cow's milk – 150 mL

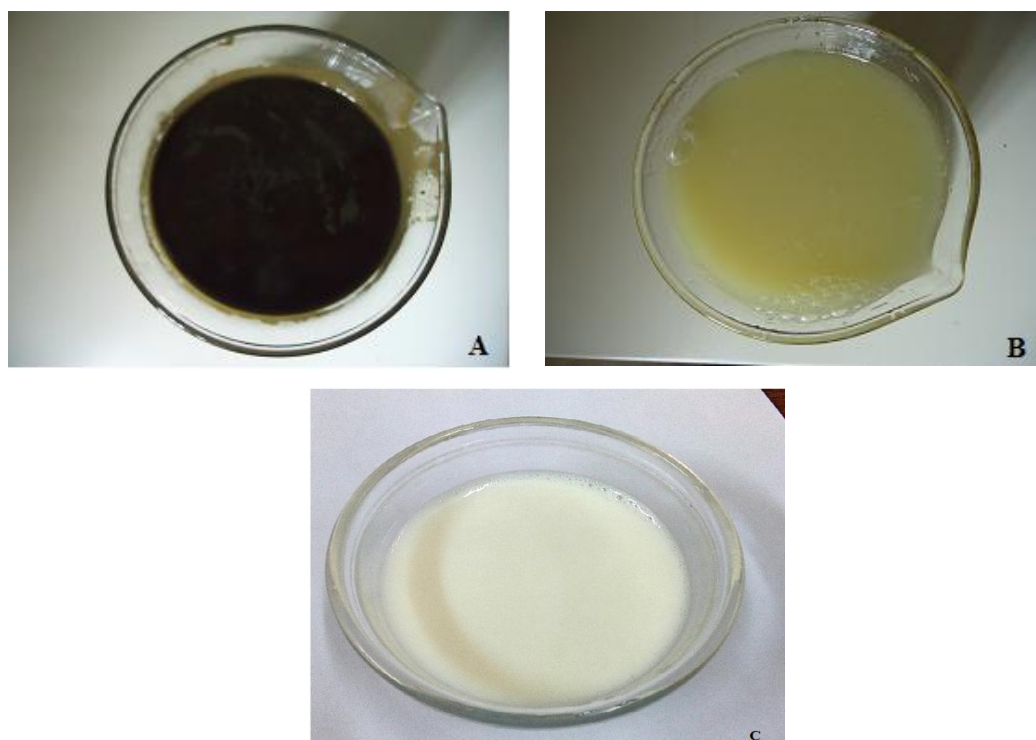


Plate 5.1.2: A denotes the Juice of Leaves of *Acalypha indica*, B denotes the Lemon juice and C denotes the Cow's milk

Method

- Cow's milk, Lemon juice and *Acalypha indica* juice were mixed well in a glass jar.
- A single piece of Lingam weighed 57.83 g was placed on a mud plate and heated over hot plate mounted on it.
- The juices in the glass jar were instilled over the Lingam drop by drop for 3 h continuously.
- After 3 h, the Lingam was allowed to cool and washed out with water and dried.



Plate 5.1.3: A denotes the raw Lingam was purified by Surukku method and B denotes the purified Lingam done by this process

5.1.6. Preparation of Linga chendhuram

Ingredients

- Purified Lingam -35g
- Juice of Citrullus colocynthis -1300mL

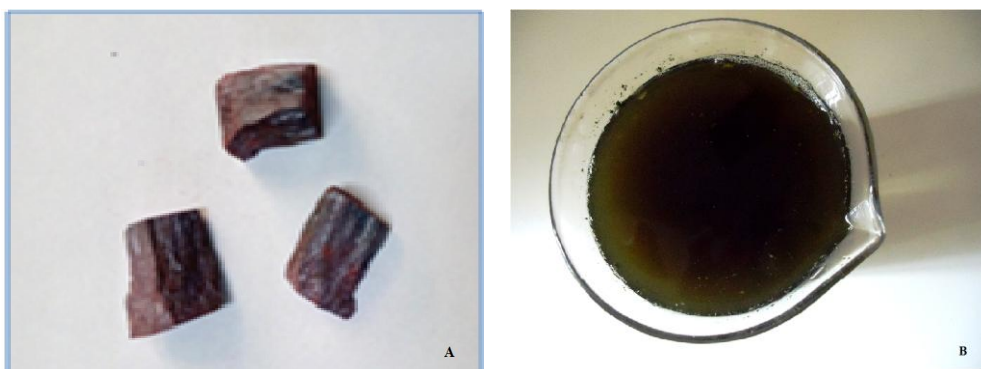


Plate 5.1. 4: A denotes the purified Lingam and B denotes the Juice of Citrullus colocynthis

Procedure

35g of purified Lingam was placed over the mud pan and heated at low flare using LPG stove. 1300 mL juice of Citrullus colocynthis (Atruthumatti) was added little by little over the Lingam simultaneously during heating. After this process, Lingam was taken to kalvam and ground into very fine powder until its luster was not appeared.

5.2. Qualitative analysis of Linga chendhuram

5.2.1 Siddha classical method

The quality of Linga chendhuram was accessed by the parameters cited in the Siddha Pharmacopoeia (47).

- Red in colour without any shiny appearance
- No taste and inodorous
- Won't redeem glint on heating repeatedly at same temperature
- Samples stay afloat on water. Did not instantly merge in water
- Illuminous
- When the sample rubbed in between Index finger and Thumb it should be infringed in the papillary ridges.

5.2.2. Organoleptic evaluation

It is the procedure of qualitative evaluation on the basis of the study of morphological and sensory profile of whole drugs.

5.2.3. Physico chemical analysis

The procedures recommended in protocol (48) for testing (Pharmacopoeial Laboratory for Indian Medicine) were followed to resolve loss on drying at 105°C, total Ash, total acid-insoluble ash and solubility in alcohol and water. All the procedures were repeatedly done for 3 times and the average values was recorded.

Loss on Drying

- 3 g of LC was kept in a previously weighed 100 mL beaker
- LC heated at an temperature of 105°C for 5 hrs in an oven and cooled and weighed.
- This method was repeated until constant weight was obtained.
- The percentage of loss in weight of the sample was calculated.

$$\% \text{ Loss on Drying at } 105^{\circ}\text{C} = \frac{\text{Loss in weight of the sample} \times 100}{\text{Weight of the LC by taken}}$$

Total Ash

- 3g of LC was weighed in a previously burned and tarred Silica dish.

- Spreaded in the dish evenly.
- The dish was ignited in a muffle furnace at 600°C until it became white i.e., Free from Carbon.
- The dish was cooled and weighed and the ash percentage was calculated.

$$\% \text{ Total Ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of the LC taken}}$$

Acid insoluble Ash

- With the collected total ash, 45 mL of 1:5 Hydrochloric acid was added in three portions of 15 mL each time.
- Boiled gently for 5 minutes
- Filtered using filter paper Whatman No: 41
- The insoluble ash found over the ash less filter paper was collected.
- Using distilled water the insoluble ash was washed until the residue was free from acid.
- The insoluble matter present in the filter paper was transferred to the original dish, dried and burned to constant weight.
- The dish was cooled and weighed.
- The percentage of Acid insoluble -ash of the air dried material was calculated.

$$\% \text{ Acid insoluble-ash} = \frac{\text{Weight of the acid insoluble residue} \times 100}{\text{Weight of the LC}}$$

Water Soluble Ash

- The total ash of LC was again obtained by the above method for total ash and weighed.
- The whole ash content was boiled for 5 min with 25 mL of distilled water.
- Filtered using filter paper Whatman No: 41
- The insoluble ash found over the ash less filter paper was collected.
- The insoluble ash was washed with hot water and transferred to the previously weighed silica crucible.
- Ignited the crucible for 15 min at a temperature not more than 450° C in muffle furnace.

- Weighed the crucible with the residue until constant weight was attained .then the weight of unsolvable ash was determined.
- The weight of the water soluble ash was determined by deducting the weight of insoluble ash from the weight of total ash.
- The percentage of water soluble ash was calculated.

$$\% \text{ Water soluble ash value} = \frac{\text{Wt. of Water soluble ash}}{\text{Wt. of crude drug taken}} \times 100$$

Alcohol Soluble Extractive Value

- 3g of LC was taken in a glass stoppered flask.
- 100 mL of 95 % distilled alcohol was added to the flask.
- The flask was shaken occasionally for 6 hours and allowed standing for 18 hours without disturbance.
- Filtered rapidly without loss of alcohol solvent.
- In a 100 ml of beaker weighed priorly 25 mL of the filtrate was pipetted.
- The beaker was evaporated to dryness on a water bath.
- Beaker kept in an air oven at 105°C for 6 h
- Cooled the beaker in a desiccator and weighed.
- Alcohol extractable matter of the LC was calculated in a percentage.

$$\% \text{ Alcohol- soluble extractive} = \frac{\text{Weight of the extract}}{25 \times \text{weight of the LC taken}} \times 100$$

Water soluble Extractive value

- 3g of LC was taken in a glass stoppered flask.
- 100 mL of distilled water was added to the flask.
- The flask was shaken occasionally for 6 h and allowed standing for 18 h without disturbance.
- Filtered rapidly without loss of alcohol solvent.
- 25 mL of the filtrate was pipetted in a previously weighed 100 mL beaker.
- The beaker was evaporated to dryness on a water bath.
- Kept the beaker in an air oven at 105°C for 6 h
- Cooled the beaker in a desiccator and weighed.

- The percentage of water extractable matter of the LC was calculated.

$$\% \text{ Water-soluble extractive} = \frac{\text{Weight of the extract}}{25 \times \text{weight of the sample taken}} \times 100$$

5.2.4. X ray diffraction analysis

The powder X-ray diffraction study was done by the instrument Bruker D8 Advance X-ray diffraction.

5.2.5. Microscopic analysis

The particle size of LC was determined by using the High Resolution Scanning Electron Microscope (HR-SEM) analysis. Carl Zeiss MA15/EVO 18 Scanning Electron Microscope was used for the analysis. A representative portion of each sample was smeared onto a double side carbon tape and mounted on aluminum stubs in order to obtain a higher quality secondary electron image for SEM examination.

Resolution: separation of gold particle 1.2 nm in size on a carbon substrate

Magnification: From a min of 12X to greater than 100000X

5.3. Quantitative analysis of Linga chendhuram

5.3.1. Atomic Absorption Spectroscopic study

Heavy metals concentration such as Lead and Cadmium were observed by Atomic Absorption Spectroscopic study. The procedures recommended for analysis of heavy metals like Lead and Cadmium in WHO, 1998 and AOAC, 2005

Instrument details

Thermo Fischer M Series, 650902 VI.27 model Atomic Absorption Spectrometer (AAS) was used for the analysis. The operating parameters were as follows:

Instrument technique	: Flame technique
Wavelength (Lead)	: 217 nm
Wavelength (Cadmium)	: 228.8 nm
Slit width	: 0.5 nm
Lamp current (Lead)	: 4.0 mA
Lamp current (Cadmium)	: 3.0 mA
Carrier gas and flow rate	: Air and Acetylene, 1.1 L/min

Flow rate : 2 ml/min

The Hollow cathode lamps for Lead and Cadmium analysis were used as light source to provide specific wave length for the elements to be determined.

5.3.2. Wavelength dispersive X-ray fluorescence spectroscopic study

Model S4 Pioneer Bruker aXS, X-ray fluorescence analysis is a quick, non-destructive and non hazardous analysis method with very high accuracy and reproducibility. All elements of periodic table from Be (Beryllium) to U (Uranium) can be measured qualitatively, semi quantitatively and quantitatively in powders, solids and liquids.

X-ray source: Rhodium was used as the standard anode material.

The tube and generator was designed for a permanent output of 4 kW.

Detector: Scintillation counter and proportional counter

Software used: SPECTRA plus Software Package for X-ray Spectrometers Version 1.6

5.3.3. Inductively Coupled Plasma – Optical Emission spectroscopic study

This study was performed using Perkin Elmer Optima 5300 DV Inductively Coupled Plasma – Optical Emission Spectrometer.

Sample preparation - Microwave Digestion.

Test sample was weighed 0.25 g and is transferred into a liner provided with the instrument. Slowly with this, add 9 mL of HNO₃ was added such that no piece of sample was stuck on the slides. Mixed well and wait for few minutes to react. Thereafter, the liner was placed in the vessel jacket and sealed the vessel and placed in the rotor fixed in microwave and 180°C was set for 5 min and hold at the same temperature for least 10 minutes. The vessel was cooled down to a vessel interior temperature attains below 60°C and vessel surface temperature attains below 50°C, then rotor was removed. The digested sample was made up to 100mL with millipore water. If visible insoluble particles exist, solution was filtered through whatman filter paper.

5.4. Safety studies of Linga chendhuram in animal model

The toxicity studies of Linga chendhuram (LC) in animal model were conducted after obtaining prior approval (1248/ac/09/CPCSEA/5-12/2011) for animal studies from CPCSEA, Government of India through the Institutional Animal Ethics Committee

(IAEC) of NIS, Chennai and conducted at animal house, NIS, Chennai, Tamil Nadu, India.

5.4.1. Experiment animals

Species/Strain: Albino rat / Wistar

Sex: Male and Female, Female rats were nulliparous and non-pregnant

Age: 8-12 weeks

Weight: 140±20 g

Source of procurement: King Institute of Preventive Medicine, Guindy, Chennai.

The animals were maintained in the animal house of NIS, Chennai following the guidelines of INSA, New Delhi.

5.4.2. Laboratory condition maintained

Room temperature: 22±2°C

Relative humidity: 40 – 65 %

Ventilation: Air cycles: 15/min; 70:30 Exchange ratio

Illumination: By Fluorescent Lamp 60 Lumens/Watt (325 Lux)

Photoperiod: 12-h light/dark cycle by time controlled lighting system

Noise control: Constructed with Concrete walls

5.4.3. Husbandry

Housing: Same sex of three animals were housed in polypropylene cages

Bedding: With husk

Feed: Amruth Rodent pellet, Pranav Agro Industries Ltd, Sangli, Maharashtra, India

Water: Purified water by Reverse Osmosis procedure was supplied ad libitum by Rodent water feeder.

5.4.4. Identification

Cage: Cage card was tagged in each cage and indicated with animal numbers, markings and sex.

Animal: Each animal has marked with picric acid on the fur for identification (Head, Neck, Body and Base of tail) and it was indicated in cage card along with number.

5.4.5. Preparation of Vehicle

The vehicle was prepared by mixing 1ml of honey with 1ml of distilled water. For each dosage of test drug, freshly prepared vehicle was used for the study.

5.4.6. Test sample

Linga chendhram (LC), 65 mg three times a day with honey

5.5. Acute oral toxicity study

5.5.1. Methodology

The acute oral toxicity test was performed following 423 guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals (49-50).

5.5.2. Procedure

Selection of Animals

Six female Wistar rats were randomly selected and acclimatized for one week prior to the study. The rats were fastened overnight before the administration of test drug.

Dose calculation

The body weight of each fasted rat was weighed and an individual dose of test drug LC was calculated.

Dosing

The acute toxicity study was done at the starting dose of 2000 mg/kg body weight of a rat as per Annexure 2d of OECD guidelines. For the first step, 2000 mg/kg LC was suspended in 2 mL vehicle and administered to three animals using oral gavage as single time on 0 day. After single time administration of LC, rats were deprived from feed and water for 4 h. The three animals were observed for mortality and abnormal clinical signs periodically for 14 days. Since there was no mortality and abnormal signs in the first step, further three animals were administered with LC at the same dosage of 2000 mg/kg as single time for second step and observed for mortality and clinical signs of toxicity. The Median Lethal dose (LD50 cut-off value) for LC was determined in accordance with

Globally Harmonized System of Classification and Labeling of Chemicals cited in Annex 2d of OECD Guidelines 423.

5.5.3. Observations

Body weight

Each animal was weighed and recorded prior to the administration of test drug (On 0 day) and again on 7 and 14 day.

Cage Side observations

After administration of LC, all animals were observed for mortality and clinical signs of toxicity and behavioral changes at 30 min, 1, 2 and 4 hours and after that once a day for the next 14 days. The observations include general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture, changes in eyes and mucous membrane of oral cavity, salivation, lethargy, sleep, coma, convulsion, tremors, diarrhea, morbidity and mortality.

Necropsy

The six animals were done for gross necropsies. The organs present in the thoracic and abdominal cavities were examined.

5.6. 28 days repeated oral toxicity

5.6.1. Methodology

A 28-day repeated oral toxicity study was performed according to the OECD guideline - 407 with minor modifications in dosage levels of test drug (51).

5.6.2. Selection of animals

Twenty male and twenty female Wistar rats were randomly selected and acclimatized for 7 days prior to the conduction of study. At the end of acclimatization period, animals were examined for good health condition.

5.6.3. Dose calculation of test drug

In the literature of Siddha, 65 mg of LC as three times a day was recommended as therapeutic dosage for adult human. For the study, the dose of LC in rat was estimated by

conversion of dose of LC in 70 kg human on the basis of relative body surface area. The therapeutic dose in rat was calculated as follows:

200 g rat dose = Daily therapeutic dose in Human x Surface area conversion factor
= 195 mg x 0.018 = 3.51 mg

1000 g rat dose = 3.51 mg x 5 = 17.55 mg (Approximately 18 mg)

In the present study, LC was administered at three dose levels as below

Low dose: The therapeutic dose of LC was fixed – 18 mg/kg (x)

Intermittent dose: 5 times the therapeutic dose of LC was fixed – 90 mg/kg (5x)

High dose: 10 times the therapeutic dose of LC was fixed – 180 mg/kg (10x)

5.6.4. Experimental design

Number of groups: 4

Number of animals in each group: 5 Male and 5 Female

Treatment period: 28 days

Frequency: Single time per day. Daily at morning time

Route: Oral

Table 5.6.1: Grouping and treatment details for 28 days repeated oral toxicity study

Group	Treatment
I – Control	Vehicle – Honey 1mL+ distilled water 1mL
II – Test group at low dose level	LC at 18 mg/kg b. wt suspended in 2 mL vehicle
III – Test group at intermittent dose level	LC at 90 mg/kg b. wt suspended in 2 mL vehicle
IV – Test group at high dose level	LC at 180 mg/kg b. wt suspended in 2 mL vehicle

5.6.5. Observations

The experimental animals in all groups were observed throughout the course of period of 28 days treatment of drug.

5.6.6. Body weight

Each animal was weighed and recorded on 0 day, at weekly intervals through-out the course of study period and at the sacrifice day. Mean body weight and percentage of body weight gain were calculated for each group of both sexes.

5.6.7. Food consumption

The quantity of food consumed by the animals in each cage was recorded at weekly intervals. The mean value of food consumption was calculated for each group of both sexes.

5.6.8. Clinical signs

After one hour of treatment on each day throughout the study course, all animals were observed for signs of toxicity. The clinical signs were examined at the same time in each day. If any clinical signs were observed, the time of onset, intensity and duration were recorded. The observations include general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes and changes in skin and fur texture, changes in eyes and mucous membrane of oral cavity, salivation, lethargy, sleep, coma, convulsion, tremors and diarrhea.

5.6.9. Mortality and Morbidity

All the experimental animals were observed for mortality and morbidity twice daily during the entire course of study.

5.6.10. Urinalysis

Animals of control and high dose groups were kept in metabolic cages in last 7 days of observation. 1 mL of urine sample was collected without fecal contamination from each animal and used for urinalysis. The following parameters were analyzed using routine appropriate methodology.

Colour

Transparency

Specific gravity

pH

Presence of Protein, Glucose, Bilirubin, Ketones, Blood and Urobilinogen

5.6.11. Terminal studies

Euthanasia

Animals of control, low, intermittent and high dose groups were fasted over-night at the end of 28 day of study period and on 29 day animals were sacrificed under excessive chloroform anesthesia.

Blood collection

After scarification, immediately a volume of 5 mL blood was collected from each animal by a sterile disposable syringe through cardiac puncture. 2 mL of blood was transferred into a tube containing anticoagulant Potassium EDTA (1.5 mg/mL) and used for haematological investigations. 3 mL of blood was transferred into a tube without anticoagulant and used for biochemical investigations.

Haematological Investigations

The following haematological parameters were analysed using Erba Mannheim® haematology analyser.

Haemoglobin (Hb)

Red Blood Cell count (RBC)

Red cell Distribution Width (RDW)

White Blood Cell count (WBC)

WBC Differential count - Lymphocyte, Monocyte and Granulocyte

Haematocrit (HCT)

Mean Corpuscular Volume (MCV)

Mean Corpuscular Haemoglobin (MCH)

Mean Corpuscular Hemoglobin Concentration (MCHC)

Platelet count, Platelet Crit (PCT)

Platelet Distribution Width (PDW)

Mean Platelet Volume (MPV)

The reticulocyte count was estimated microscopically.

Biochemical Investigations

The following biochemical parameters were analysed using Erba system Pack kits in Fully Automated Biochemistry analyzer.

Glucose

Cholesterol

Triglyceride (TG)

Protein

Ure

Creatinine

Bilirubin

Serum glutamic-oxaloacetic transaminase (SGOT)

Serum glutamic pyruvic transaminase (SGPT)

Alkaline Phosphatase (ALP)

Necropsy study

After blood collection, body weights of all animals were recorded. All rats were dissected for gross necropsy study. Cranial, thoracic and abdominal cavities were opened and viscera's were dissected out. Organs such as brain, trachea, lungs, heart, liver, kidney, stomach, spleen, intestine, testis, uterus and ovaries were studied for gross study by viewed under magnification glass to find the presence of macroscopic pathological lesion. Each organ was weighed and expressed in terms of absolute organ weight. With respect to body weight, relative organ weight was calculated by the below formula.

% Relative organ weight = [Absolute organ weight / Body weight] x 100

5.6.12. Histo-pathological study

Collection of organs

Since no abnormalities found during necropsy study, the organs of one animal showing high value of blood renal and hepatic parameters among the animals from control, high dose were subjected to histo-pathological studies. Organs such as brain, liver, kidney, lungs, stomach, heart, spleen, and femorotibial joints were collected and placed in 10 % Neutral buffered Formalin. Further below procedures were carried out in Liveon Biolabs Pvt. Ltd. Tumakuru, Karnataka, India.

Collection of tissues

Thin pieces of 3 – 5 mm thickness of tissues were cut from the organs collected.

Fixation

The collected tissues were kept in 10 % Formalin at room temperature for 48 h to harden the tissues by coagulating the cell protein, to prevent the structure and to prevent the shrinkages. The volume of formalin added was 10 times the volume of the tissues.

Hydration

After fixation, the tissues were washed completely in running water.

Dehydration

The tissues were dehydrated by passing it in ascending grades of alcohol as below to prevent undue shrinkage of tissues.

Ethyl alcohol 50 % for 8 h

Ethyl alcohol 70 % for 2 h

Ethyl alcohol 90 % for 2 h

Absolute alcohol – I for 1 h

Absolute alcohol – II for 1 h

Clearing

After dehydration, the tissues were cleared from alcohol by keeping in Xylol – I and II each with 30 min.

Infiltration

The tissues were completely impregnated with Paraffin wax (Melting point 50 - 56°C) kept in the cups and melted in a paraffin oven. The tissues were kept for 30 min in each cup.

Embedding

Two L shaped moulds were arranged in the form of a rectangle over a porcelain slab. The melted paraffin was poured into the mould and the tissue was so oriented that the cutting surface of the tissue faces the porcelain slab. The moulds were removed as soon as paraffin sets and the blocks were sectioned.

Sectioning

The blocks were trimmed by removing the excess paraffin all around. Sections of 4 – 5 μm thickness were cut in a Rotary microtome. The sections were transferred from the cutting edge of the microtome knife with the help of spatula to a tissue floatation bath having warm water (40 - 45°C). Then the sections were spread out uniformly and were taken on the clean glass slides coated with Meyer's albumin – glycerine mixture.

Staining of sections

Haematoxylin and eosin method of staining (H & E) was employed.

H & E staining procedure

1. Deparaffinised the sections by passing in Xylol for 5 to 10 min.
2. Removed the Xylol by passing in Absolute alcohol.
3. Washed in tap water.
4. Stained with haematoxylin for 3 to 4 min.
5. Washed in tap water.
6. Allowed the sections in acid alcohol for 15 to 30 sec.
7. Washed in tap water for 5 to 10 min.
8. Counterstained with 0.5 % Eosin until the sections appeared light pink(15-30 sec).
9. Washed in tap water.
10. Dehydrated in alcohol.
11. Cleared with Xylol for 15 to 30 sec.
12. Mounted in DPX mountant
13. Slides were dried and cover slipped without the presence of air bubble.
14. Examined under a light microscope and microscopic features were observed.

5.6.13. Statistical analysis

All data were expressed as mean \pm standard deviation (S.D). The test groups were compared with control for testing significance by one way ANOVA followed by Dunnet test using GRAPH PAD INSTAT version 3 software programme. P values less than 0.05 were considered as significant.

5.7. Efficacy studies of Linga chendhuram in animal model

The efficacy studies of Linga chendhuram (LC) in animal model were conducted after obtaining prior approval (KMCRET/MD(S)/04/2014-15) for animal studies from CPCSEA, Government of India through the Institutional Animal Ethics Committee (IAEC) of KMCH college of Pharmacy, Coimbatore and conducted at animal house, KMCH college of Pharmacy, Coimbatore, India

5.7.1. Experiment animals

Species/Strain: Albino rat / Wistar, Swiss mice

Sex: Male and Female, Female rats were nulliparous and non-pregnant

Age: 8-12 weeks

Weight: Mice-15-30g, Rat- 120- 160 g

Source of procurement: Sri Venkateshwara Traders, Bangalore

The animals were maintained in the animal house of KMCH of Pharmacy, Coimbatore, following the guidelines of INSA, New Delhi.

5.7.2. Laboratory condition maintained

Room temperature: 22±2°C

Relative humidity: 40 – 65 %

Ventilation: Air cycles: 15/min; 70:30 Exchange ratio

Illumination: By Fluorescent Lamp 60 Lumens/Watt (325 Lux)

Photoperiod: 12-h light/dark cycle by time controlled lighting system

Noise control: Constructed with Concrete walls

5.7.3. Husbandry

Housing: Same sex of three animals were housed in polypropylene cages

Bedding: With husk

Feed: Sri Venkateshwara Traders, Bangalore.

Water: Purified water by Reverse Osmosis procedure was supplied ad libitum by Rodent water feeder.

5.7.4. Identification

Cage: Cage card was tagged in each cage and indicated with animal numbers, markings and sex.

Animal: Each animal has marked with picric acid on the fur for identification (Head, Neck, Body and Base of tail) and it was indicated in cage card along with number.

5.7.5. Test sample

Linga chendhram (LC), 65 mg, three times a day with honey

5.7.6. Preparation of test sample

195 mg LC was suspended with 10mL of diluted honey (5mL honey +5mL distilled water) for the preparation of test sample. 1mL test sample constituted 19.5 mg of LC. The test sample was prepared freshly on each day of dosing.

5.7.7. Dosage allocation for test drug groups

The human intended therapeutic dosage for LC mentioned in the literature is 65 mg/dosage and three times daily. The daily dosage of LC should be 195 mg for human. On the basis of body surface area ratio between 70 kg human and 200 g rat, the dosage of LC for rat has been arrived as 17.55 mg/kg closely to 18 mg/kg. The therapeutic dose 18 mg/kg (x) was fixed as low dose for the study. The average dose 90 mg/kg (5x) was fixed as intermittent dose and 180 mg/kg (10x) was fixed as high dose for the studies.

5.8. Experimental details of Analgesic activity

5.8.1. Centrally acting Analgesics by hot plate method

This study was done by the method of EM Franzotti et al., 2000 (52). The paws of mice are very sensitive to heat at temperatures which won't damage the skin. The responses are jumping, withdrawal of the paws and licking of the paws. Nociceptive response is the responses of licking/shaking of hind paw/jumping when the animal is placed on the surface of the hot plate.

The hot plate, which is commercially available, consists of an electrically heated surface. The temperature is maintained at 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time till licking or jumping occurs is recorded by a stop-watch. One day before the experiment, three basal readings (of nociceptive response) in each mouse were recorded. The animals showing the basal reading below 8 seconds and above 16 seconds were excluded from the study. Food was withdrawn 12 hours prior to drug administration until completion of experiment. The animals were weighed and numbered appropriately. All animals received the following treatment

Experimental design

Animal: Swiss albino mice were divided into 5 groups (6 mice /group)

Table 5.8.1: Grouping and treatment details for centrally acting Analgesics by hot plate method

Group	Test drug	Dosing	Route	Frequency
A – Vehicle Control	Diluted honey	1.0 mL	Oral	Single time
B - Standard	Aspirin	10 mg/kg suspended in 1.0 mL of distilled water	Oral	Single time
C – Test group I	LC at Low dose	18 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time
D – Test group II	LC at Mid dose	90 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time
E- Test group III	LC at high dose	180 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time

Thirty min post-treatment, animals were placed one after another on hot plate maintained at $55 \pm 0.5^\circ\text{C}$ at 0, 30, 60, 90, 120 & 150 min and the pain reaction time (PRT) or latency period determined with a stop watch was recorded which represents the time taken for the mice to react to the pain stimulus. The response to pain stimulus considered included; jumping, raising and licking of hind foot. The cut off time was fixed for 15 seconds to avoid paw injury.

5.8.2. Peripherally acting analgesics by writhing test

This study was done by the method of Heng-Yuan Chang et al., 2011 (53).

Experimental design

Animal: Swiss albino mice were divided into 5 groups (6 mice /group)

Table 5.8.2: Grouping and treatment details for peripherally acting analgesics by writhing test

Group	Test drug	Dosing	Route	Frequency
A – Vehicle Control	Diluted honey	1.0 mL	Oral	Single time
B - Standard	Aspirin	10 mg/kg suspended in 1.0 mL of distilled water	Oral	Single time
C – Test group I	LC at Low dose	18 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time
D – Test group II	LC at Mid dose	90 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time
E- Test group III	LC at high dose	180 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time

Induction of writhing

Writhing means constriction of abdominal muscles along with the stretching of hind limbs. Writhing can be induced by an irritant such as phenylquinone, acetic acid etc. 60 min post treatment, all groups were administered with 0.1 mL/10 g acetic acid solution (10 mL/kg) as intra peritoneal injection.

Observations

After 5 min of injection, total number of writhing movements () was recorded over a period of 10 min and percentage of inhibition of abdominal writhing was calculated as follow.

$$\% \text{ inhibition of abdominal writhing} = \frac{(W_c - W_t)}{W_c} \times 100$$

W_c - No. of writhing in control group, W_t - No. of writhing in test group

5.9. Screening methods for Anti-inflammatory activity.

5.9.1. Carrageenan induced acute hind paw inflammation

This study was done by the method of Heng-Yuan Chang et al., 2011 (54).

Experimental design

Animal: Wistar rats were divided into 5 groups (6 rat /group)

Table 5.9.1: Grouping and treatment details for carrageenan induced acute hind paw inflammation

Group	Test drug	Dosing	Route	Frequency
A – Vehicle Control	Diluted honey	1.0 mL	Oral	Single time
B - Standard	Diclofenac sodium	10 mg/kg suspended in 1.0 mL of distilled water	Oral	Single time
C – Test group I	LC at Low dose	18 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time
D – Test group II	LC at Mid dose	90 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time
E- Test group III	LC at high dose	180 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time

After above treatment acute paw oedema was developed in all rats by injecting 0.1 mL of 1% (w/v) Carrageenan solution prepared in normal saline in sub plantar region of the left hind paw of all rats. The volume paw was measured at 0, 1, 2, 3, 4, 5 and 6 h using digital Plethysmometer after the administration of Carrageenan.

$$\% \text{ Paw edema inhibition} = \frac{A - B}{A} \times 100$$

Where, A denotes the mean increase in paw edema of Control group, B denotes the mean increase in paw edema of standard / test groups

5.9.2. Cotton pellet granuloma method

This study was done by the method of Kaneria MS et al (54)

Induction of granuloma formation

Adsorbent cotton wool was cut into pieces weighing 20±1 mg and made into pellets. The pellets were then sterilized in a hot air oven at 120° for 2 h. The abdomen was shaved cleanly, swabbed with 70% ethanol and two sterilized cotton pellets were implanted subcutaneously, one on each side of the abdomen of the animal under light ether anaesthesia. After implantation, all animals received the following treatment.

Experimental design

Animal: Wistar rats were divided into 5 groups (6 rat /group).

Table 5.9.1: Grouping and treatment details for Cotton pellet granuloma method

Group	Test drug	Dosing	Route	Frequency
A – Vehicle Control	Diluted honey	1.0 mL	Oral	Single time
B - Standard	Dexamethasone	10 mg/kg suspended in 1.0 mL of distilled water	Oral	Single time
C – Test group I	LC at Low dose	18 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time
D – Test group II	LC at Mid dose	90 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time
E- Test group III	LC at high dose	180 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time

Observations

On the 8th day after implantation, rats were anaesthetized with pentobarbital sodium. The pellets were dissected and dried at 60° for 24 h, weighed after cooling. The mean weight of the cotton pellets of the control group as well as experimental groups was calculated. The percentage inhibition of the dry and wet weight of the granuloma were calculated and compared.

5.10. Anti-pyretic activity

This study was done by the method of Vogel, G.H. (2002) (55) Wistar rats having normal rectal temperature checked using digital thermometer were selected for the study. Following body weight measurements and 15 min acclimatization to procedure room, pyrexia was induced in all rats by subcutaneous injection of 20% aqueous suspension of brewer's yeast (*Saccharomyces cerevisiae*) 10 mL/kg. All animals were fasted with access to only water, after injection of yeast for one day. After that, the rectal temperature of each rat was recorded and pyrexia was confirmed by an increase in temperature more than 1°C, while animals showing less than 1°C rise in temperature were excluded from the experiment.

Experimental design

Animal: Wistar rats were divided into 5 groups (6 rat /group).

Table 5.9.1: Grouping and treatment details for Anti-pyretic activity

Group	Test drug	Dosing	Route	Frequency
A – Vehicle Control	Diluted honey	1.0 mL	Oral	Single time
B - Standard	Paracetamol	10 mg/kg suspended in 1.0 mL of distilled water	Oral	Single time
C – Test group I	LC at Low dose	18 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time
D – Test group II	LC at Mid dose	90 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time
E- Test group III	LC at high dose	180 mg/kg of LC suspended in 1.0 mL of honey	Oral	Single time

The rectal temperature of the groups was recorded at 1 h intervals for 5 h.

5.11. CLINICAL STUDY

5.11.1. Registration:

The clinical protocol of this study has been approved by the Human Ethical Committee of National Institute of Siddha, Chennai, India, No: NIS/IEC/7/2013-14/23, dt: 24-12-2013 and it was registered in Clinical Trial Registry – India, No: CTRI/2014/04/004576

5.11.2. Study Type : Retrospective

5.11.3. Study Design : Allocation: Non Randomized,

End point classification: Safety and efficacy study

Group: Single

Masking: Open label

Centre: Single

5.11. 4. Study place : Ayothidoss Pandither Hospital,

National Institute of Siddha, Chennai.

5.11.5. Study population:

A sample size of 145 patients were chosen for the study among the total population who were reported having fever at the outpatient department of Pathology (Noi Naadal) and Casuality (Avasara Maruthuvam) of Ayothidoss Pandither Hospital, National Institute of Siddha Chennai, India within the age group of 21 – 60 years. The patients were undergone screening for the fulfillment of selection criteria for the enrollment in the study.

5.11.6. Test drug details:

Test drug - Linga chendharam

Dose - 65mg,

Vehicle - Honey 5ml.

Route of administration - Oral

Frequency and period of administration- Three times a day for 5 days

5.11.7. Selection and Withdrawal of Subjects

Eligibility

Age :	21 to 60 years
Genders Eligible for Study :	Both
Accepts Healthy Volunteers :	No

Inclusion criteria:

- Males and non pregnant females
- Patients Diagnosed as Suram having the symptoms of increased body temperature along with burning sensation of eye, body pain and nausea
- Body temperature range: 100°F - 103 ° F
- Patients given informed consent
- Patients complied to the study procedures

Exclusion Criteria

- Patients received antipyretic's, NSAID's, Corticosteroids, antimalarial and antibiotic drugs for current episode of fever
- Temperature more than 103 ° F and below 100°F.
- Pregnant and nursing women
- Terminally ill patients (or) patients with severe cardiac, hepatic (Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) values > 3 folds of upper limit of normal laboratory values), Renal (Increased urea and creatinine level), Cerebro vascular disease, malignancy, hematological disorders and chronic uncontrolled systemic diseases
- Patients already participated in a new drug study in the past 3 months.
- Subject withdrawal criteria:
- Subject undergone other treatment without informing the Investigator.
- Clinical failure after 2 days of treatment as worsening or no amelioration of signs & symptoms.
- Request from Subject.
- Repeated protocol criteria violation and non-compliance with its specification.

- Serious adverse events / reactions / Intercurrent illness where continuation of study possessed serious risk to the subject
- Subject developed hypersensitivity drug reaction

5.11.8. Subject enrolment

For the study, we screened 145 patients having symptom of fever and observed 100 patients were fulfilled major inclusion criteria and eligible for the study. Among 100, 75 patients gave willingness to participate in the study and 75 patients were enrolled.

5.11.9. Examination

General examination such as Body weight, Height, Body temperature, Pulse rate, Heart rate, Respiratory rate, Blood pressure, Pallor, Icterus, Clubbing, Cyanosis, Lymph node enlargement, Pedal Oedema and examination of vital organs were carried out on before and after treatment.

5.11.10. Envagai thervu and Uyir thathukkal examination:

Envagai thervu (Eight folds of examination) were carried out and observed the signs of variations in Naa (Tongue), Niram (Colour), Mozhi (Speech), Vizhi (Eye), Malam (Stools), Moothiram (Urine), Naadi (Pulse), Sparism (Touch) on before and after treatment. The features of Uyir thathukkal (Humours) viz., Vali, Azhal and Iyyam were observed before and after treatment.

5.11.11. Investigations:

The following investigations were carried out at the beginning of the study and ruled out for serious medical illnesses and also at the end of medication period to assess the tolerability of LC.

Haematology:

Haemoglobin, Total leucocyte count, Differential leucocyte count, Total RBC count, Platelet count and ESR.

Biochemistry:

(Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), Total Bilirubin, Alkaline phosphatase, Random Blood sugar, Serum Creatinine, Blood Urea Nitrogen (BUN) & Serum electrolytes.

Urinalysis: Routine & Microscopic examinations

5.11.12. Case sheet proforma:

Patients were given unique registration number. The screening forms were filled separately such as complaints and duration, history of present and past illness, family history, Physical examinations findings, objectives findings, investigations, drug compliance and dietary advices, informed consent, adverse events were systematically recorded in the case sheet proforma for analysis. The case taking proforma was elaborately designed for the purpose of incorporating the Siddha methods of diagnosis as mentioned in the Siddha literature. After enrolling the patient in the study, a separate file for the each patient was opened and all the forms were kept in the file confidentially

5.11.13. Review of the patients:

The patients were requested to visit for review daily for 5 days. The patients were examined vital sign, Envagai thervu and any adverse event occurred were recorded in the CRF.

5.11.14. Procedure for monitoring patient compliance:

Medicine intake chart and thermometer were provided to the patients for entry the medicine intake time and temperature by them. On every visit, the detail in the chart given to the patient were added in the CRF

5.11.15. Adverse event:

Any serious adverse event and unexpected adverse drug reaction was observed during the treatment period and planned to report the event to the pharmacovigilance committee of the study centre if it was happened.

5.11.16. Clinical assessment:

65 mg LC suspended with 5 ml honey was administered 8 h once for 5 days. After administration of LC, the temperature was recorded in each 30 min interval for first 4 h then 3 h interval for further period of 5 days. The pain score was recorded in each 3 h interval for the period of 5 days using Visual Analogue Scale (VAS)

5.11.17. Out come:

Primary: Reduction of the raised oral temperature recorded using graphical dot plotting method.

Secondary: Improving the other manifestations such as any inflammation, joint pain, etc. Reducing the intensity of joint pain and headache, on a Visual Analogue Scale (VAS) for pain (0 = no pain to 100mm =severe pain at day 0, 1, 2, 3, 4, 5, 6)

5.11.18. Data management:

After enrollment of the patient in the study, a separate file for each patient was opened and all forms were filed. Study No. and Patient No. were entered on the top of file for easy identification and arranged in a separate rack at the concerned OPD unit. Whenever study patient visits OPD during the study period, the respective patient file was taken and necessary recordings were made at the assessment form or other suitable form. The screening forms were filed separately. At last, Data recordings were monitored for completion. All collected data were entered in excel format using Microsoft office excel and analysis for statically significance.

5.11.19. Statistical Analysis:

All data were expressed as Mean \pm Standard Deviation (SD). P value was calculated using paired 't' test using GRAPH PAD INSTAT version 3 software programs. P value less than 0.05.

6.1. PHYSICO CHEMICAL CHARACTERIZATION OF LINGA CHENDHURAM

The finished test drug was stored in an air tight sterile glass container.

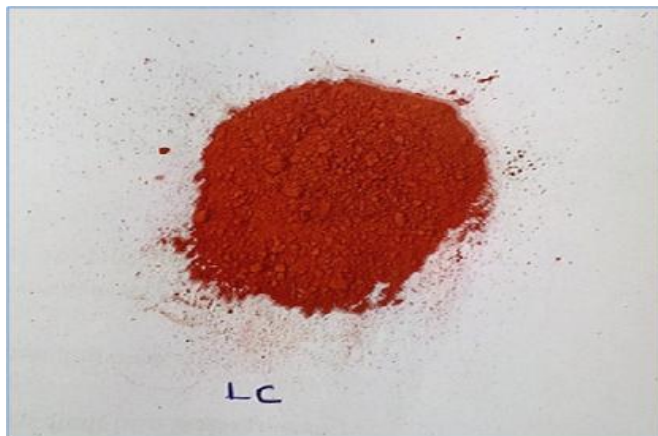


Plate 6.1.1.: Prepared the Linga chendhuram

6.1.1. Quality assessment

The Table 6.1.1 show LC satisfies all the quality parameters mentioned in the literature and proves that the LC prepared by the above process was well finished.

Table 6.1.1: Quality analysis of *Linga Chendhuram*

S. No	<i>Siddha</i> classical method	Results
1	Red colour without shining appearance	Positive
2	No taste	Positive
3	No odour	Positive
4	No luster formation on heating again at same temperature	Positive
5	Sample floats on water.	Positive
6	Not translucent	Positive
7	Impinged in the papillary ridges of Thumb and Index finger	Positive

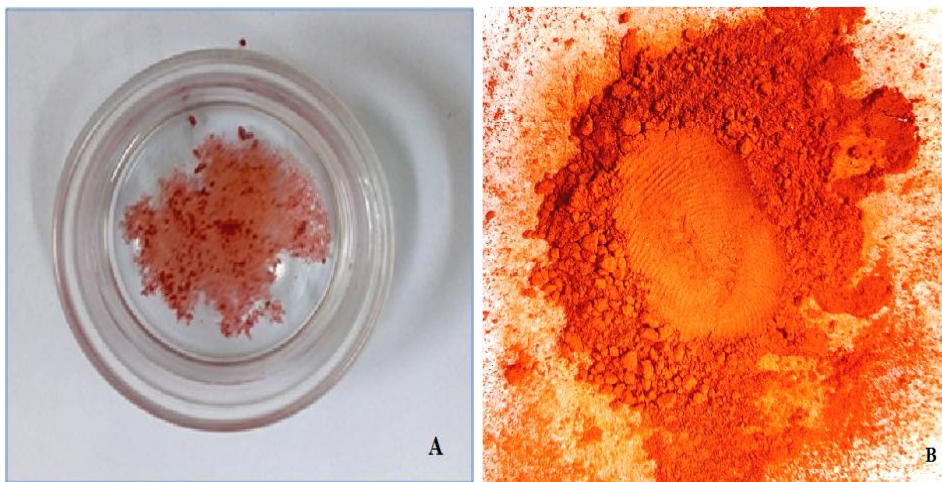


Plate 6.1.2: A denotes the LC floats on water and B denotes the papillary ridges of Thumb finger

6.1.2. Organoleptic characters of *Linga chendhuram*

The organoleptic characters of the Linga Chendhuram were as shown in table 6.1.2. LC was brick red smooth fine powder with characteristic odour slightly pungent and no taste.

Table 6.1.2: Organoleptic characters of *Linga chendhuram*

S. No	Parameter	<i>Linga chendhuram</i>
1	Colour	Brick red
2	Taste	Nil
3	Odour	Slightly pungent
4	Magnetism	Nil
5	Reaction to HCL	No effervescence
6	Luminescence	Non fluorescent

6.1.3. Physicochemical analysis

Ash values

The results of ash values were shown in the table 6.1.3 & 4. LC has low value of total ash and inferred that the presence of more organic compounds and it has better purity.

Table 6.1.3: Total ash value of *Linga chendhuram*

Weight of <i>Linga chendhuram</i> taken (g)	Percentage w/w Total ash	Mean \pm S.D %
3.0	0.42	0.55 \pm 0.13
3.0	0.55	
3.0	0.68	

Table 6.1.4: Acid insoluble ash value of *Linga chendhuram*

Weight of <i>Linga chendhuram</i> taken (g)	Percentage w/w acid insoluble ash	Mean \pm S.D %
3.0	0.011	0.016 \pm 0.007
3.0	0.014	
3.0	0.025	

Extractive values

The results were shown in the table 6.1.5 & 6 and inferred that LC has poor extractive values and poor solubility in solvents.

Table 6.1.5: Alcohol soluble Extractive value of *Linga chendhuram*

Solvent (100 mL)	Weight of <i>Linga chendhuram</i> taken (g)	Extractive Value (%)	Mean \pm S.D %
Ethanol	5.0	1.20	1.3 \pm 0.123
Ethanol	5.0	1.27	
Ethanol	5.0	1.44	

Table 6.1.6: Water soluble Extractive value of *Linga chendhuram*

Solvent (100 ml)	Weight of <i>Linga chendhuram</i> taken (g)	Extractive Value (%)	Mean \pm S.D %
Water	5.0	2.10	2.24 \pm 0.15
Water	5.0	2.24	
Water	5.0	2.40	

Loss on drying value

From the results of table 6.1.7, it is inferred that LC had low moisture content and has better higher stability.

Table 6.1.7: Moisture content of *Linga chendhuram*

Weight of <i>Linga chendhuram</i> taken (g)	Loss of weight at 105°C (g)	Mean \pm S.D %
2.0	0.018	0.236 \pm 0.005
2.0	0.024	
2.0	0.029	

Physical Parameters

The concise results of physical parameters of LC were shown in the table 6.1.8.

Table 6.1.8: Physical Parameters of *Linga chendhuram*

S. No	Parameter	Result % (Mean±S.D)
1	Loss on drying at 105°C	0.236±0.005
2	Total Ash Value	0.55±0.13
3	Acid Insoluble ash	0.016±0.007
4	Alcohol soluble extract	1.3±0.123
5	Water soluble extract	2.24±0.15

6.1.4. X ray diffraction analysis

The XRD patterns of the LC are shown in Fig. 6.1.1. LC was present in crystalline form with characteristics peaks near to 26°, 31°, 37°, 43°, 45°, 53° and 56° 2-Theta. XRD finger print of LC was found to be similar to the previous work of Sudha et al., 2009 [7]. The peak value in between 26° - 27° and 31°-32° corresponds to the standard peaks for HgS. The remaining peaks indicate the presence of other compound formed due to processing using herb.

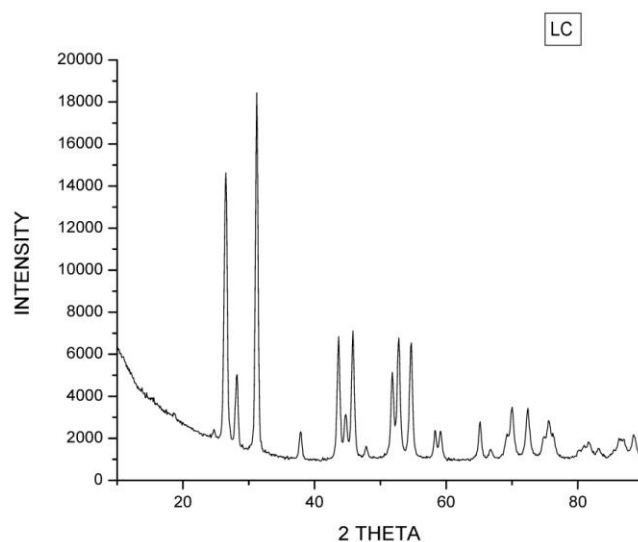


Figure.6.1.1: XRD image of LC

6.1.5. Microscopic analysis

SEM images of LC showed difference in size from 1 – 10 μm and agglomeration of the particles (6.1.2 & 3). Constant addition of herbal juice and grinding during process of LC made agglomeration of the particles.

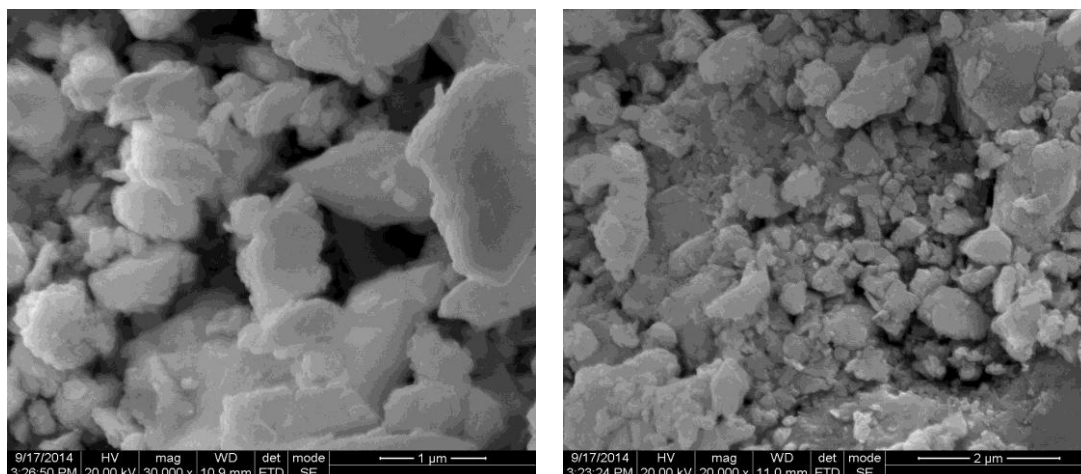


Plate 6.1.2- SEM image of LC showing particle sizes of 1 μm and 2 μm

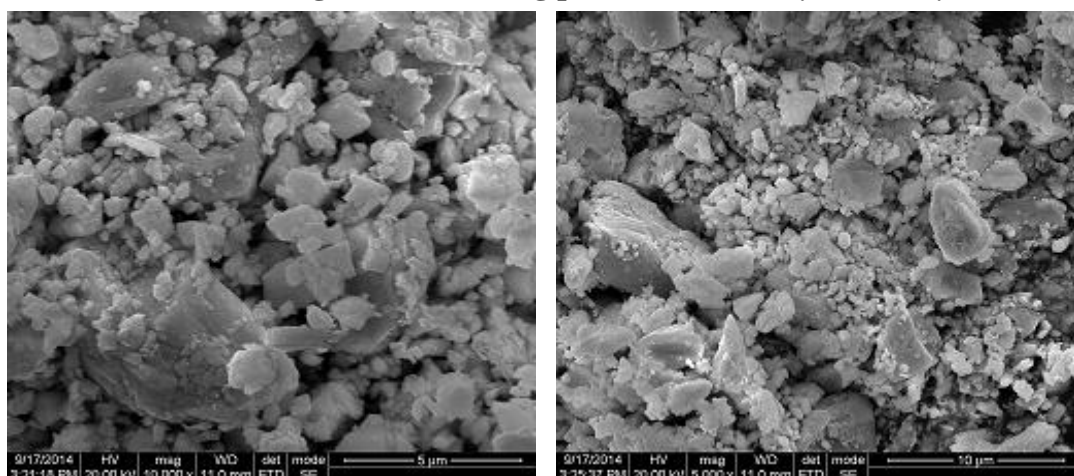


Plate 6.1.3- SEM image of LC showing particle sizes of 5 μm and 10 μm

6.1.6. Concentration of Elements in Oxide form

The elements found in the oxide form were depicted in the table 6.1.9. The LC has major concentration of Sulphur trioxide. The other oxides were found to be in traceable amount.

Table 6.1.9: Concentration of Elements in Oxide form of *Linga Chendhuram*

Element in oxide form	Concentration (%)	Elemental form	Concentration (%)
Sulphur trioxide	26.89	Mercury	84.64
Arsenic trioxide	3.83	Sulphur	10.77
Magnesium oxide	0.45	Arsenic	2.90
Silicon dioxide	0.32	Magnesium	0.27
Lead oxide	0.28	Lead	0.26
Manganese oxide	0.22	Manganese	0.17
Thallium	0.15	Thallium	0.15
Iron(III) oxide	0.13	Silicon	0.15
Calcium oxide	0.11	Chloride	0.11
Sodium oxide	0.11	Iron	0.09
Chloride	0.11	Potassium	0.09
Potassium oxide	0.11	Zinc	0.08
Zinc oxide	0.10	Sodium	0.08
Selenium dioxide	0.08	Calcium	0.08
Aluminium oxide	0.07	Selenium	0.06
Platinum	0.04	Platinum	0.04
Copper(II) oxide	0.02	Aluminium	0.04
		Copper	0.02

6.1.7. Elemental concentration

The concentration of trace elements including heavy metals were observed by Inductively Coupled Plasma Optical Emission Spectroscopic study was shown in the table 6.1.10 and 11. The concentration of heavy metals such as Lead and Cadmium in LC were below the WHO permissible limit (Table 6.1.11).

Table 6.1.10: Trace Elements of *Linga Chendhuram*

S. No	Elemental symbol	Wavelength (nm)	Concentration (ppm)
1	Arsenic	188.979	BDL
2	Cadmium	228.802	BDL
3	Chromium	267.716	0.023 mg/L (0.023026273 ppm)
4	Mercury	253.652	3.021 mg/L (3.024450897 ppm)
5	Nickel	231.604	BDL
6	Lead	220.353	BDL
7	Sulphur	180.731	71.524 mg/L (71.60570208ppm)
8	Vanadium	313.07	BDL

Table 6.1.11: Heavy Metals Concentration in *Linga Chendhuram*

S. No	Heavy metal	Concentration	Methods/Instrument used
1	Lead	0.3098 ppm	WHO, 1998 & AOAC, 2005/Thermo Fisher AAS
2	Cadmium	Not detected	

6.2. ACUTE TOXICITY STUDY OF *LINGA CHENDHURAM*

All animals were survived throughout the observation period of 14 days at the single dose of LC at 2000 mg/kg and they were in good health and active. All animals got comparable body weight gain over the period of 14 days. Gross necropsy study was not performed due to the absence of mortality and morbidity.

Table 6.2.1: Allocation of Animal Identification code for acute toxicity study

Step	Animal code
I	LC/F1
	LC/F2
	LC/F3
II	LC/F4
	LC/F5
	LC/F6

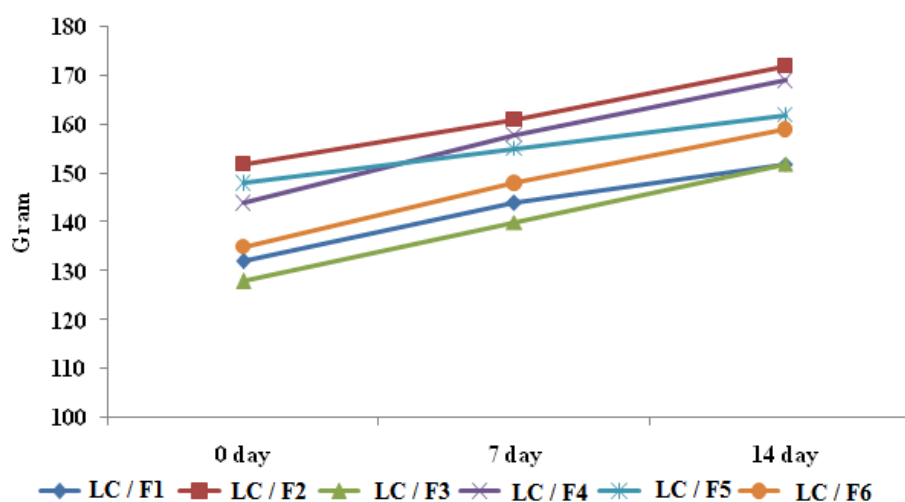


Figure 6.2.1: Effect of *Linga Chendhuram* on body weight of Wistar rats at single dose of 2000 mg/kg

Table 6.2.2: Cage side observation for the effect of *Linga Chendhuram* at 2000 mg/kg

Observation	LC/F1	LC/F2	LC/F3	LC/F4	LC/F5	LC/F6
General behaviour	N	N	N	N	N	N
Respiration	N	N	N	N	N	N
Cardiovascular signs	N	N	N	N	N	N
Motor activities	N	N	N	N	N	N
Skin	N	N	N	N	N	N
Fur	N	N	N	N	N	N
Eyes	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N
Salivation	N	N	N	N	N	N
Lethargy	Nil	Nil	Nil	Nil	Nil	Nil
Sleep	N	N	N	N	N	N
Coma	Nil	Nil	Nil	Nil	Nil	Nil
Convulsion	Nil	Nil	Nil	Nil	Nil	Nil
Tremors	Nil	Nil	Nil	Nil	Nil	Nil
Diarrhoea	Nil	Nil	Nil	Nil	Nil	Nil
Morbidity	Nil	Nil	Nil	Nil	Nil	Nil
Mortality	Nil	Nil	Nil	Nil	Nil	Nil

N – Normal

6.3. SUB-ACUTE ORAL TOXICITY STUDY

6.3.1. Randomization, Numbering and Grouping of Wistar Rats

The rats allotted to different groups are noted in the table 6.3.1.

Table 6.3.1: Allocation of Animal Identification code for 28 day Repeated oral toxicity study

Group	Sex	Animal code
I – Control	Male	LC/C/M/1 - LC/C/M/5
	Female	LC/C/F/6 - LC/C/F/10
II – Test group at low dose level	Male	LC/LD/M/1 - LC/LD/M/5
	Female	LC/LD/F/6 - LC/LD/F/10
III – Test group at intermittent dose level	Male	LC/MD/M/1 - LC/MD/M/5
	Female	LC/MD/F/6 - LC/MD/F/10
IV – Test group at high dose level	Male	LC/HD/M/1 - LC/HD/M/5
	Female	LC/HD/F/6 - LC/HD/F/10

6.3.2. Body weight

All animals involved in the study gained comparable body weight throughout the study period. But no significant change in the body weight (Figure 6.3.1 & 6.3.2) or lost in the treated test groups were observed compared with control group during the study.

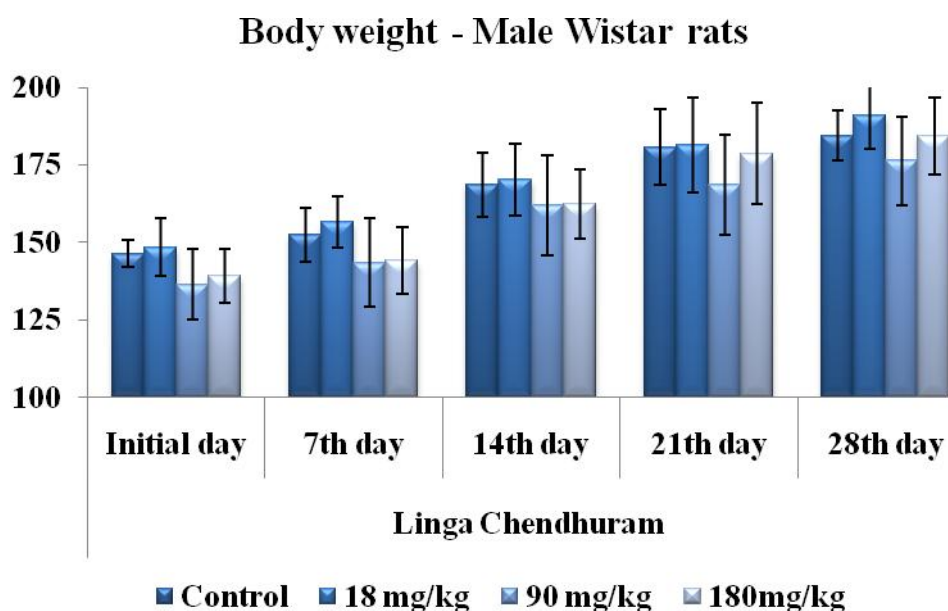


Figure 6.3.1: Effect of *Linga chendhuram* on body weight in male wistar albino rats - 28 day repeated oral toxicity study

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test.

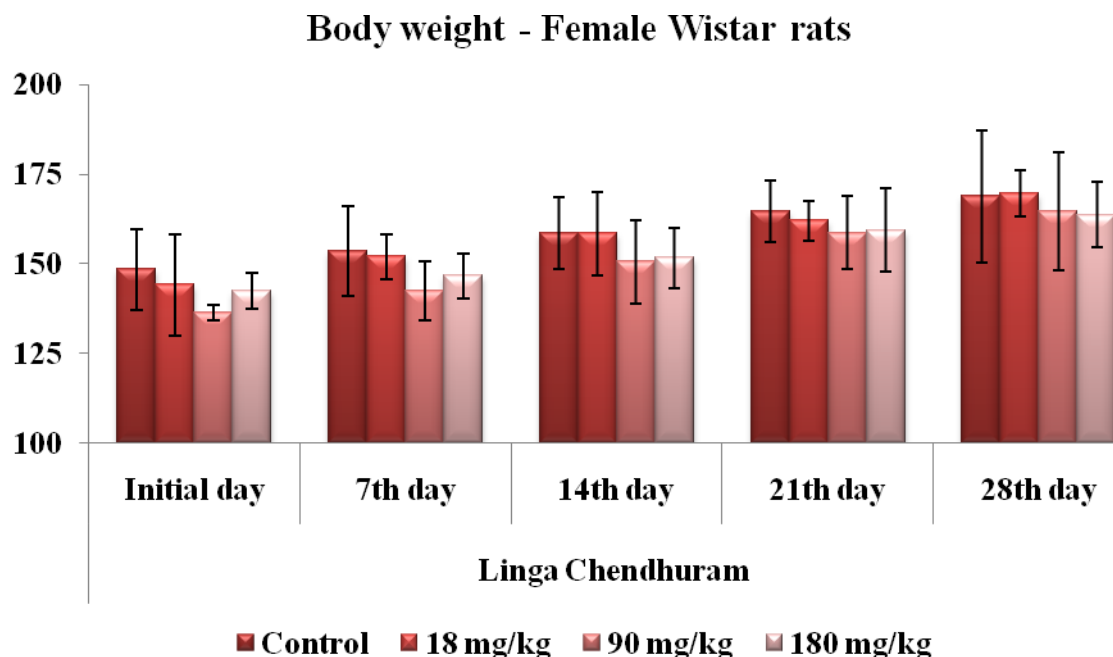


Figure 6.3.2: Effect of *Linga chendhuras* on body weight in female wistar albino rats - 28 day repeated oral toxicity study

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test

6.3.3. Food consumption

The amount of pellets consumed by Wistar rats from different test dose groups during the period of 28 days was found to be comparable with that by control group. Data were not shown here since no significant difference was observed.

6.3.4. Clinical observations

All male and female rats of Group I – IV were free from abnormal clinical signs throughout the period of test drug administration period of 28 days.

6.3.5. Mortality and morbidity

All male and female rats of Group I – IV were survived and had good health throughout the period of test drug administration period of 28 days.

6.3.6. Urine analysis

Dark coloured urine was observed in four rats of control group and two rats of high dose group. The specific gravity more than 1.02 was observed in three urine samples of control and two of high dose. pH was found higher than 7.4 in five urine samples of control and three of high dose. Trace amount of protein was observed in four urine samples of control and four of high dose. The glucose, bilirubin, ketones, blood and urobilinogen were found nil in the urine samples of three groups. The data of urinalysis of control and high dose were not shown since any specific abnormalities not observed.

6.3.7. Haematology

The results of analyzes of haematological parameters were shown in the figures 6.3.5 to 6.3.15. The results obtained reflected some significant changes in the values of various parameters assayed when compared with those of corresponding controls. Nevertheless, the decrease or increase in the values obtained was within normal physiological limits and the effect was not observed to be dose dependent.

Male Wistar rats

- Decreased values of MCH were obtained for rats in the dose groups administered 18 mg/kg b.wt ($p < 0.01$), 90 mg/kg b.wt ($p < 0.05$) and 180 mg/kg b.wt ($p < 0.01$) of LC sacrificed on day 29 (Figure 6.3.10)
- Decreased values of MCHC were obtained for rats in the dose groups administered 18 mg/kg b.wt ($p < 0.05$), 90 mg/kg b.wt ($p < 0.05$) and 180 mg/kg b.wt ($p < 0.01$) of LC sacrificed on day 29 (Figure 6.3.13)

Female Wistar rats

- Decreased values of Monocyte were obtained for rats in the dose group administered 90 mg/kg ($p < 0.05$) of LC sacrificed on day 29 (Figure 6.3.4)
- Increased values of Hemoglobin were obtained for rats in the dose group administered 18 mg/kg b.wt ($p < 0.01$) of LC sacrificed on day 29 (Figure 6.3.5)
- Increased values of Total RBC were obtained for rats in the dose groups administered 18 mg/kg b.wt ($p < 0.01$), 90 mg/kg b.wt ($p < 0.05$) and 180 mg/kg b.wt ($p < 0.05$) of LC sacrificed on day 29 (Figure 6.3.6)

- Increased values of RDW were obtained for rats in the dose groups administered 18 mg/kg b.wt ($p<0.05$) and 180 mg/kg b.wt ($p<0.05$) of LC sacrificed on day 29 (Figure 6.3.7)
- Increased values of Hematocrit were obtained for rats in the dose group administered 18 mg/kg b.wt ($p<0.01$) of LC sacrificed on day 29 (Figure 6.3.8)
- Decreased values of MCH were obtained for rats in the dose group administered 180 mg/kg b.wt ($p<0.01$) of LC sacrificed on day 29 (Figure 6.3.10)
- Increased values of Platelet were obtained for rats in the dose groups administered 18 mg/kg b.wt ($p<0.01$) and 90 mg/kg b.wt ($p<0.01$) of LC sacrificed on day 29 (Figure 6.3.12)
- Increased values of Platelet crit were obtained for rats in the dose groups administered 18 mg/kg b.wt ($p<0.01$) and 90 mg/kg b.wt ($p<0.01$) of LC sacrificed on day 29 (Figure 6.3.13)

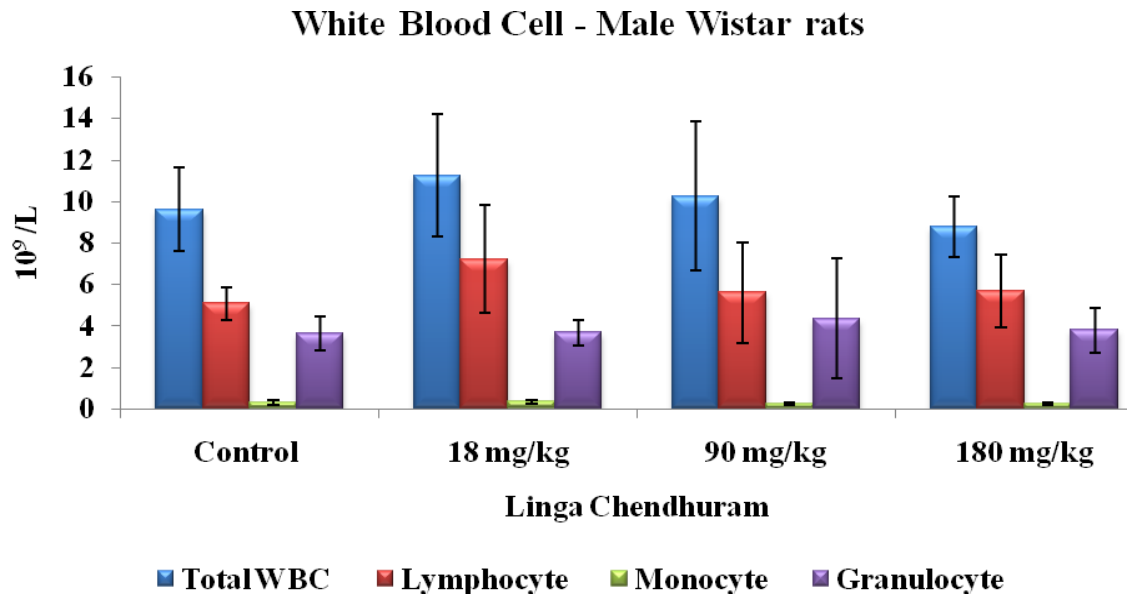


Figure 6.3.3: Effect of *Linga Chendhuram* on White blood Corpuscles in male Albino Wistar rats - 28 day repeated oral toxicity study.

WBC: White blood count

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test.

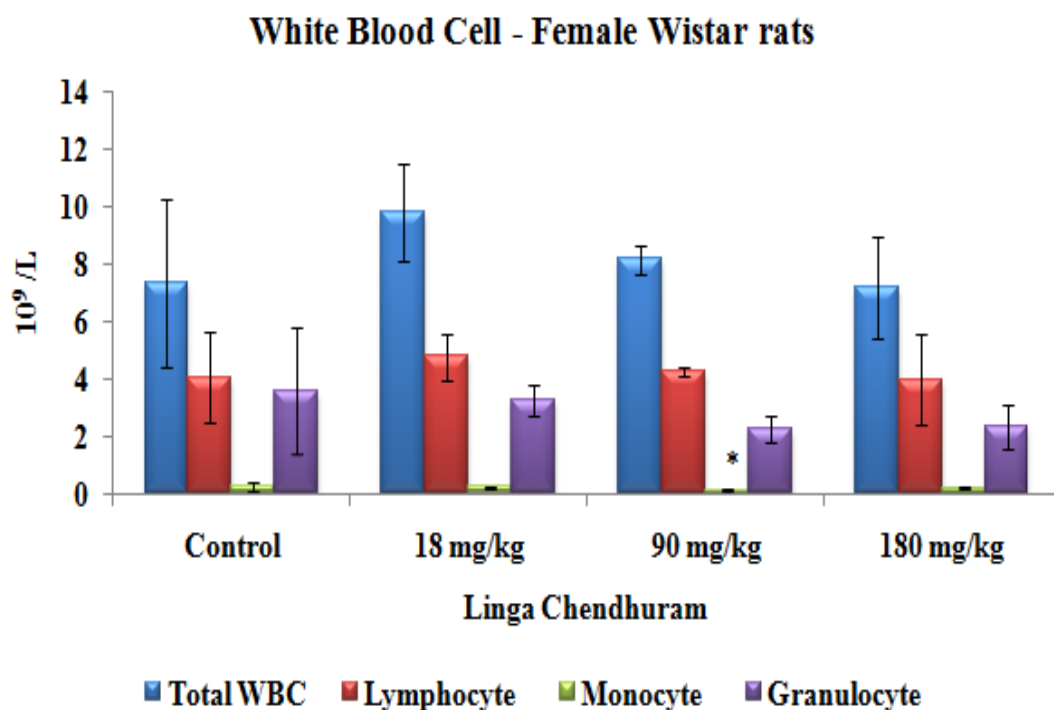


Figure 6.3.4: Effect of *Linga Chendhuram* on White blood Corpuscles in female Albino Wistar rats - 28 day repeated oral toxicity study.

WBC: White blood count

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as *p < 0.05 on compared with control group.

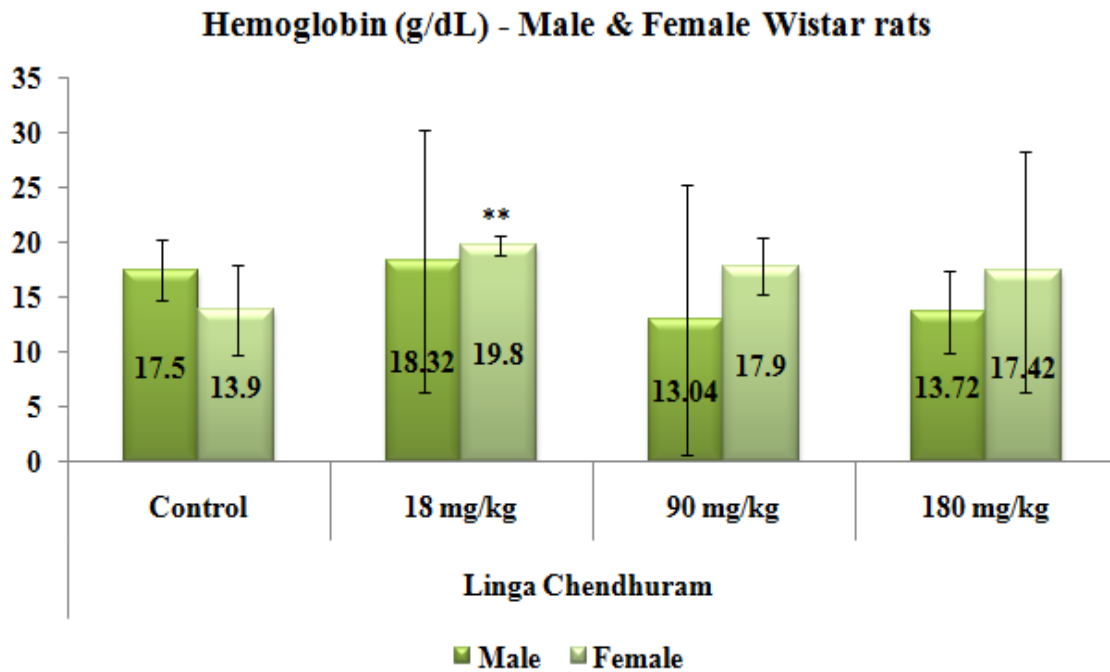


Figure 6.3.5: Effect of *Linga Chendhuram* on Haemoglobin in male and female Albino Wistar rats - 28 day repeated oral toxicity study

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as **p < 0.01 on compared with control group.

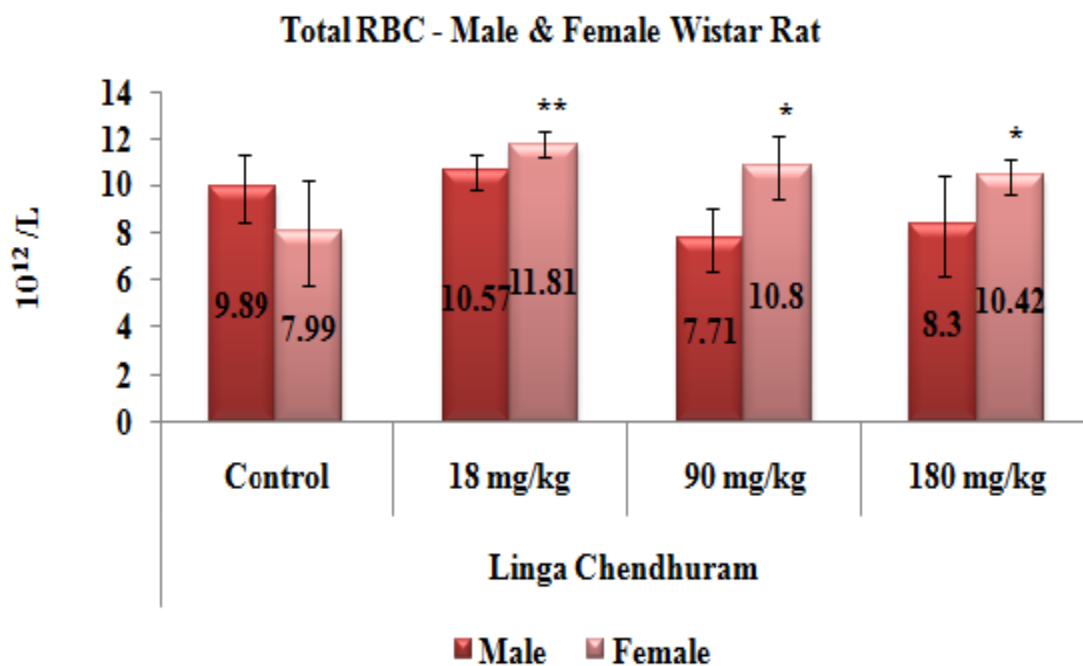


Figure 6.3.6: Effect of *Linga Chendhuram* on Red blood Corpuscles in male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as *p < 0.05 and **p < 0.01 on compared with control group.

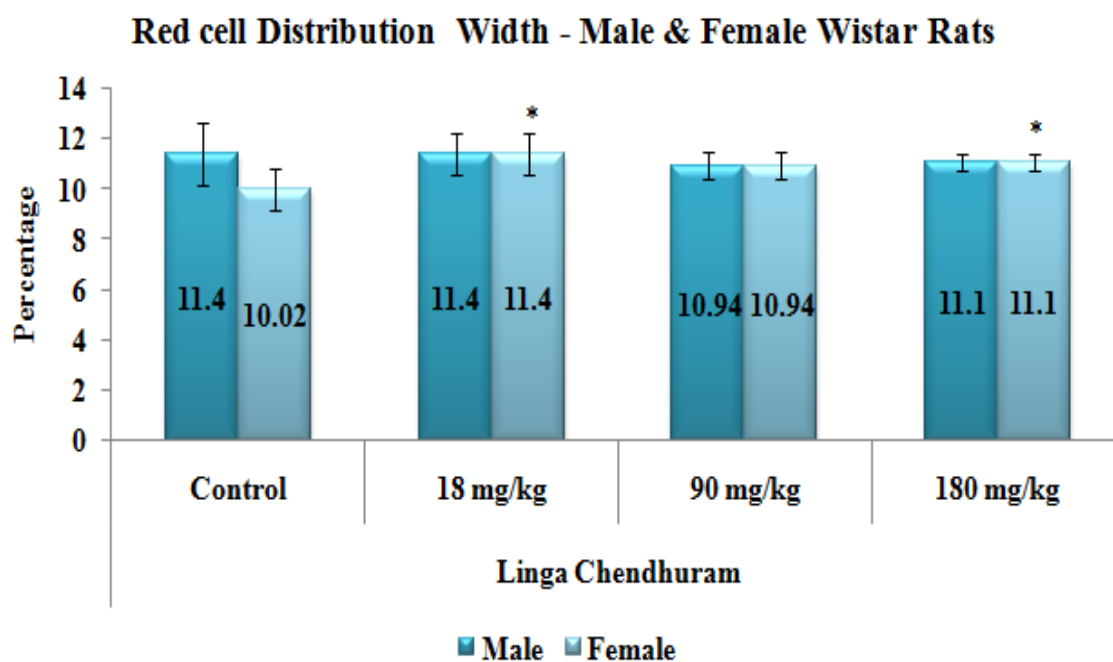


Figure 6.3.7: Effect of *Linga Chendhuram* on Red cell distribution width in male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as *p < 0.05 on compared with control group.

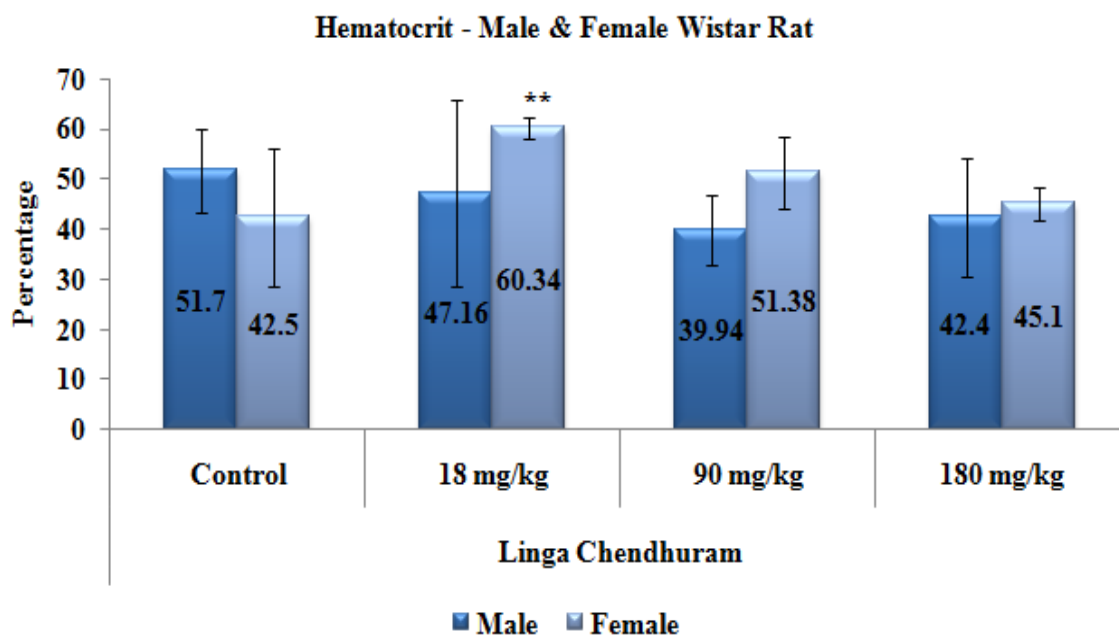


Figure 6.3.8: Effect of *Linga Chendhuram* on Hematocrit value in male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as **p < 0.01 on compared with control group.

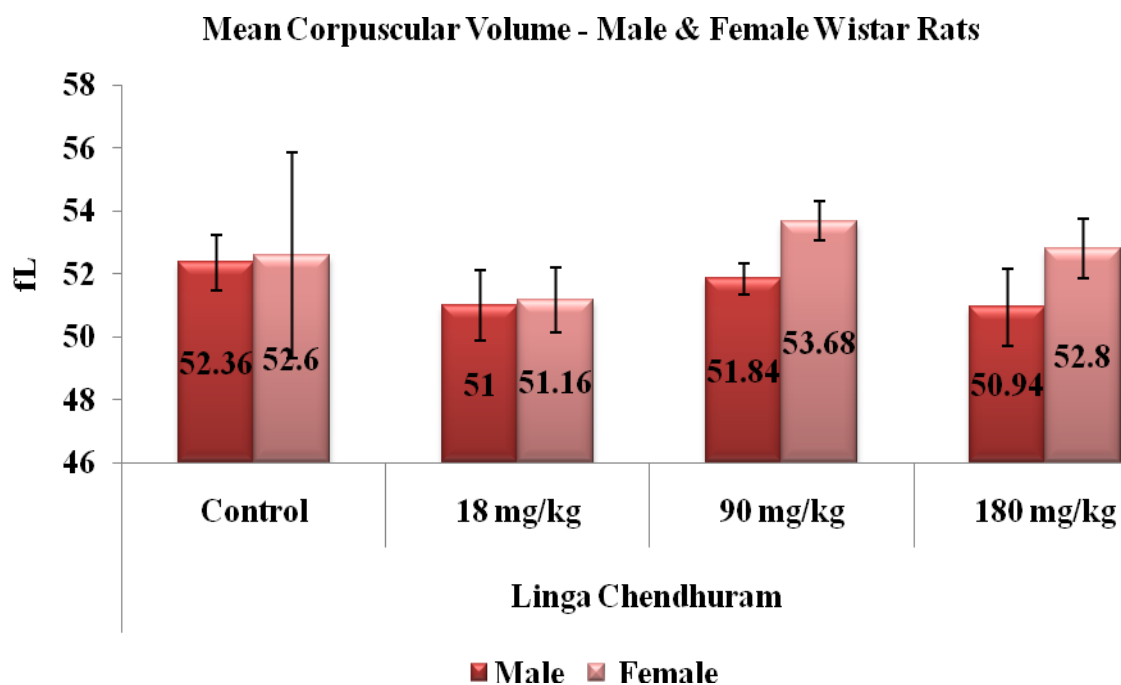


Figure 6.3.9: Effect of *Linga Chendhuram* on mean corpuscular volume in male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test.

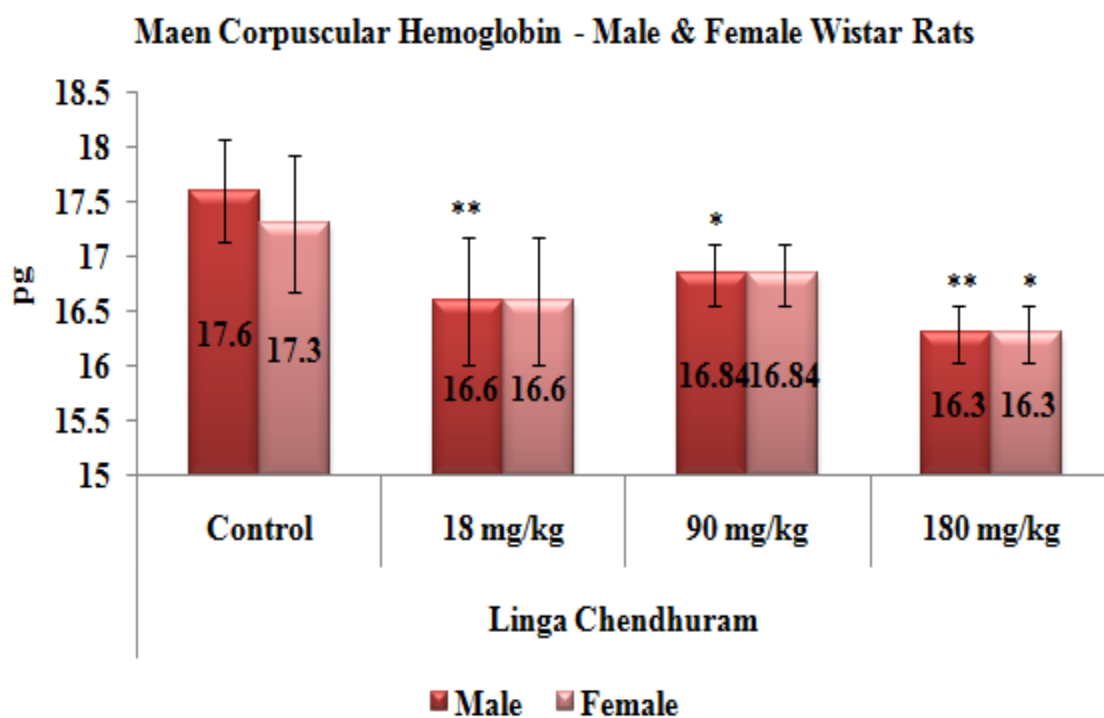


Figure 6.3.10: Effect of *Linga Chendhuram* on mean corpuscular hemoglobin in male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as *p < 0.05 and **p < 0.01 on compared with control group.

**Mean Corpuscular Hemoglobin Concentration - Male & Female
Wistar Rats**

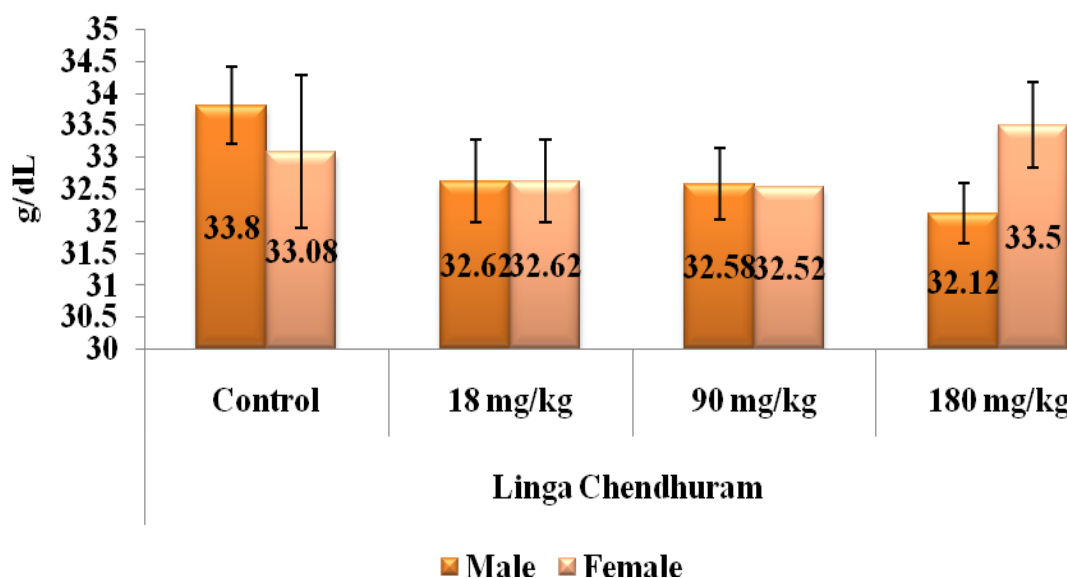


Figure 6.3.11: Effect of *Linga Chendhuram* on mean corpuscular haemoglobin concentration in male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as *p < 0.05 and **p < 0.01 on compared with control group.

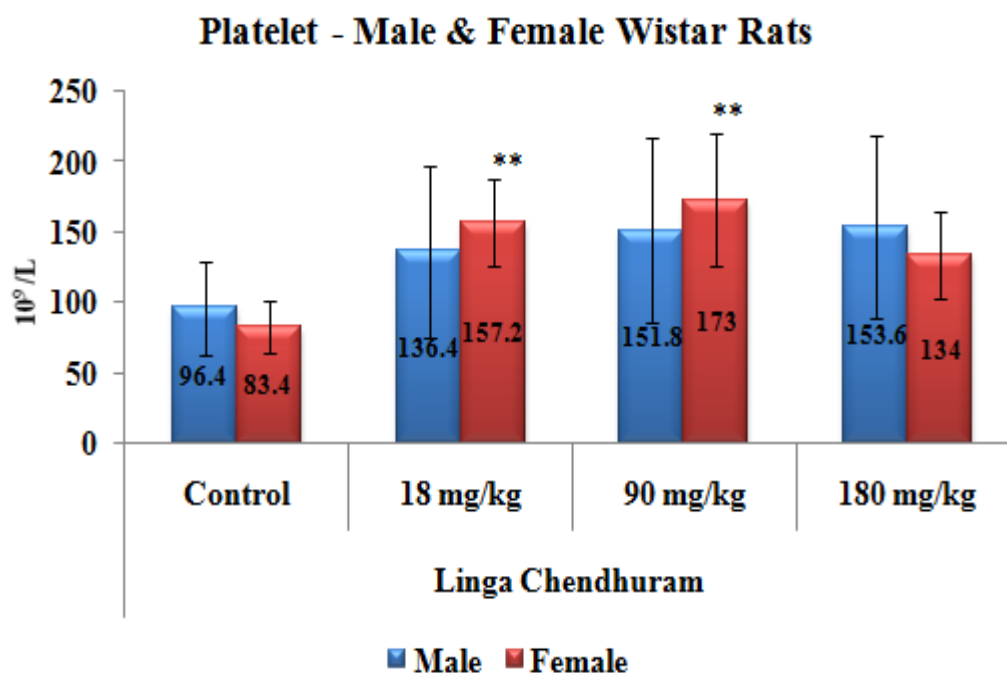


Figure 6.3.12: Effect of *Linga Chendhuram* on platelet count in male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as **p < 0.01 on compared with control group.

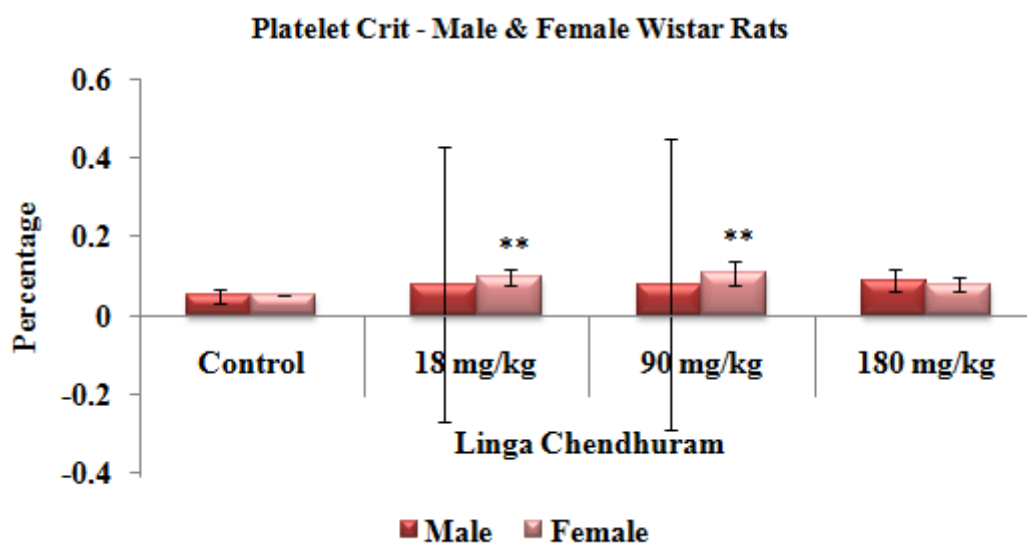


Figure 6.3.13: Effect of *Linga Chendhuram* on Platelet Crit in male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as **p < 0.01 on compared with control group.

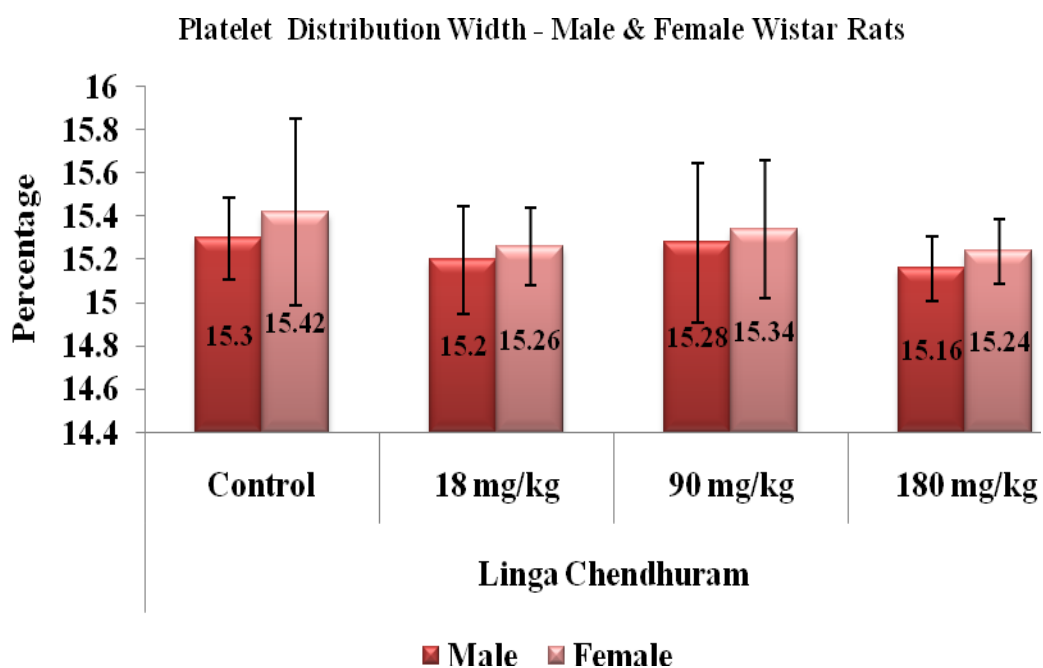


Figure 6.3.14: Effect of *Linga Chendhuram* on platelet distribution width in male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test.

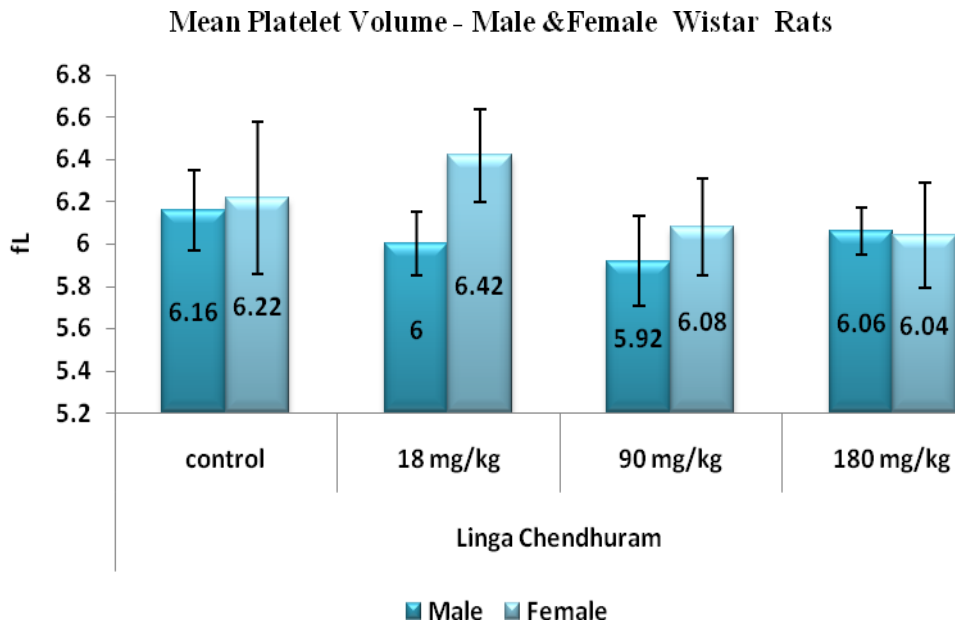


Figure 6.3.15: Effect of *Linga Chendhuram* on mean platelet volume in male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. No significant difference was observed among the groups.

6.3.7. Serum Biochemistry

The results of analyzes of biochemical parameters were shown in the figures 6.3.16 to 26. The results obtained reflected some significant changes in the values of various parameters assayed when compared with those of corresponding controls. Nevertheless, the decrease or increase in the values obtained was within normal physiological limits and the effect was not observed to be dose dependent.

Male Wistar rats

- Increased values of Triglyceride were obtained for rats in the dose groups administered 18 mg/kg b.wt ($p < 0.01$) and 180 mg/kg b.wt ($p < 0.05$) of LC sacrificed on day 29 (Figure 6.3.18)
- Decreased values of Urea were obtained for rats in the dose group administered 18 mg/kg ($p < 0.05$) of LC sacrificed on day 29 (Figure 6.3.19)
- Decreased values of Creatinine were obtained for rats in the dose groups administered 18 mg/kg ($p < 0.05$) and 90 mg/kg b.wt ($p < 0.05$) of LC sacrificed on day 29 (Figure 6.3.20)
- Increased values of Bilirubin were obtained for rats in the dose group administered 18 mg/kg b.wt ($p < 0.05$) of LC sacrificed on day 29 (Figure 6.3.21)
- Decreased values of SGPT were obtained for rats in the dose group administered 180 mg/kg ($p < 0.05$) of LC sacrificed on day 29 (Figure 6.3.22)
- Decreased values of ALP were obtained for rats in the dose groups administered 18 mg/kg ($P < 0.05$) and 90 mg/kg ($P < 0.01$) of LC sacrificed on day 29 (Figure 6.3.23)

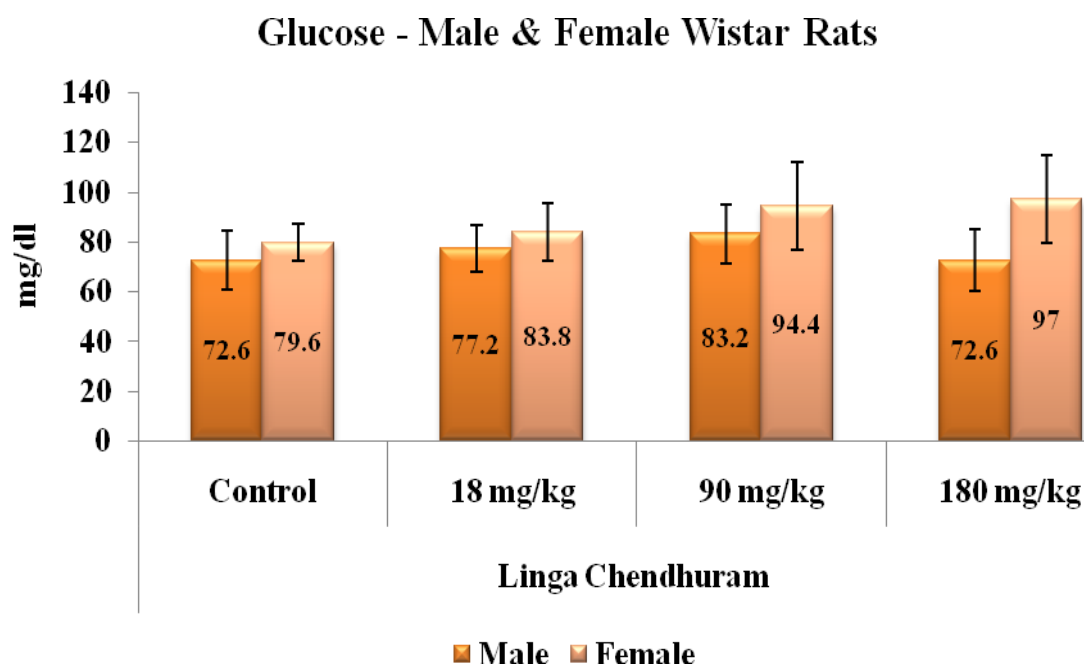


Figure 6.3.16: Effect of *Linga Chendhuram* on Glucose value in male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test.

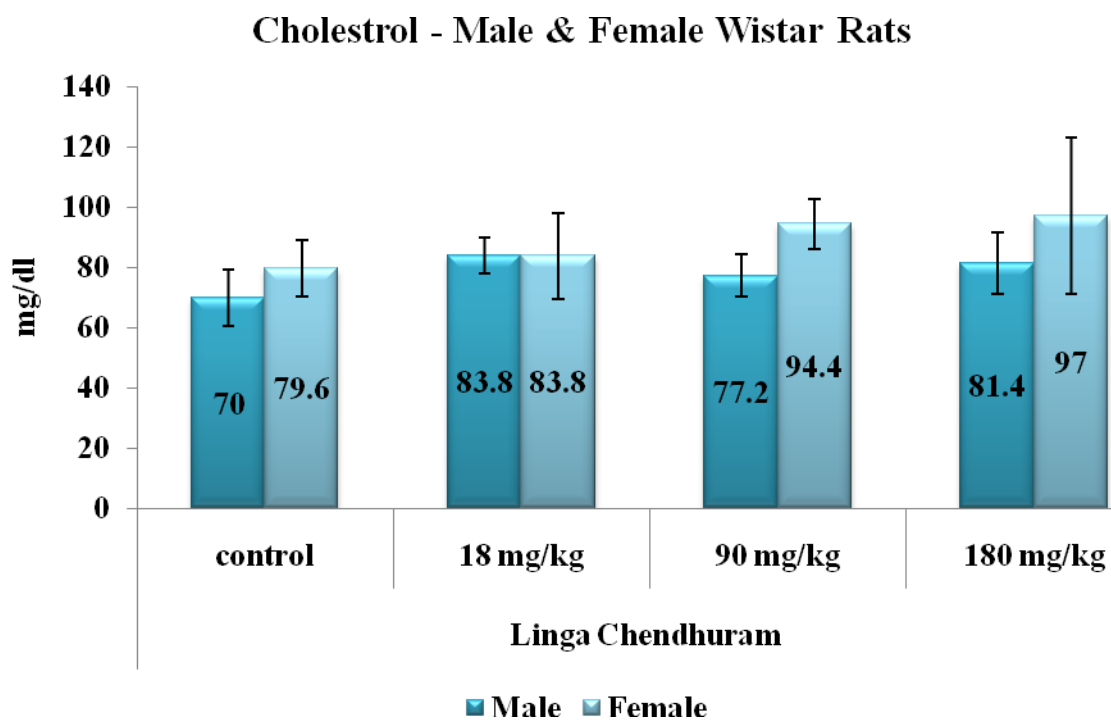


Figure 6.3.17: Effect of *Linga Chendhuram* on Cholesterol value male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test.

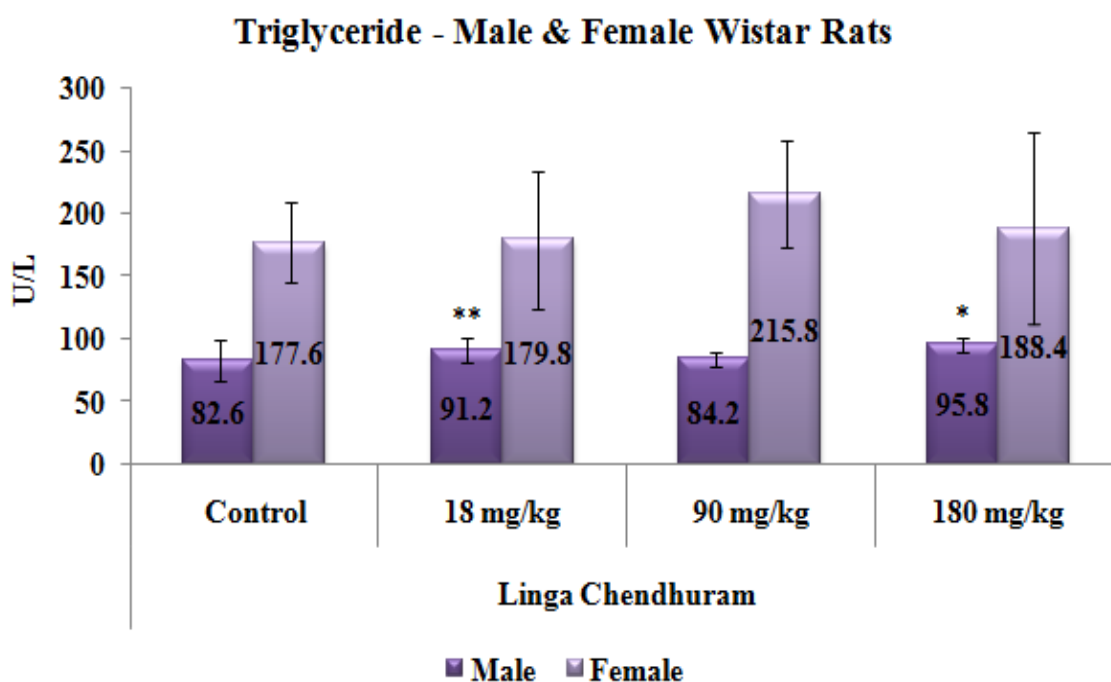


Figure 6.3.18: Effect of *Linga Chendhuram* on Triglyceride value male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as *p < 0.05 and **p < 0.01 on compared with control group.

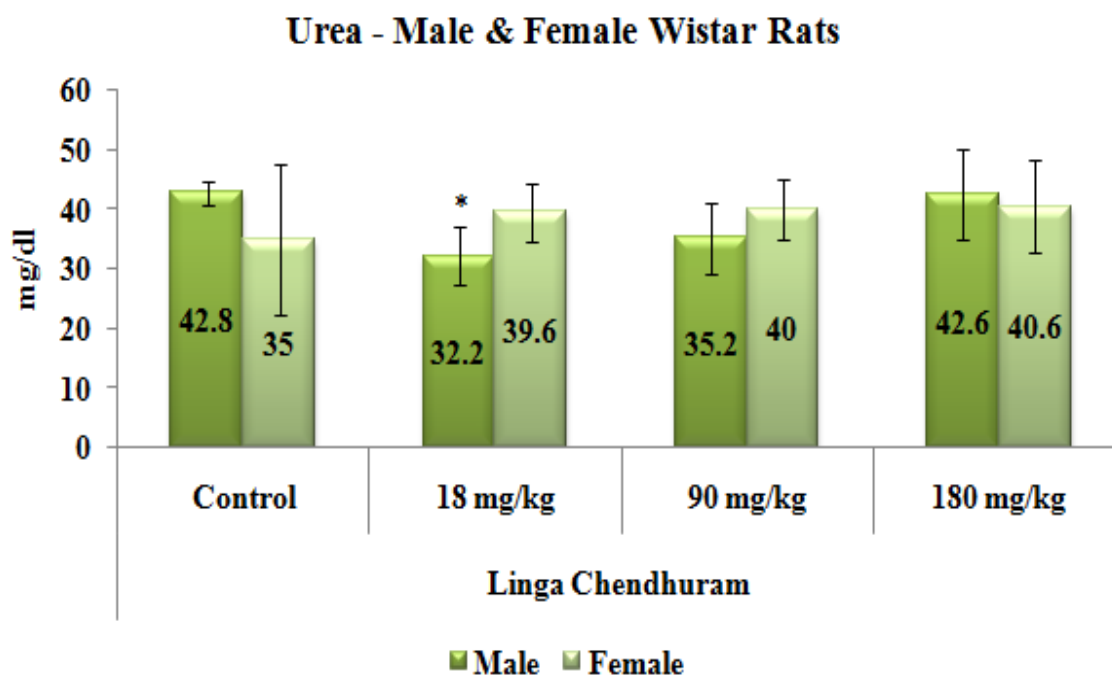


Figure 6.3.19: Effect of *Linga Chendhuram* on Urea value male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as *p < 0.05 on compared with control group.

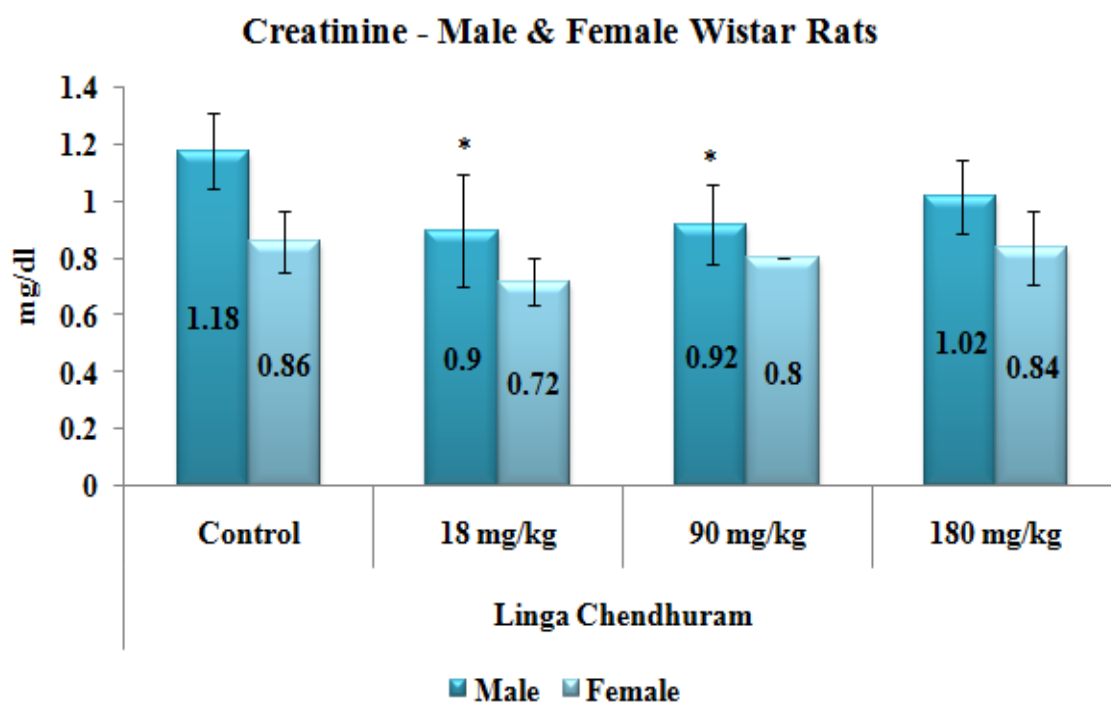


Figure 6.3.20: Effect of *Linga Chendhuram* on Creatinine value male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as *p < 0.05 on compared with control group.

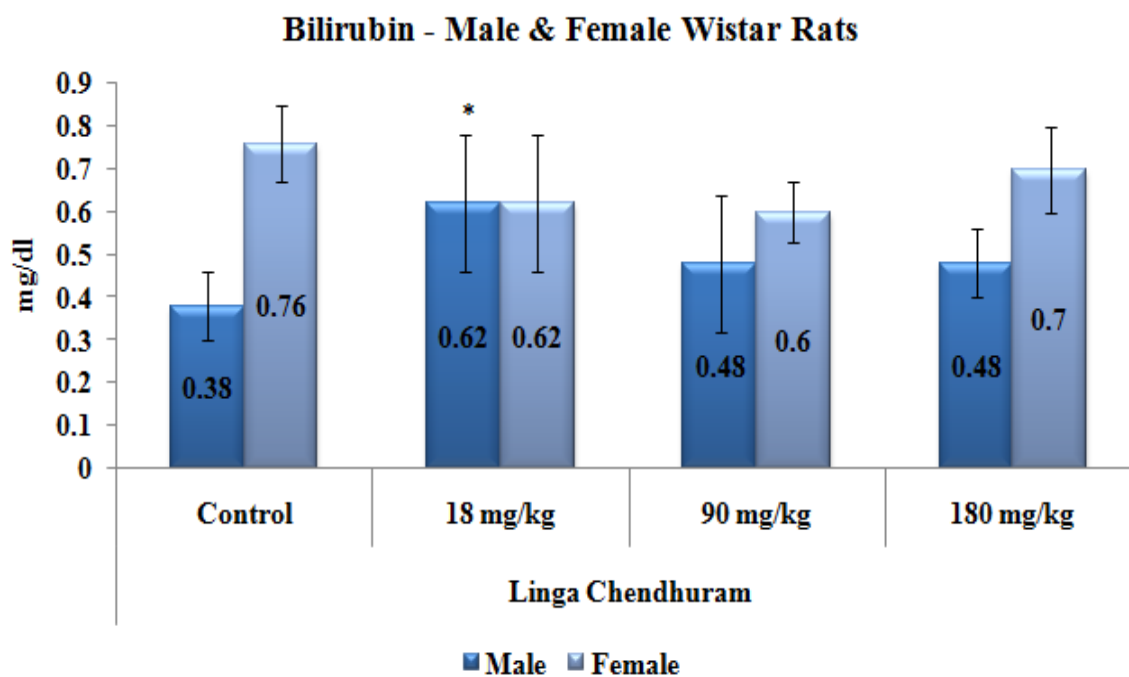


Figure 6.3.21: Effect of *Linga Chendhuram* on Bilirubin value male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as *p < 0.05 on compared with control group.

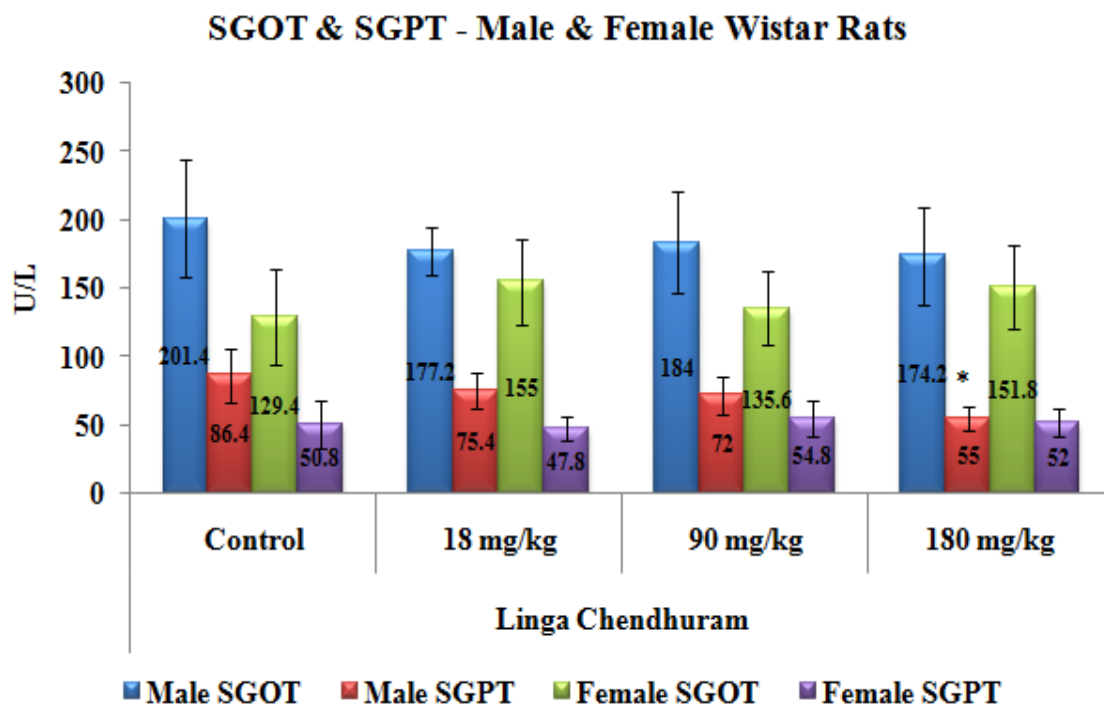


Figure 6.3.22: Effect of *Linga Chendhuram* on SGOT & SGPT value male and female Albino Wistar rats - 28 day repeated oral toxicity study.

SGOT- Serum Glutamic Oxaloacetic Transaminase

SGPT- Serum Glutamic Pyruvic transaminase

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as *p < 0.05 on compared with control group.

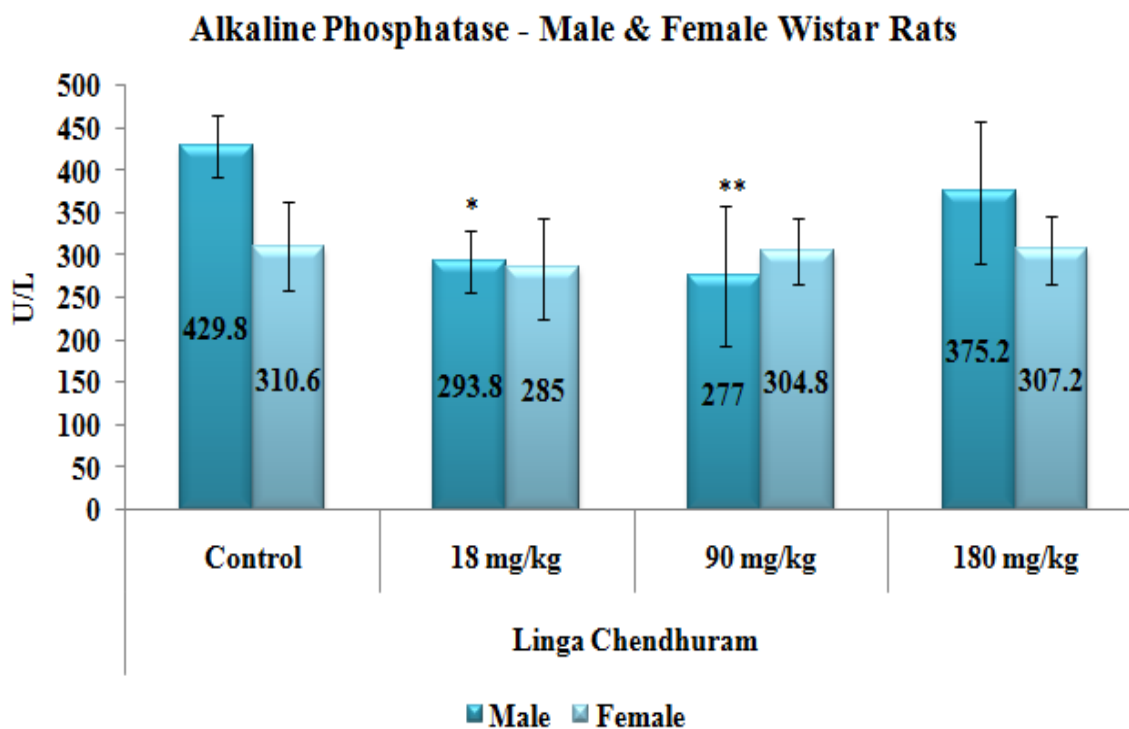


Figure 6.3.23: Effect of *Linga Chendhuram* on Alkaline phosphatase value male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test. Significance was indicated as *p < 0.05 and **p < 0.01 on compared with control group.

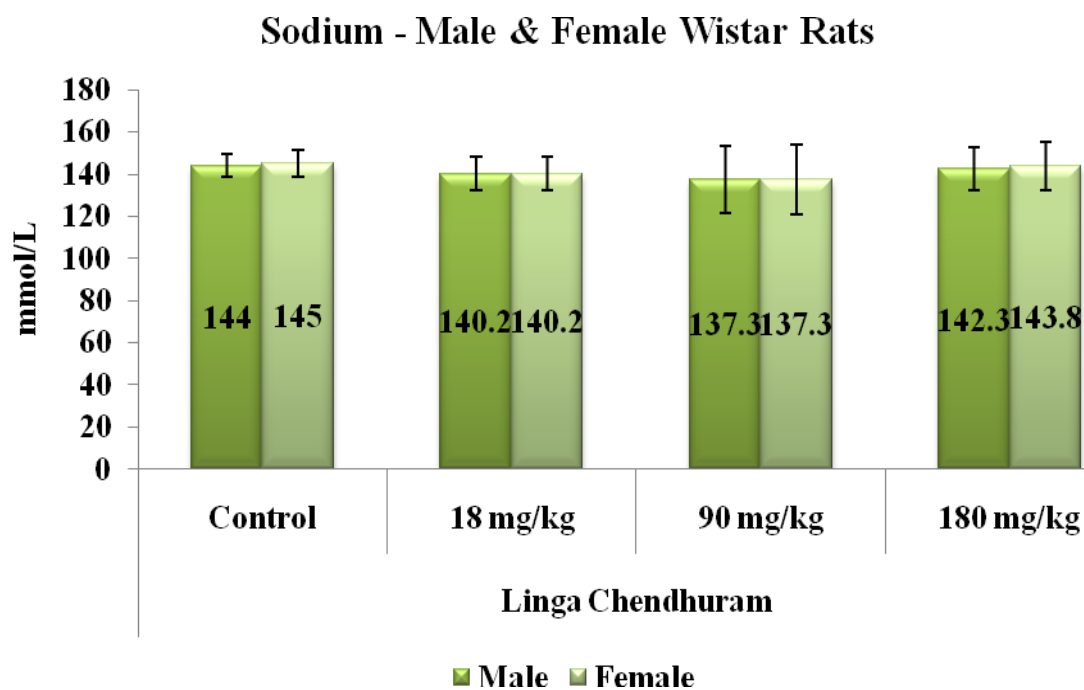


Figure 6.3.24: Effect of *Linga Chendhuram* on Sodium value male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test.

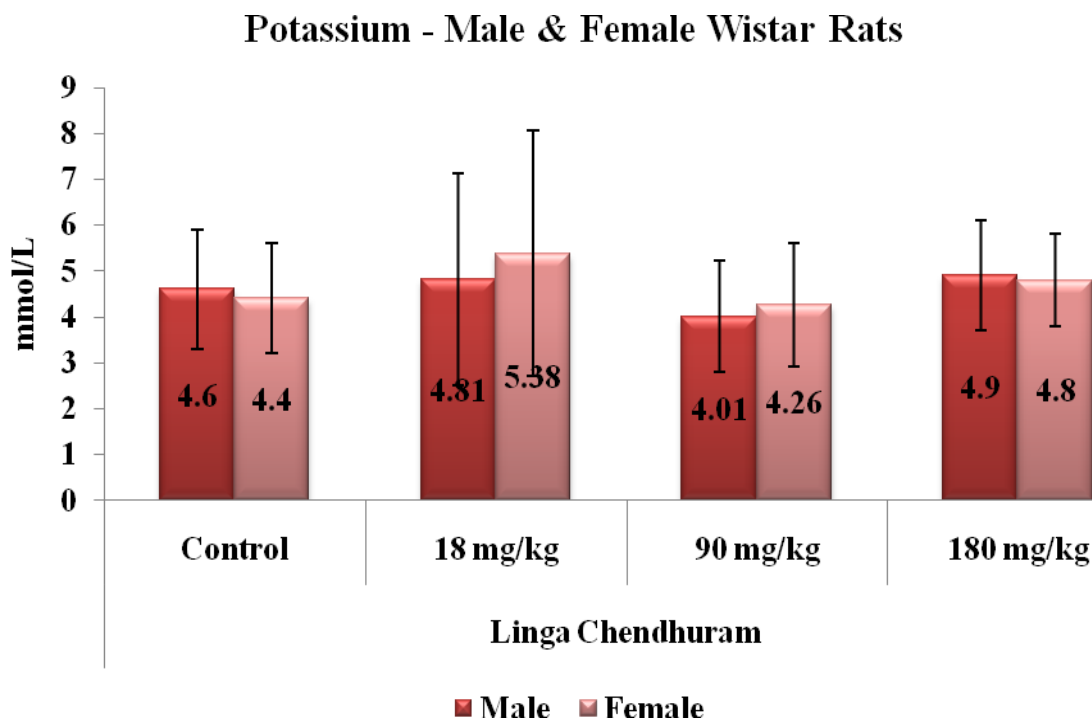


Figure 6.3.25: Effect of *Linga Chendhuram* on Potassium value male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test.

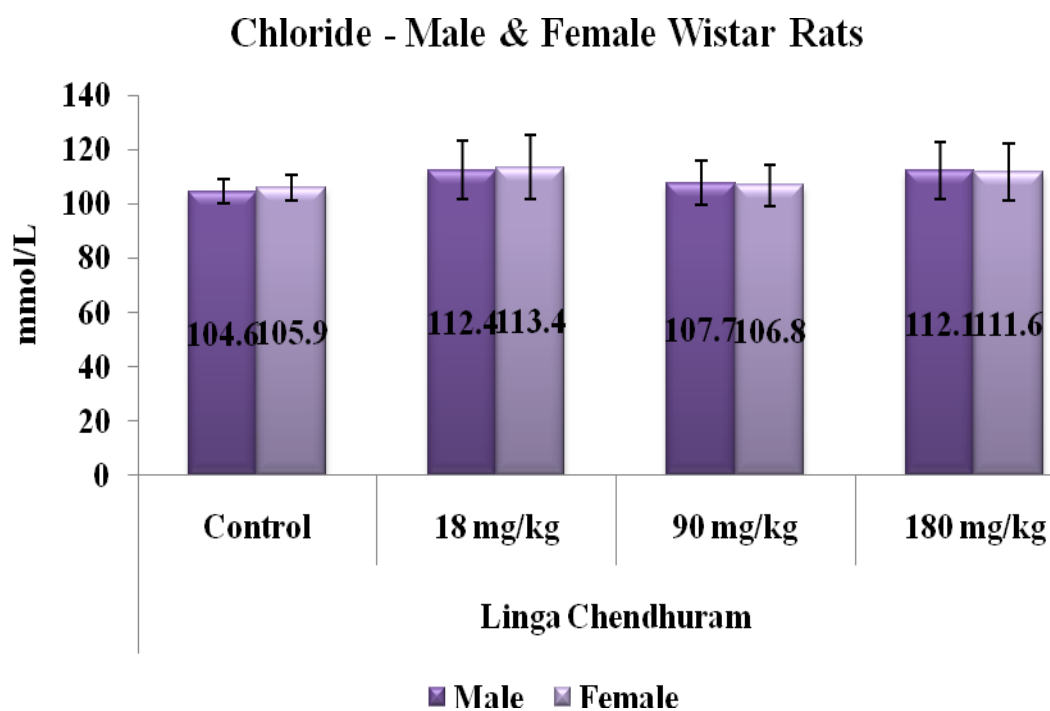


Figure 6.3.26: Effect of *Linga Chendhuram* on Chloride value male and female Albino Wistar rats - 28 day repeated oral toxicity study.

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test.

6.3.8. Necropsy study

Gross pathological examination of organs such as brain, trachea, lungs, heart, liver, kidney, stomach, spleen, intestine, testis, uterus and ovaries of Wistar rats in all groups did not reveal any abnormalities. Particularly, the gross necropsy study on the organs of control and high dose revealed no abnormal pathological morphology.

6.3.9. Organ weight

The results of absolute and relative organ weight gained of Wistar rats in all groups were shown in the figure 6.3.9. The organ weights of Wistar rats treated with LC at different dosages and were found to be comparable with control rats.

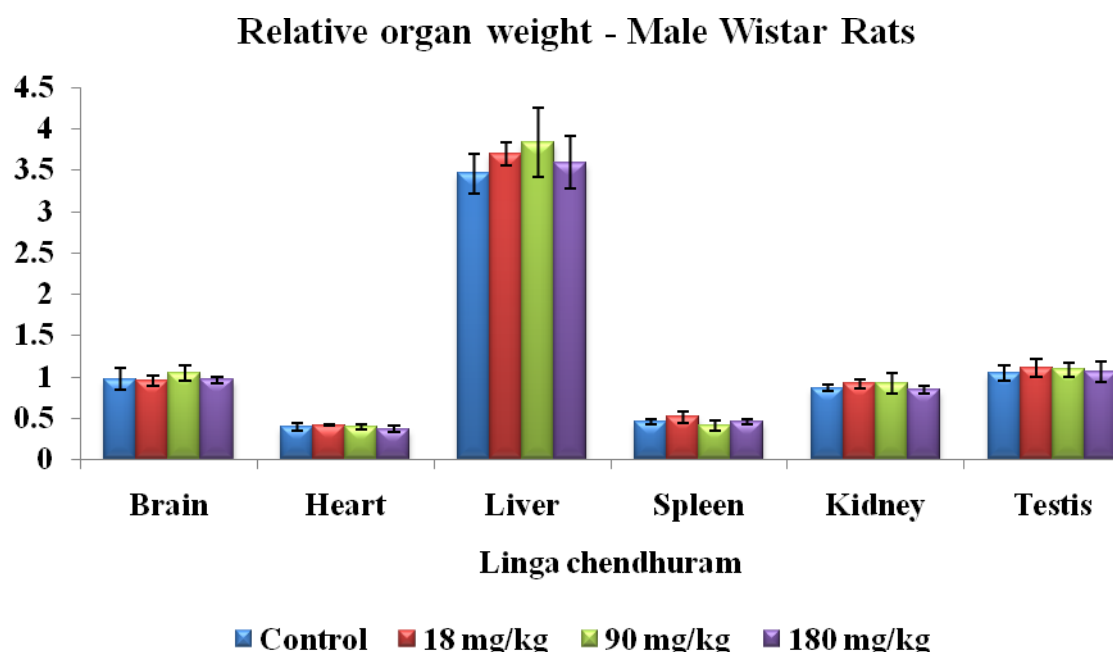


Figure 6.3.27: Effect of *Linga chendhuram* on relative organ weight in male Wistar albino rats - 28 day repeated oral toxicity study

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test

Relative organ weight - Female Wistar Rats

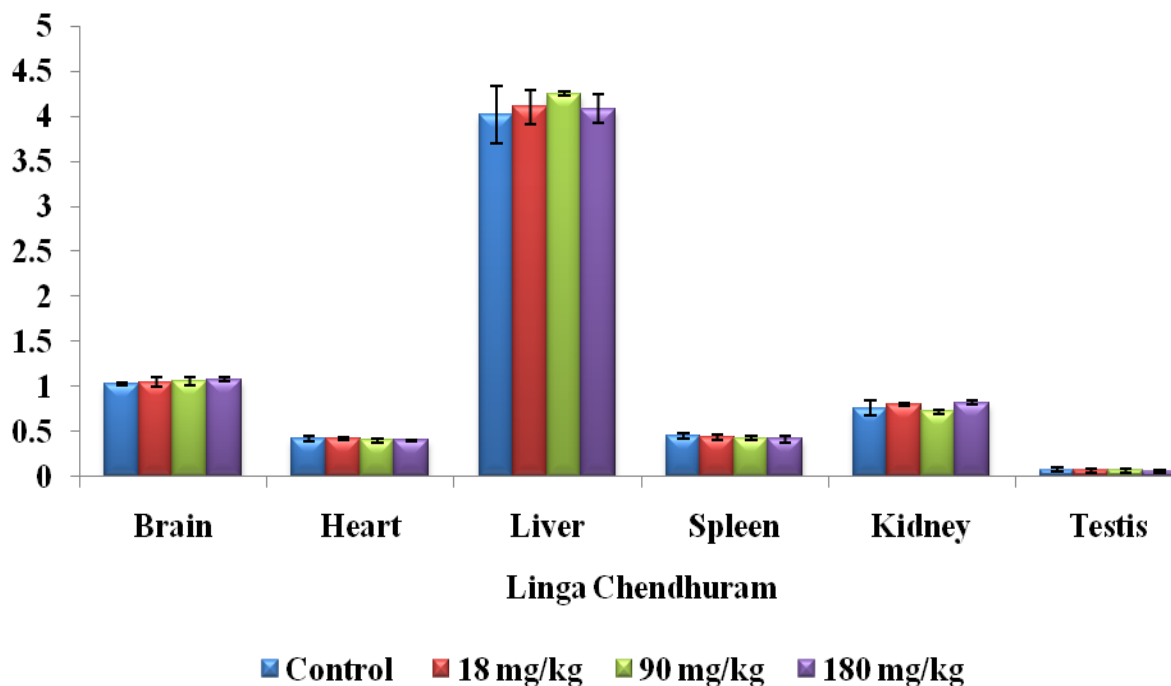


Figure 6.3.28: Effect of *Linga chendhuram* on relative organ weight in female Wistar albino rats - 28 day repeated oral toxicity study

Values were expressed as mean \pm S.D (n = 5). P value was calculated using one way ANOVA followed by Dunnett test

6.3.10. Histopathology

The results of histopathological changes observed during examinations were depicted in the table. The lesions observed were occurred spontaneously, and considered incidental and physiology related since abnormal changes were not established in blood parameters. H&E slice of organs were shown in the plates

Table 6.3.2: Effect of *Linga chendhuram* on histopathological changes in organs of Wistar rats - 28 day repeated oral toxicity study

Organ	Control	<i>Linga chendhuram</i> 180 mg/kg
Liver	NAD	NAD
Kidneys	NAD	Minimal mononuclear cell infiltration
Lungs	NAD	NAD
Stomach	NAD	NAD
Heart	NAD	NAD
Spleen	NAD	NAD
Brain	NAD	NAD
Femoro-tibial joint	NAD	NAD

NAD – No Abnormality Detected

Histopathology slide

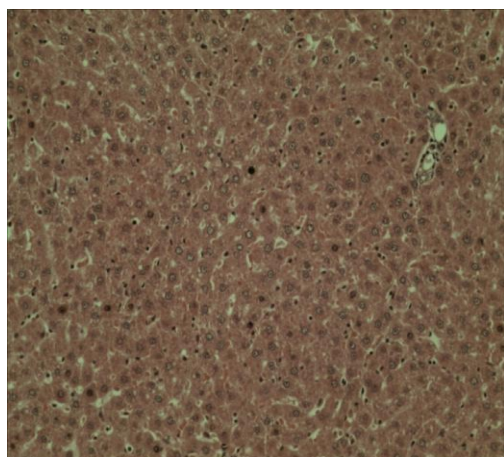


Plate 6.3.2

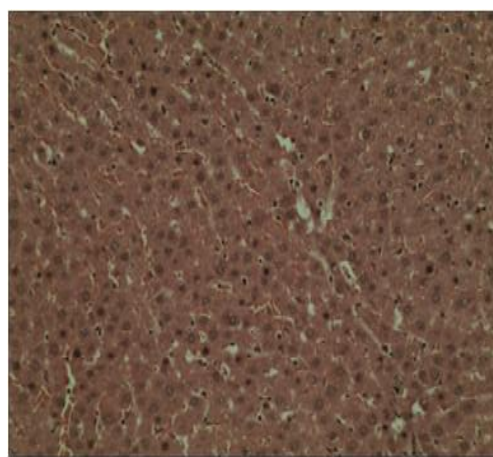


Plate 6.3.2A

Plate 6.3.2: H&E sliced Liver of test group of male rat treated with LC at 180 mg/kg showing normal architecture (10X)

Plate 6.3.2A: H&E sliced Liver of control group showing normal architecture (10X)

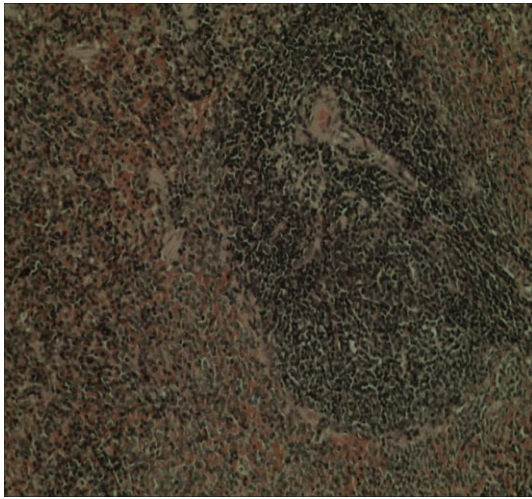


Plate 6.3.3

Plate 6.3.3: H&E sliced Spleen of test group of male rat treated with LC at 180 mg/kg showing normal architecture (10X)

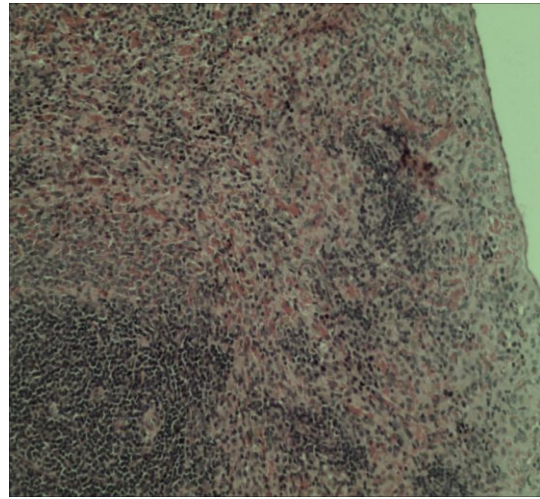


Plate 6.3.3 A

Plate 6.3.3A: H&E sliced Spleen of control group showing normal architecture (10X)

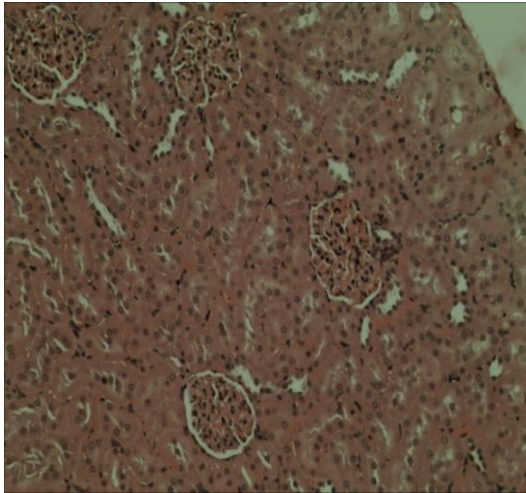


Plate 6.3.4

Plate 6.3.4: H&E sliced kidney of test group of male rat treated with LC at 180 mg/kg showing normal architecture (10X)

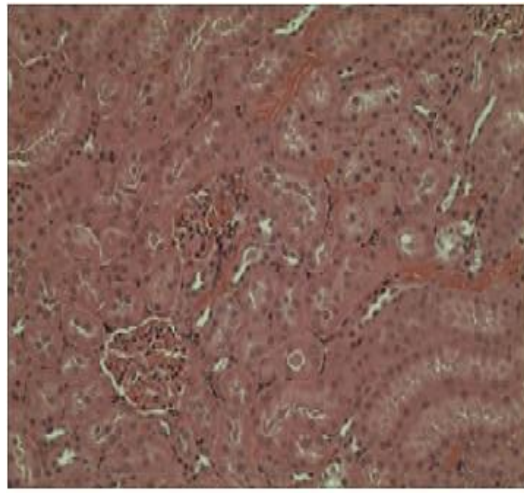


Plate 6.3.4A

Plate 6.3.4A- H&E sliced kidney of control group showing normal architecture (10X)

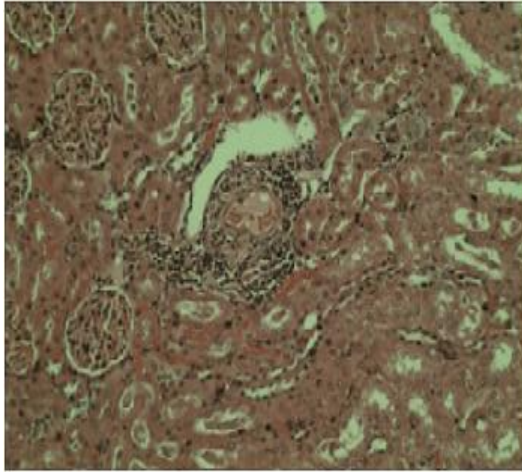


Plate 6.3.5

Plate 6.3.5: H&E sliced kidney of test group of female rat treated with LC at 180 mg/kg showing infiltration of mononuclear cells (10X)

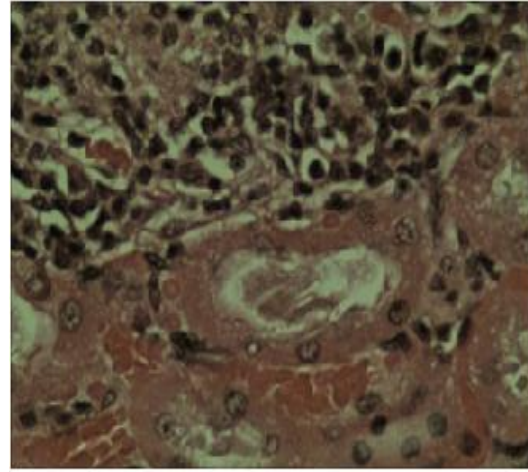


Plate 6.3.5A

Plate 6.3.5A: H&E sliced kidney of test group of female rat treated with LC at 180 mg/kg showing infiltration of mononuclear cells (40X)

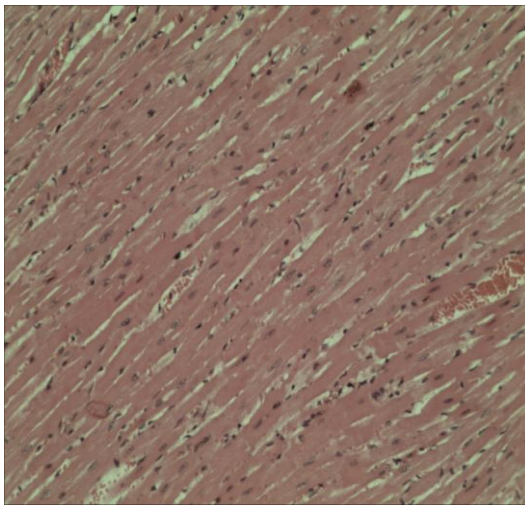


Plate 6.3.6

Plate 6.3.6: H&E sliced Heart of test group of female rat treated with LC at 180 mg/kg showing normal architecture (10X)

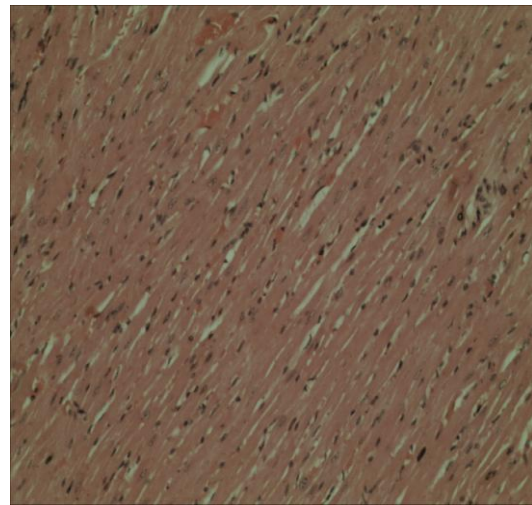


Plate 6.3.6A

Plate 6.3.6A: H&E sliced Heart of control group showing normal architecture (10X)

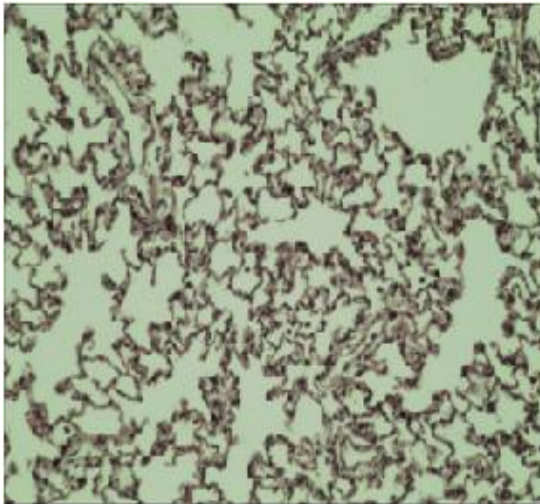


Plate 6.3.7

Plate 6.3.7: H&E sliced Lung of test group of female rat treated with LC at 180 mg/kg showing normal architecture (10X)

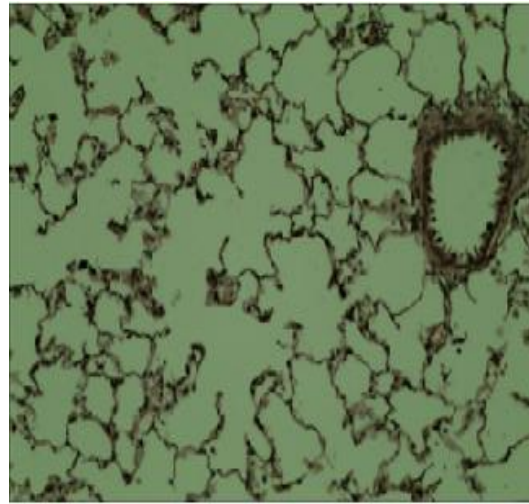


Plate 6.3.7A

Plate 6.3.7A: H&E sliced Lung of control group showing normal architecture (10X)

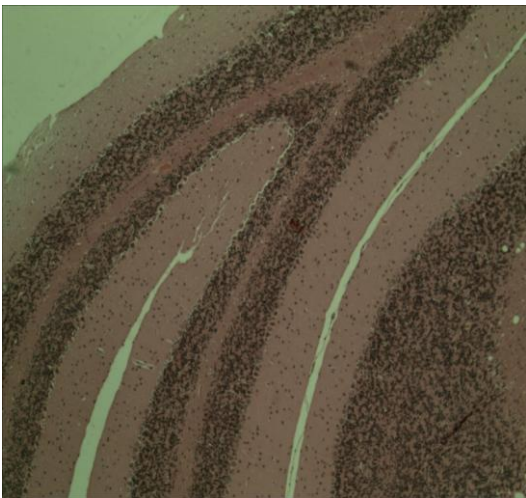


Plate 6.3.8

Plate 6.3.8: H&E sliced Cerebellum of test group of female rat treated with LC at 180 mg/kg showing normal architecture (10X)



Plate 6.3.8A

Plate 6.3.8A: H&E sliced Cerebellum of control group showing normal architecture (10X)

6.4. Analgesic activity

6.4.1. Hot plate method

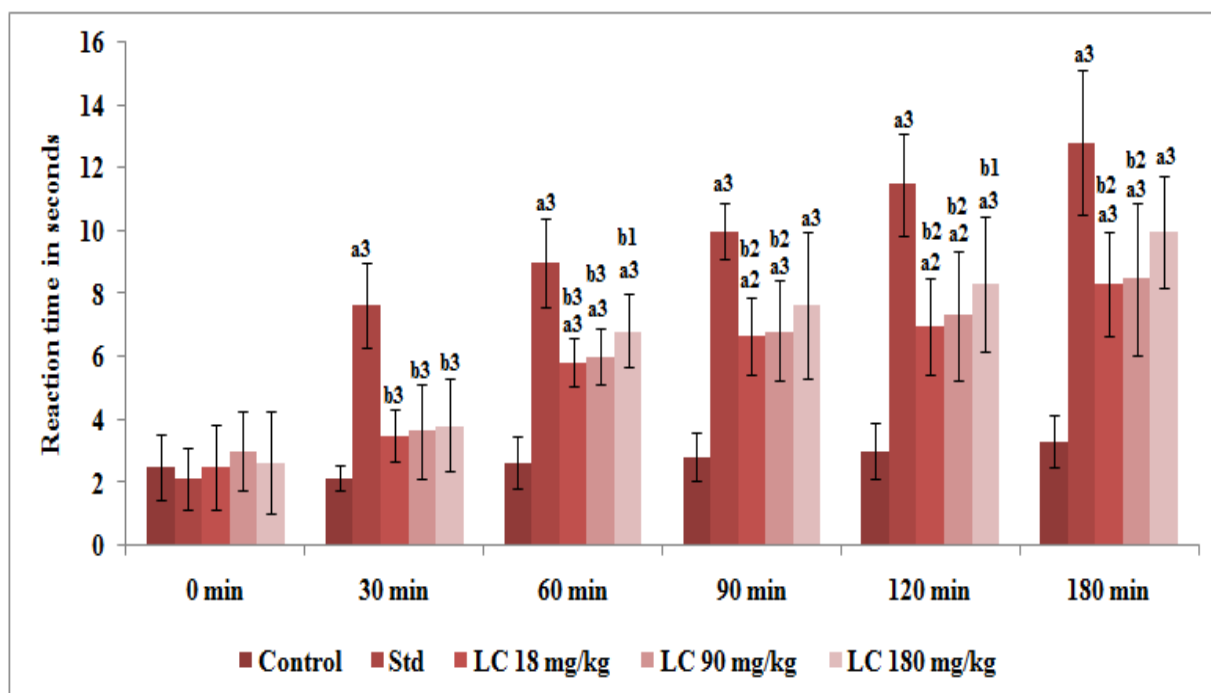


Figure 6.4.1: Effect of *Linga Chendhuram* on the central analgesic activity by hot plate method in Swiss mice of control and experimental groups.

Data were expressed as mean \pm S.D for 6 mice; One way ANOVA: Tukey's multiple comparison tests.

^{a2} $p < 0.01$ significantly different compared with control mice treated with diluted honey.

^{a3} $p < 0.001$ significantly different compared with control mice treated with diluted honey.

^{b1} $p < 0.05$ significantly different compared with standard group of mice treated with Aspirin.

^{b2} $p < 0.01$ significantly different compared with standard group of mice treated with Aspirin.

^{b3} $p < 0.001$ significantly different compared with standard group of mice treated with Aspirin.

6.4.2. Writhing test

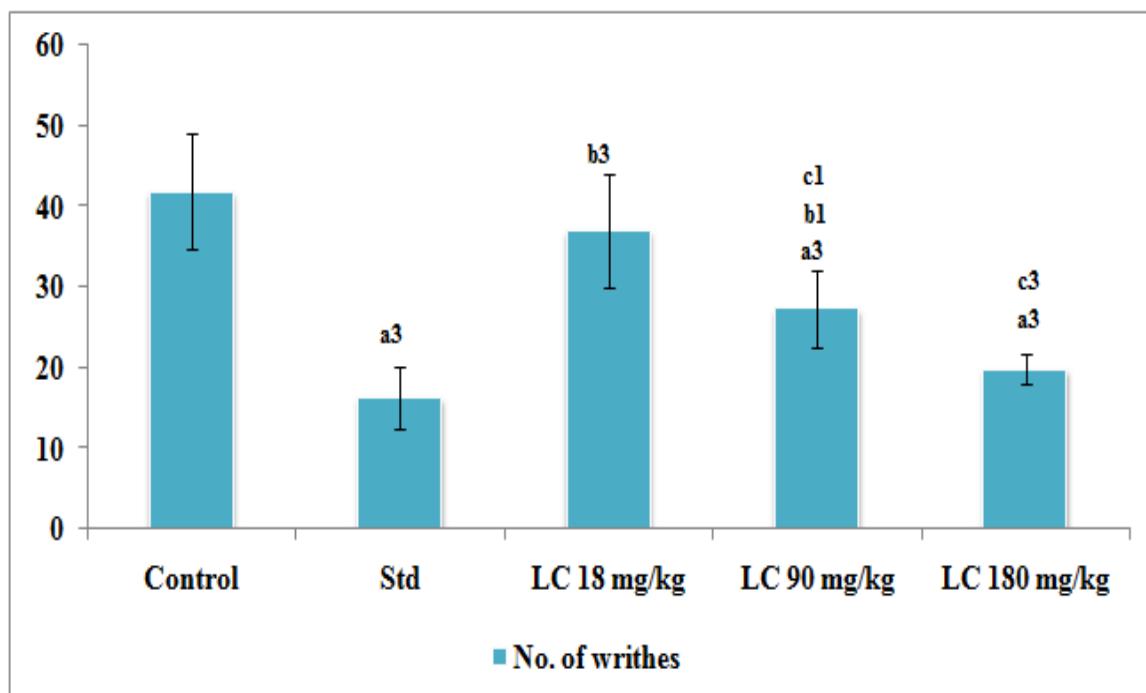


Figure 6.4.2: Effect of *Linga Chendhuram* on the peripheral analgesic activity by writhing method in Swiss mice of control and experimental groups.

Data were expressed as mean \pm S.D for 6 mice; One way ANOVA: Tukey's multiple comparison tests.

^{a3} $p < 0.001$ significantly different compared with control mice treated with diluted honey.

^{b1} $p < 0.05$ significantly different compared with standard group of mice treated with Aspirin.

^{b3} $p < 0.001$ significantly different compared with standard group of mice treated with Aspirin.

^{c1} $p < 0.05$ significantly different compared with test group of mice treated with LC at 18 mg/kg.

^{c3} $p < 0.001$ significantly different compared with test group of mice treated with LC at 18 mg/kg.

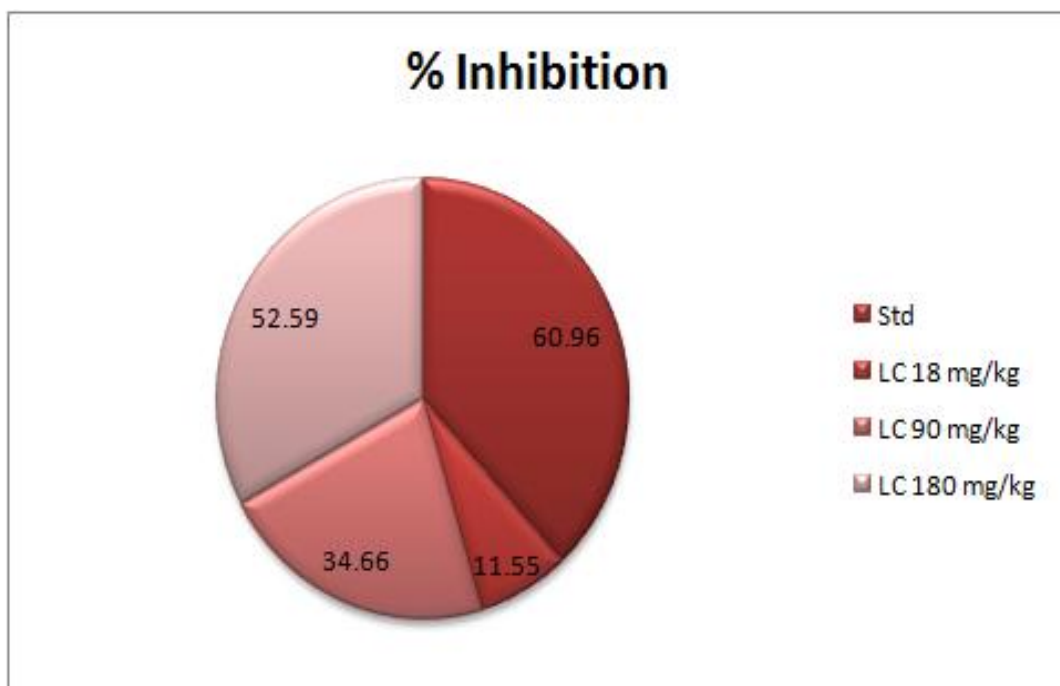


Figure 6.4.3: Effect of *Linga Chendharam* on inhibition of writhing in Swiss mice of experimental groups.

Among the experimental groups, LC at the dose of 180 mg/kg exhibited better inhibition of writhing but less effective than Aspirin.

6.5. Anti inflammatory activity

6.5.1. Carrageenan induced acute hind paw inflammation

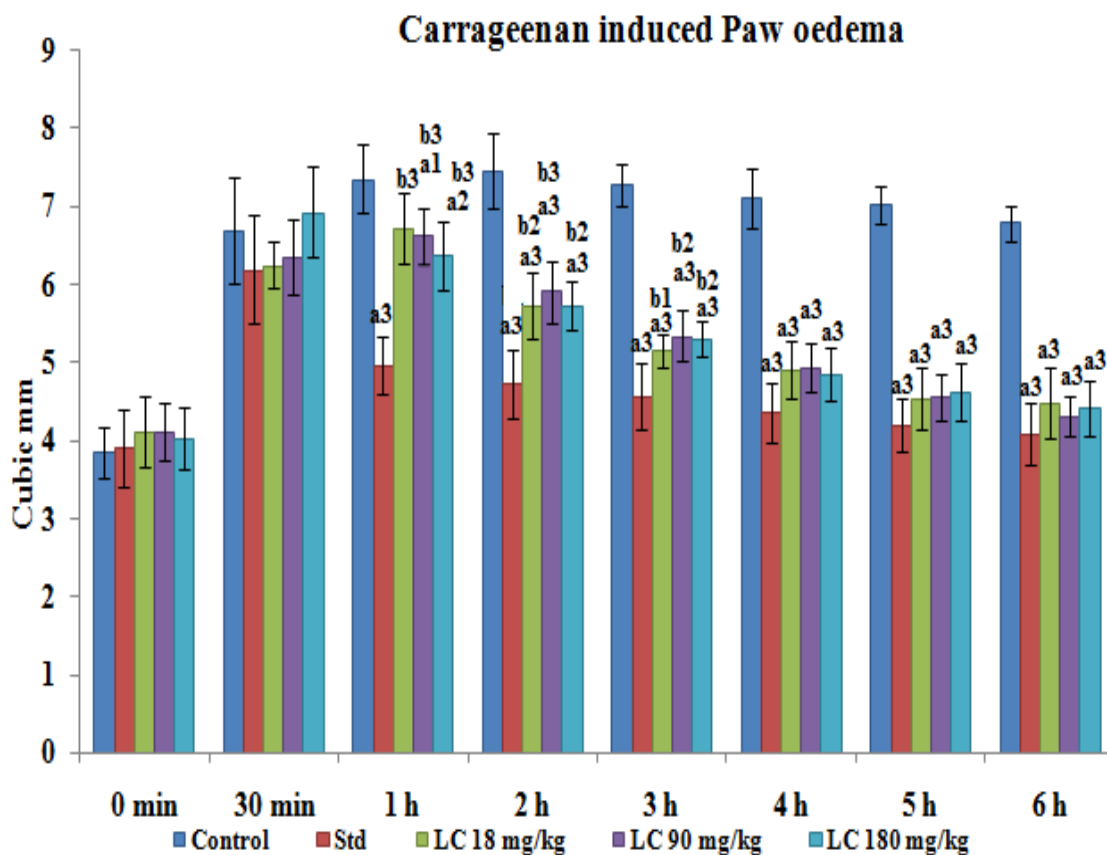


Figure 6.5.1: Effect of *Linga Chendhuram* on the acute hind paw inflammation by Carrageenan induced paw oedema method in Wistar rats of control and experimental groups.

Data were expressed as mean \pm S.D for 6 rats; One way ANOVA: Tukey's multiple comparison tests.

^{a1} $p < 0.05$ significantly different compared with control rats treated with diluted honey.

^{a2} $p < 0.01$ significantly different compared with control rats treated with diluted honey.

^{a3} $p < 0.001$ significantly different compared with control rats treated with diluted honey.

^{b1} $p < 0.05$ significantly different compared with standard group of rats treated with Diclofenac.

^{b2} $p < 0.01$ significantly different compared with standard group of rats treated with Diclofenac.

^{b3} $p < 0.001$ significantly different compared with standard group of rats treated with Diclofenac.

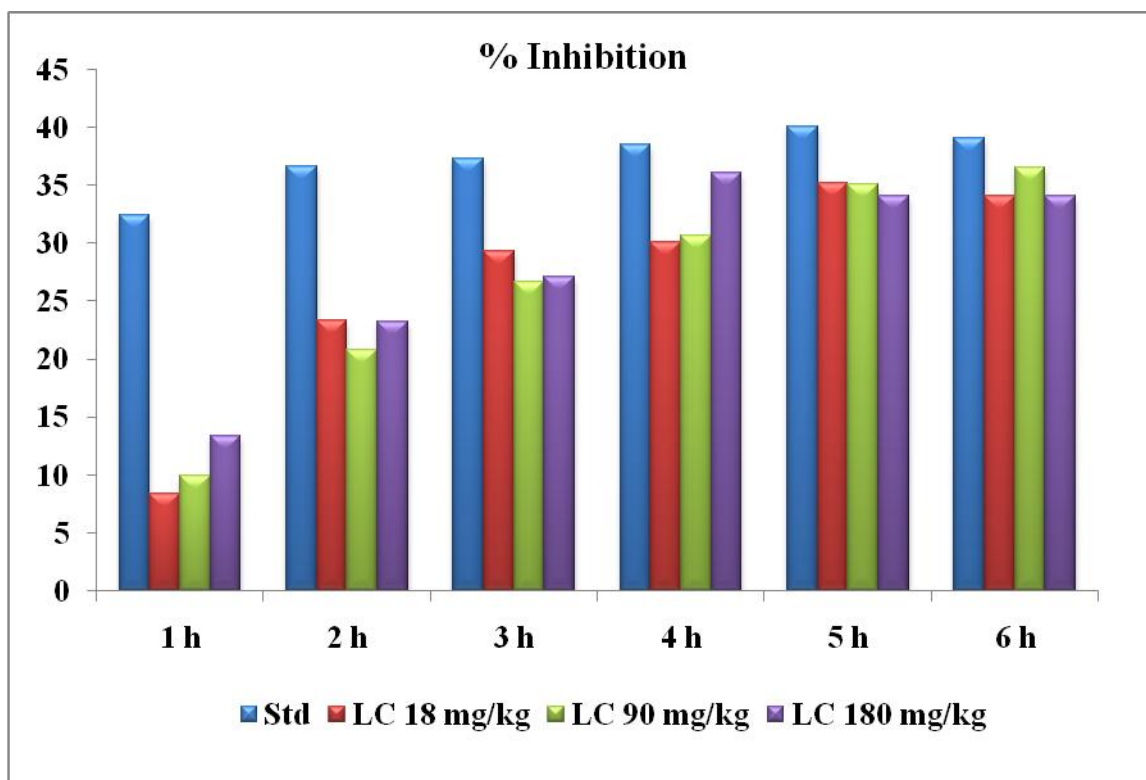


Figure 6.5.2: Effect of *Linga Chendhuram* on inhibition of paw oedema in Wistar rats of experimental groups.

Among the experimental groups, LC at the dose of 180 mg/kg exhibited 36% better inhibition of paw oedema after 4 h treatment. LC at the dose of 90 mg/kg exhibited 37% better inhibition of paw oedema after 6 h treatment. LC at the dose of 18 mg/kg exhibited 35% better inhibition of paw oedema after 5 h treatment. Diclofenac exhibited 40% better inhibition of paw oedema after 5 h treatment.

6.5.2. Cotton pellet granuloma method

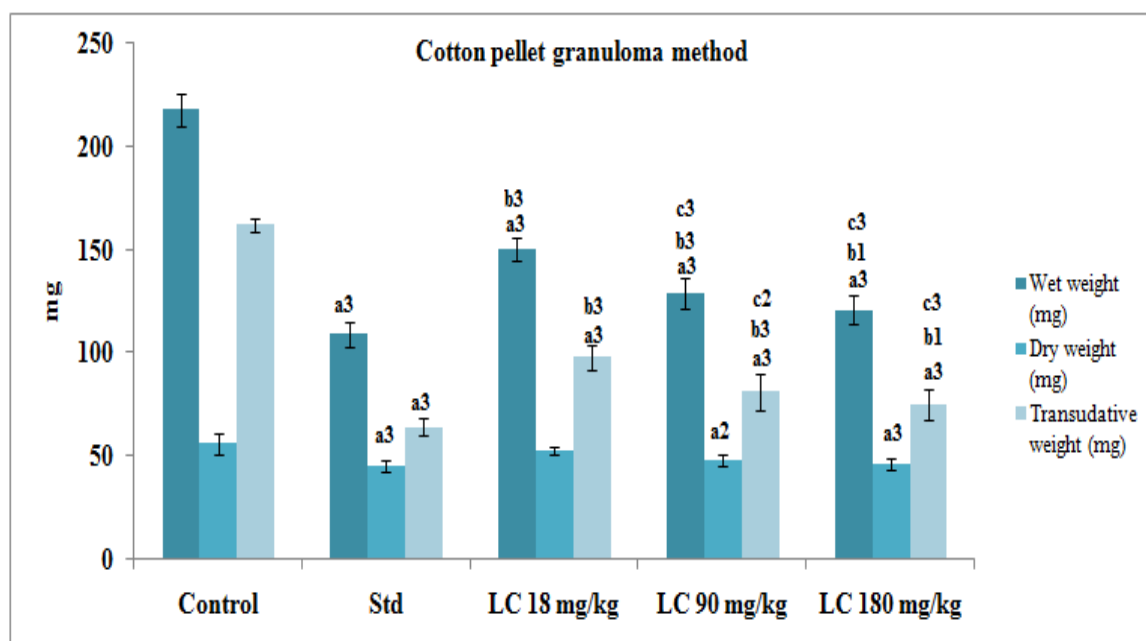


Figure 6.5.3: Effect of *Linga Chendhuram* on granuloma formation by Cotton pellet method in Wistar rats of control and experimental groups.

Data were expressed as mean \pm S.D for 6 rats; One way ANOVA: Tukey's multiple comparison tests.

^{a2} $p < 0.01$ significantly different compared with control rats treated with diluted honey.

^{a3} $p < 0.001$ significantly different compared with control rats treated with diluted honey.

^{b1} $p < 0.05$ significantly different compared with standard group of rats treated with Dexamethasone.

^{b2} $p < 0.01$ significantly different compared with standard group of rats treated with Dexamethasone.

^{b3} $p < 0.001$ significantly different compared with standard group of rats treated with Dexamethasone.

^{c1} $p < 0.05$ significantly different compared with test group of rats treated with LC at the dose of 18 mg/kg.

^{c2} $p < 0.01$ significantly different compared with test group of rats treated with LC at the dose of 18 mg/kg.

^{c3} $p < 0.001$ significantly different compared with test group of rats treated with LC at the dose of 18 mg/kg.

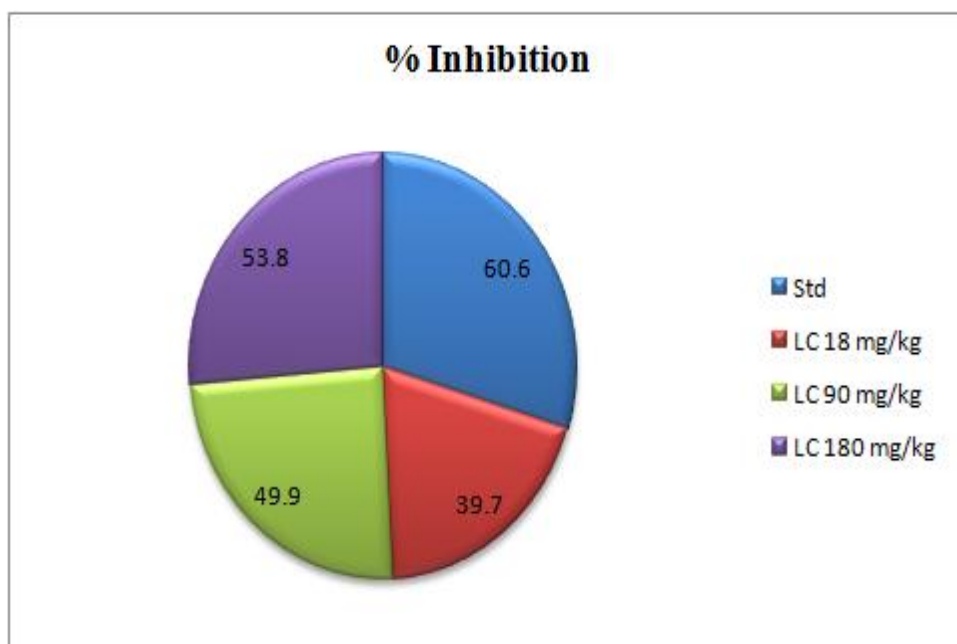


Figure 6.5.4: Effect of *Linga Chendharam* on inhibition of granuloma formation in Wistar rats of experimental groups.

Among the experimental groups, LC at the dose of 180 mg/kg exhibited better inhibition of granuloma formation but less effective than Dexamethasone.

6.6. Brewer's yeast induced pyrexia

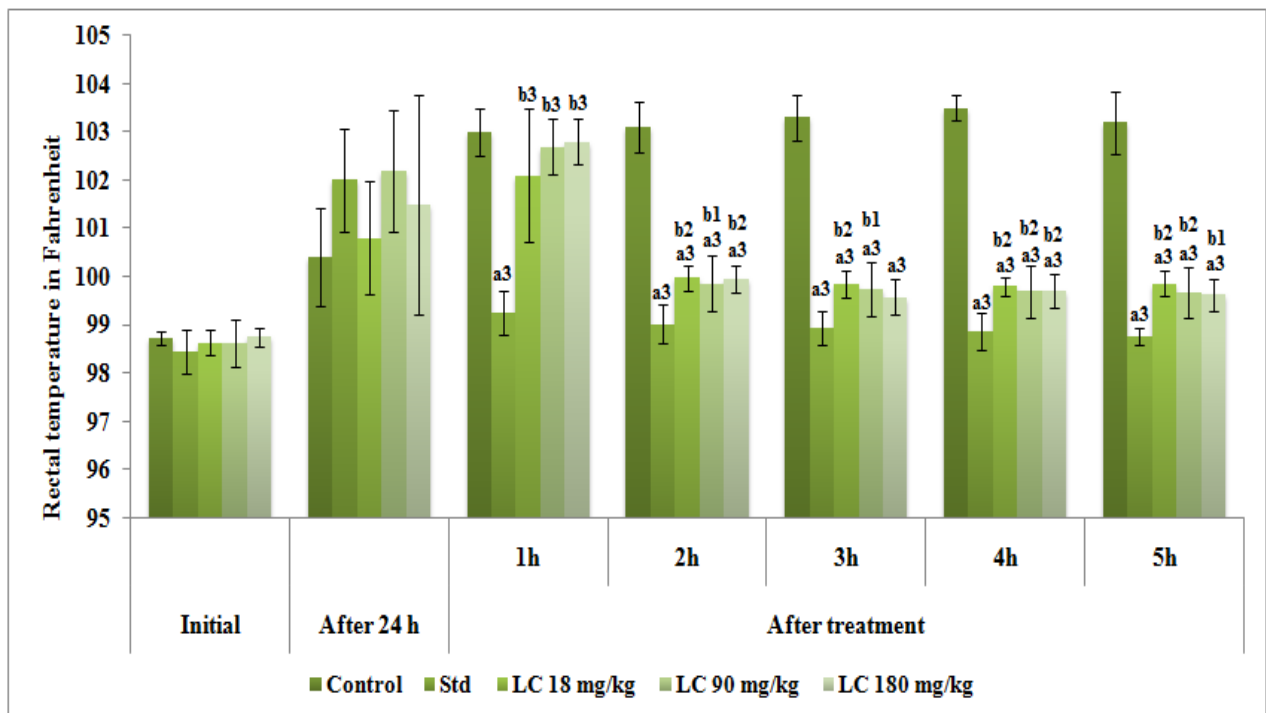


Figure 6.6.1: Effect of *Linga Chendhuram* on pyrexia induced by Brewer's yeast in Wistar rats of control and experimental groups.

Data were expressed as mean \pm S.D for 6 rats; One way ANOVA: Tukey's multiple comparison tests.

^{a3} $p < 0.001$ significantly different compared with control rats treated with diluted honey.

^{b1} $p < 0.05$ significantly different compared with standard group of rats treated with Paracetamol.

^{b2} $p < 0.01$ significantly different compared with standard group of rats treated with Paracetamol.

^{b3} $p < 0.001$ significantly different compared with standard group of rats treated with Paracetamol.

6.7. CLINICAL STUDY

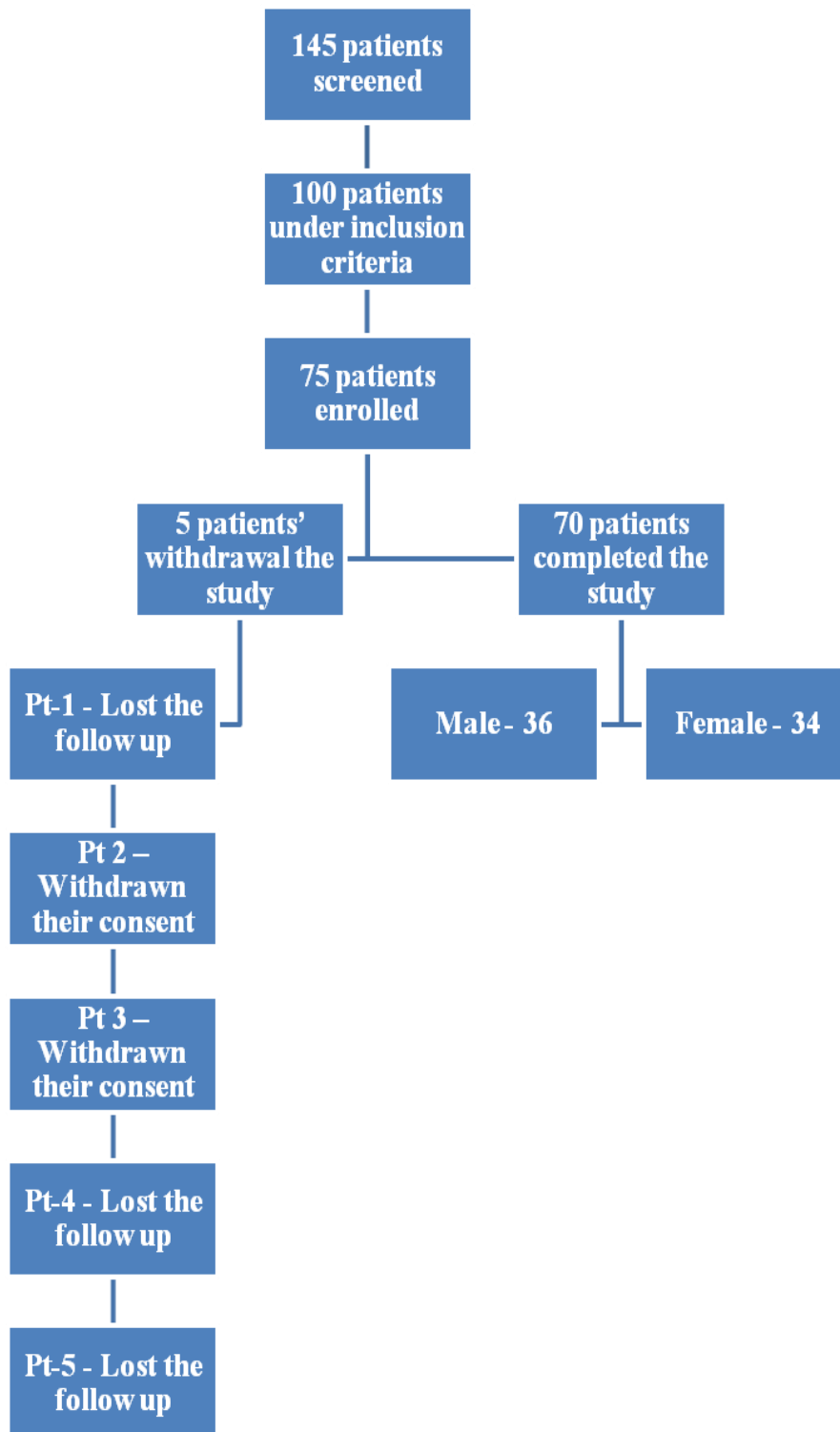


Chart 6.7.1: Flow chart of patient recruitment for clinical study

The observations were made and figured with regards to the following features:

- Gender distribution
- Age distribution
- Age distribution according to Siddha Aspect
- Occupational status
- Food habits
- Food habits
- Distribution of thinai
- Kaalam distribution
- Iymporigal and Iympulungal (Penta sensors and its modalities)
- Kanmenthiriyangal (Motor machinery and its execution)
- Gunam (Character)
- Envagai thervugal
Naadi, Naa, Niram, Mozhi, Meikuri, Vizhi, Malam and Mothiram (Neerkuri & Neikuri)
- Distribution of mukkutram
Deranged Vali, Deranged Pitham and Deranged Kabam
- Temperature distribution
- Reduction of fever after treatment
- Effect of LC on Reduction of Temperature within 120 min among 28 patients
- Effect of LC on temperature pattern of 28 patients
- Effect of LC on Reduction of Temperature within 240 min among 20 patients
- Effect of LC on temperature pattern of 20 patients
- Effect of LC on Reduction of Temperature after 240 min among 22 patients
- Visual analogue scale
- Grade of Pain scale
- Effect of LC on 70 *Suram* patients to assess the primary and secondary outcomes
- Incidence no of patients had relapse of fever after 120min & 240 min

6.7.1. Gender distribution

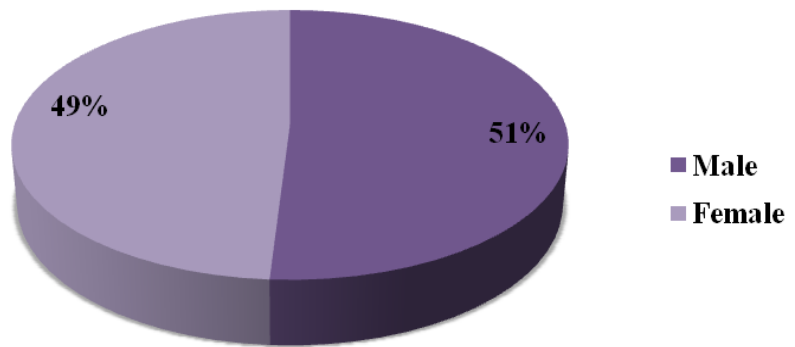


Figure 6.7.1: Gender distribution

- Among 70 patients enrolled, 36 were male (51%) and 34 were female (49%).

6.7.2. Age distribution

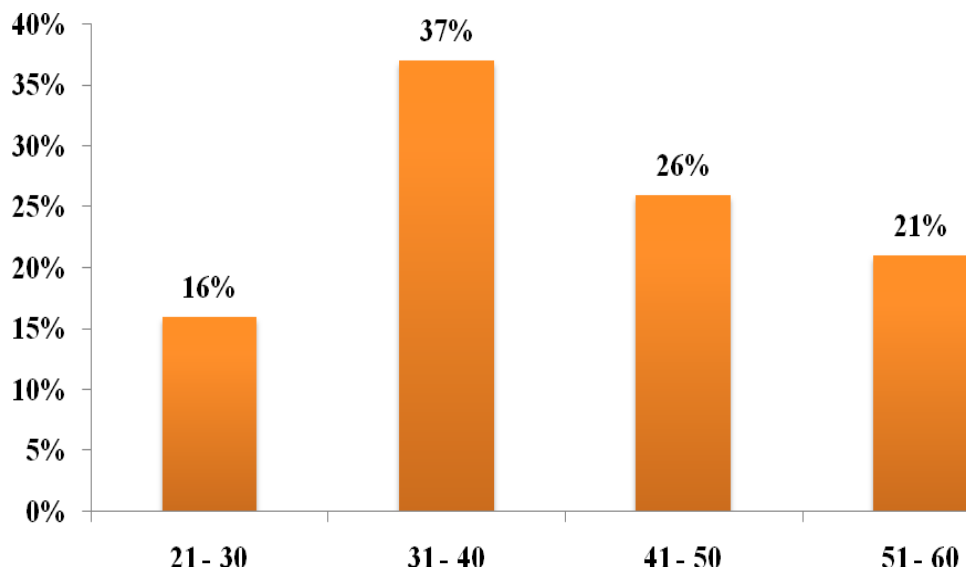


Figure 6.7.2: Age distribution

- Among the 70 cases, it was observed that more number of patients enrolled for Suram were between 31-40 years.

6.7.3. Age distribution according to *Siddha* Aspect

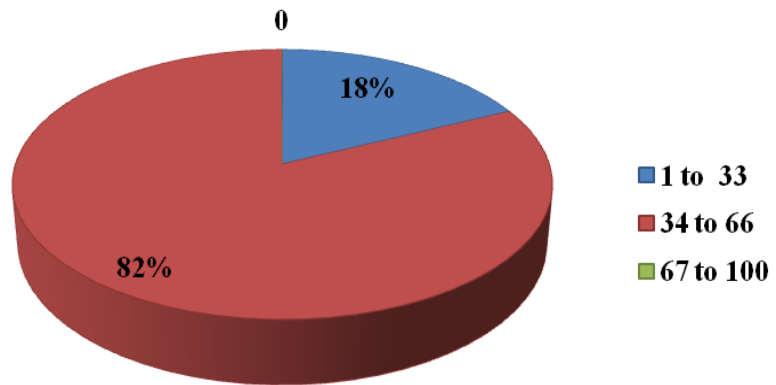


Figure 6.7.3: Age distribution according to *Siddha* Aspect

- Among the 70 cases, it was observed that more number (82 %) of patients enrolled in Pitha kalam

6.7.4. Occupational status

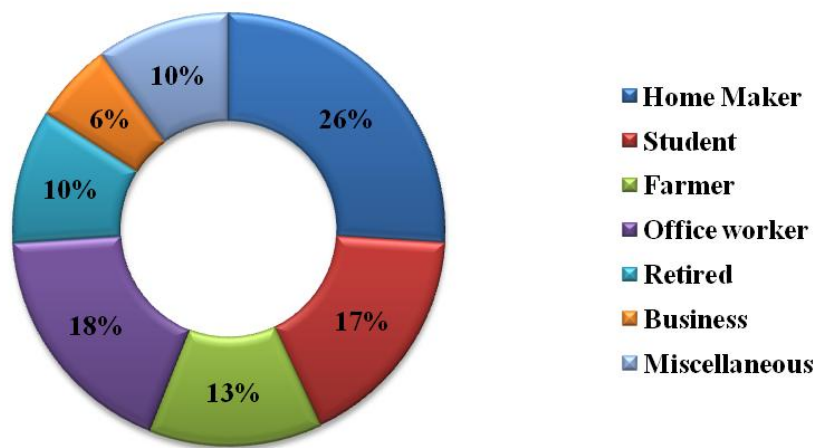


Figure 6.7.4: Occupational status

- Among the 70 cases, 26% of cases are home makers, 17% of cases are Students, of 13% cases are Farmer, 18% of cases are Office based workers, 10% of cases are Retired persons and 6% of cases are Business people.

6.7.5. Food habits

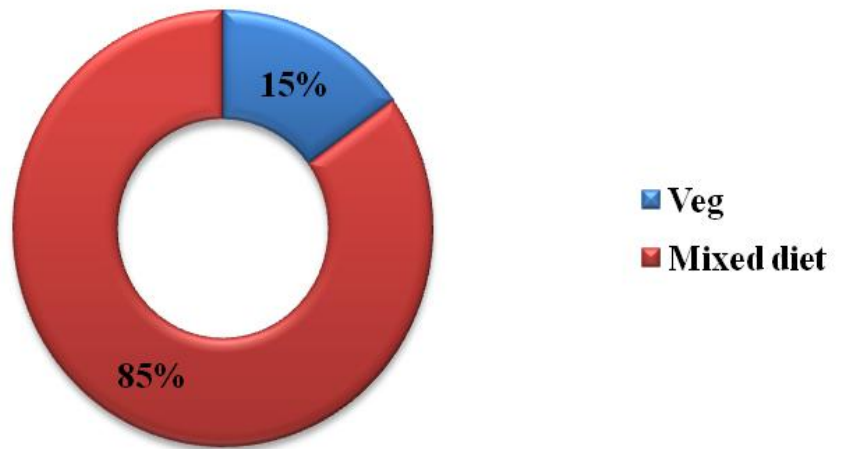


Figure 6.7.5: Food habits

- Among 70 patients enrolled, 85% were mixed diet and 15% were vegetarian diet.

6.7.6. Income status

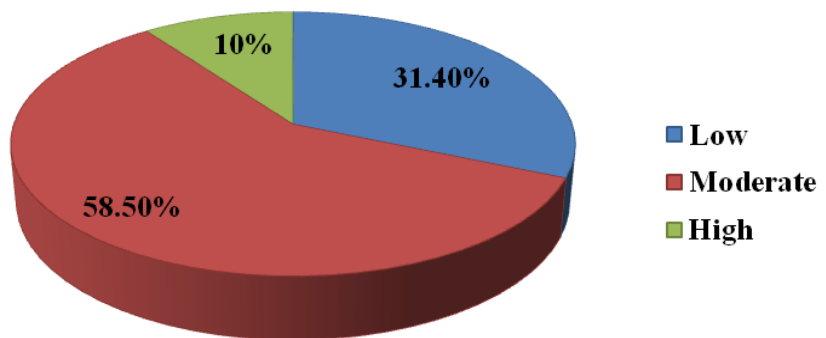


Figure 6.7.6: Income status

- Among the 70 cases, 58.5% of cases are moderate income group, 31.4% of cases are low income group and 10% of cases are high income group

6.7.7. Noi ultra nilam

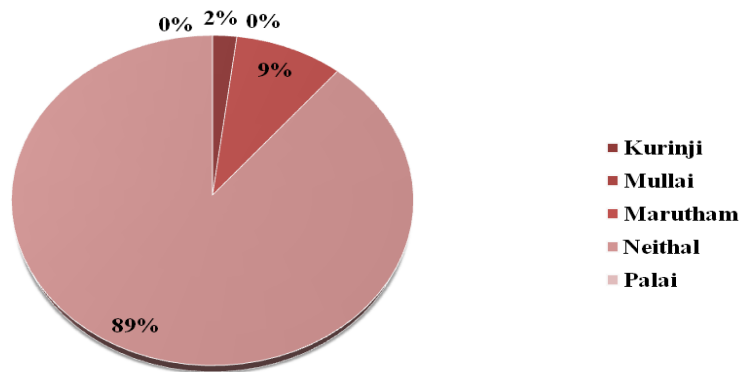


Figure 6.7.7: Noi ultra nilam

In this study patients were belongs to, 89% of the patients were belong to the land tract of Neithal (Coastal region), 9% of the patients were belong to the land tract of Marutham (Cultivable land) and 2% of the patients were belong to the land tract of Kurunji (Hilly area)

6.7.8. Noi ultra kalam

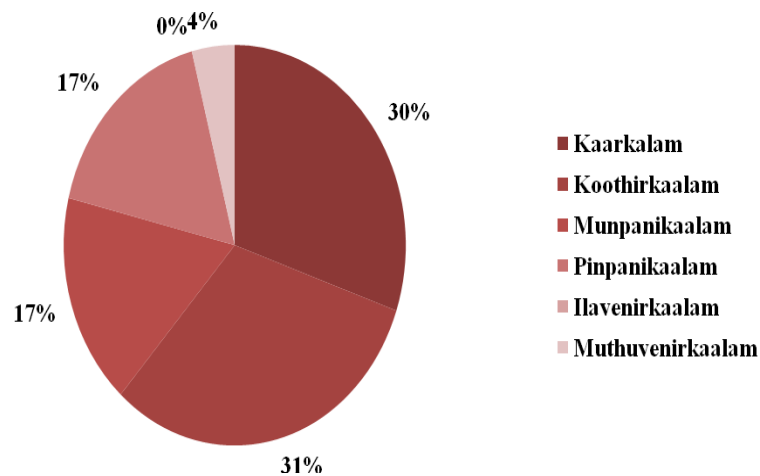


Figure 6.7.8: Noi ultra kalam

In this study patients were suffered from fever, 30% of the patients were affected during Kaar kalam (Mid August to Mid October), 31% of the patients were affected during Koothir kalam (Mid October to Mid December), 17% of the patients were affected during Munpani kalam (Mid December to Mid February) and Pinpani kalam (Mid February to Mid April) and 4 % of the patients were affected during Muthuvenir kalam (mid june to mid august)

6.7.9. Iymporigal and Iympulangal (Penta sensors and its modalities)

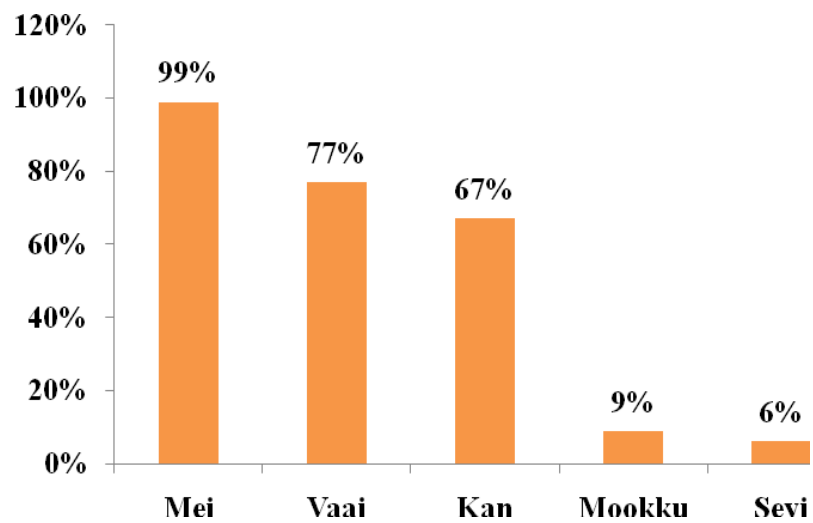


Figure 6.7.9: Iymporigal and Iympulangal (Penta sensors and its modalities)

Among the 70 cases, 99% cases had affected in Mei, 77% cases had affected in Vai, 67% cases had affected in Kan, 9% cases had affected in Mookku and 6% cases had affected in Sevi

6.7.10. Kanmenthiryangal (Motor machinery and its execution)

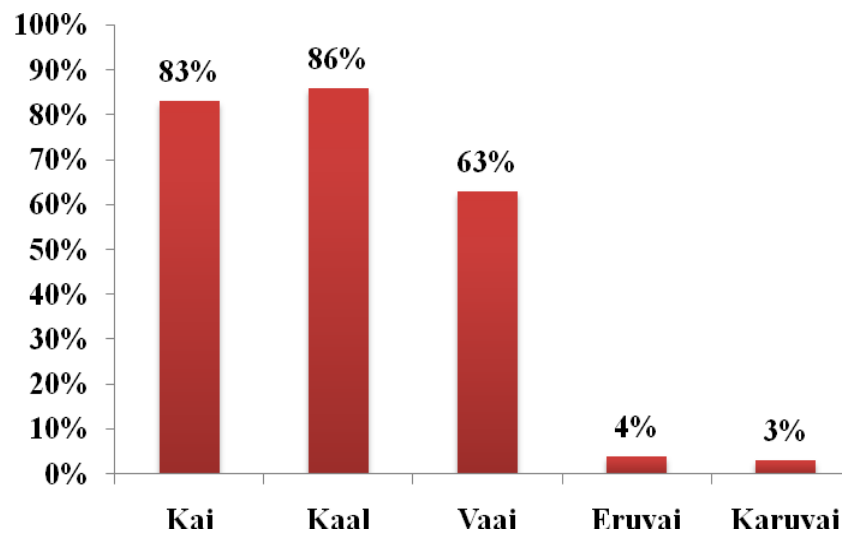


Figure 6.7.10: Kanmenthiryangal (Motor machinery and its execution)

Among the 70 cases, 83% cases had affected in Kai. 86% cases had affected in Kaal, 63% cases had affected in Vaai, 4% cases had affected in Eruvai and 3% cases had affected in Karuvai

6.7.11. Gunam

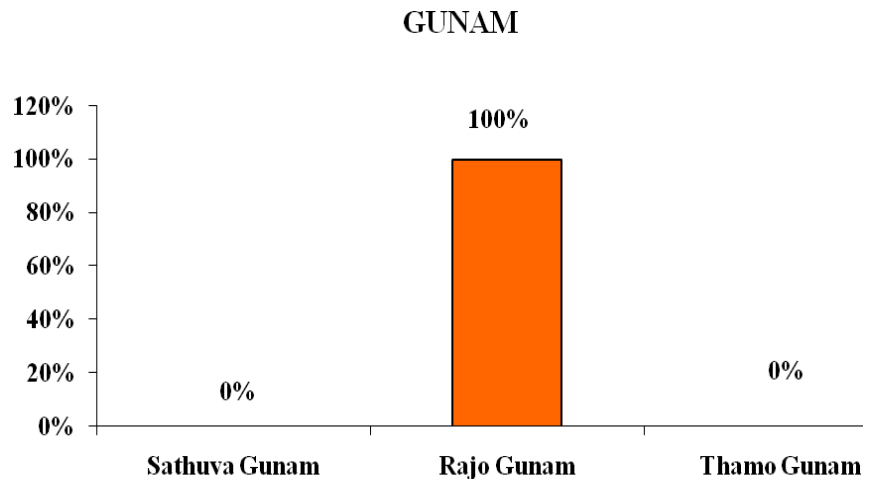


Figure 6.7.11: Gunam

Among the 70 cases, 100% cases had belongs to Rajo gunam

6.7.12. Uyir thatukkal - Vali

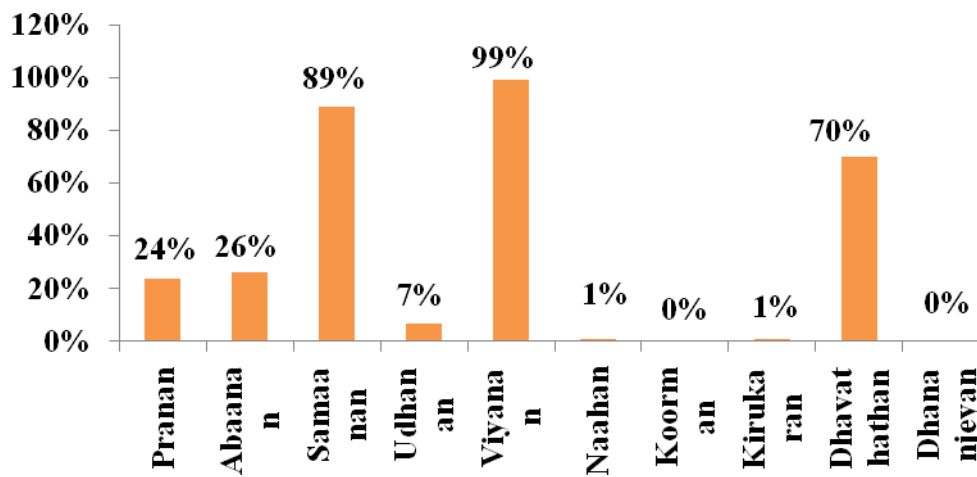


Figure 6.7.12: Uyir thatukkal - Vali

Among the 70 cases, 99%cases had deranged Viyanan, 89%cases had deranged Samanan, 70% cases had deranged Devathathan, 99%cases had deranged Viyanan, 26%cases had deranged Abaanana, 24%cases had deranged Pranana, 7%cases had deranged Udhanan and 1%cases had deranged Nathan and Kirukaran

6.7.13. Uyir thatukkal - Azhal & Iyyam

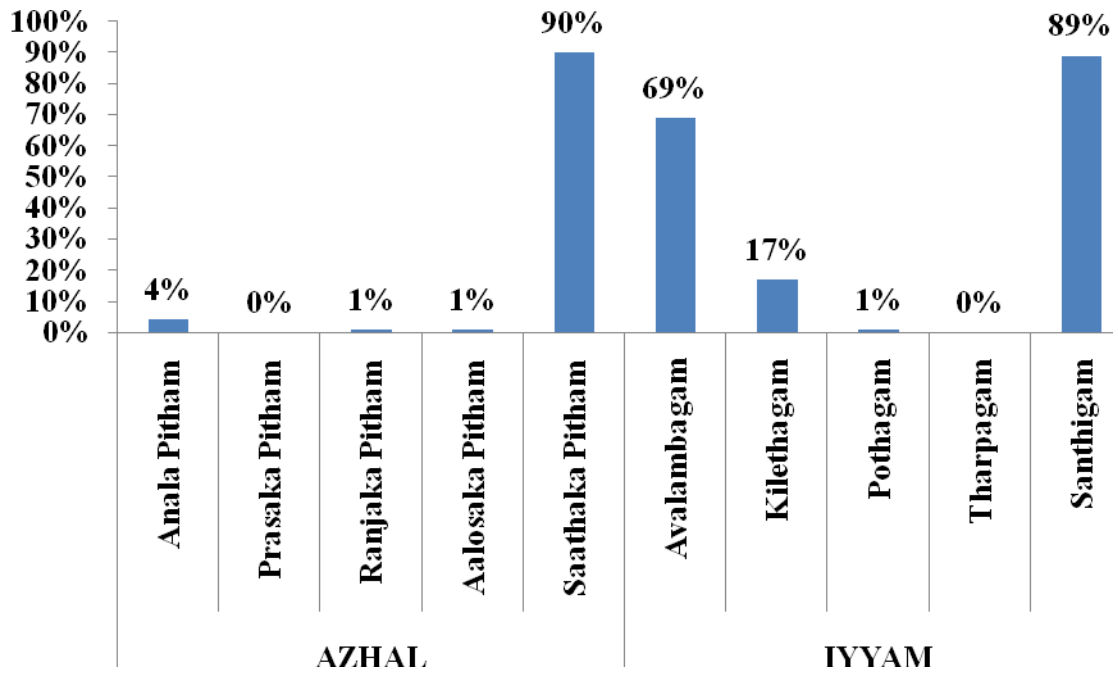


Figure 6.7.13: Uyir thatukkal - Azhal & Iyyam

In Azhal

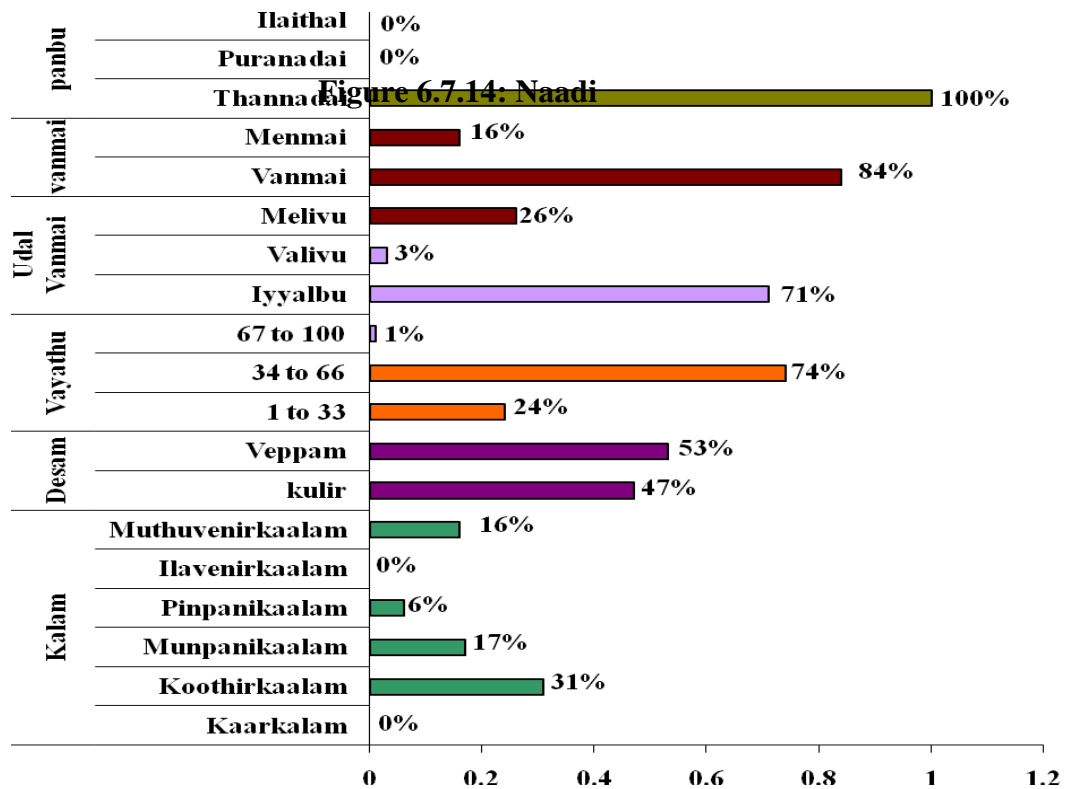
- Out of 70 cases, 90% cases had deranged Saathaka pitham, 4% cases had deranged Saathaka pitham and 1% cases had deranged Ranjaka pitham and Aalosaka pitham

In Iyyam

- Out of 70% cases 89% cases had deranged Santhigam, 69% cases had deranged Avalambagam, 17% cases had deranged Kilethagam and 1% cases had deranged Pothagam

6.7.14. ENVAGAI THERVU (EIGHT FOLD EXAMINATION)

NAADI



Pulse reading season (*Kalam*)

- Among 70 cases, 31% cases had pulse seen in Koothirkalm, 17% cases had pulse seen in Munpanikalam, 16% cases had pulse seen in Muthirvenirkalm and 6% cases had pulse seen in Pinpanikalam

Climate of the patient's habitat (*Desam*)

- Among 70 cases, 53% cases had pulse seen in hot climate of the patient's habitat and 47% cases had pulse seen in temperate climate of the patient's habitat

Age (*Vayathu*)

- Among 70 cases, 24% cases had between 1 to 33 years, 74% cases had between 34 to 66 years and 24% cases had between 1 to 33 years

Expansile nature (*Vanmai*)

- Among 70 cases, 84% cases had expansile nature (Vanmai) and 14% cases had menmai

Habit (*Panbu*)

- Among 70 cases, 100% cases had naadi seen in playing in (Thannadai)

NAADI NADAI (*PULSE PLAY*)

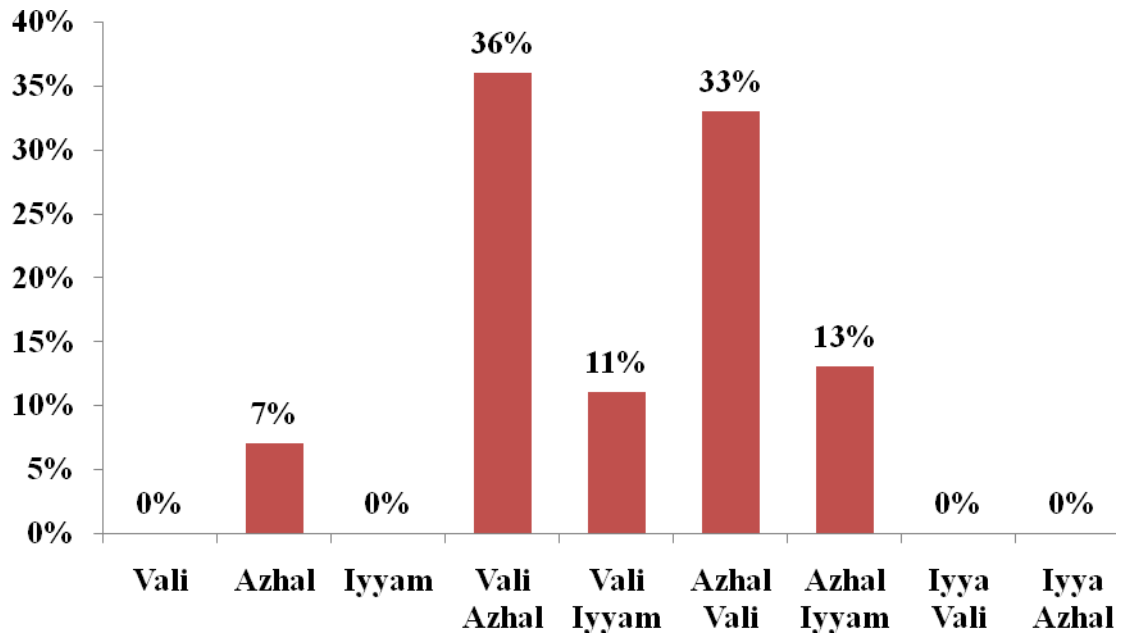


Figure 6.7.15: Naadi nadai

Among the 70 cases, 36% cases had Valiazhal naadi, 33% cases had Azhalvali naadi, 13% cases had Azhaliyyam naadi, 11% cases had Valiyyam naadi and 36% cases had azhal naadi

Naa (Tongue)

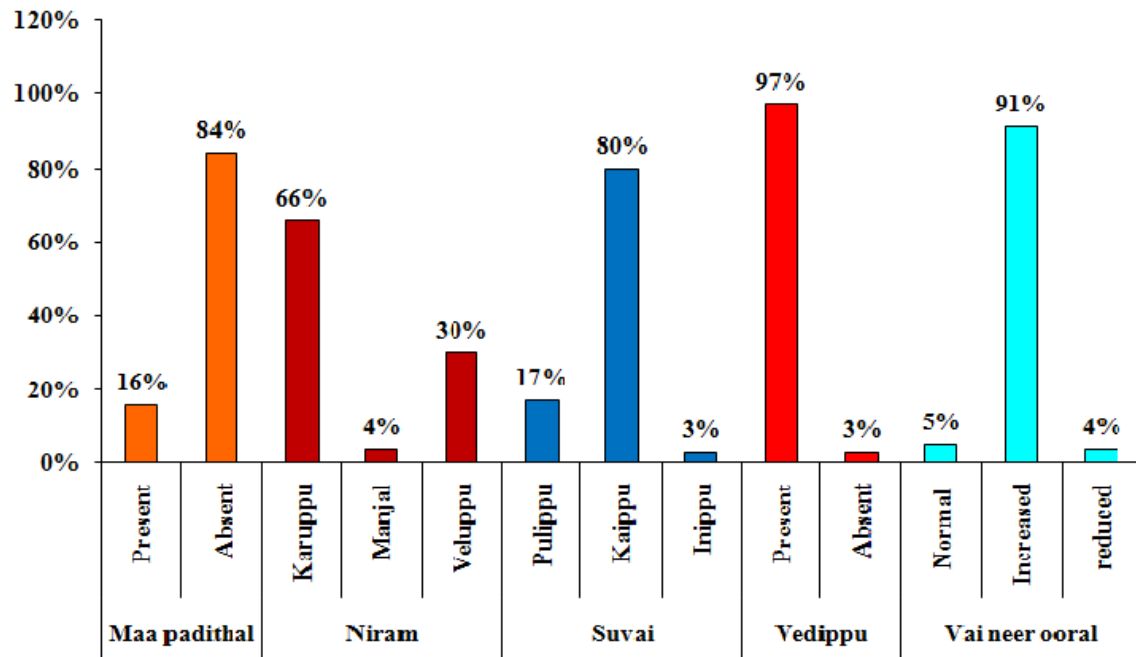


Figure 6.7.16: *Naa (Tongue)*

Coatedness (Maa padinthiruthal)

- Among 70 cases, 77% cases had normal tongue and 16% cases had coatedness

Colour of the tongue (Niram)

- Among 70 cases, 66% cases had dark colour (Karuppu) tongue, 30% cases had pale colour (Velluppu) tongue and 4% cases had yellow colour (Manjal) tongue

Taste sensation of the tongue (Suvai)

- Among 70 cases, 80% cases had bitter taste (Kaippu) tongue, 17% cases had sour taste (Pulippu) tongue and 3% cases had sweet taste (Inippu) tongue

Fissure of the tongue (Vedippu)

- Among 70 cases, 97% cases had present the fissure and 3% cases had no fissure

Salivation of the tongue (Vai neer ooral)

- Among 70 cases, 91% cases had increased salivation, 5% cases had no salivation and 4% cases had reduced salivation

Niram and Mozhi

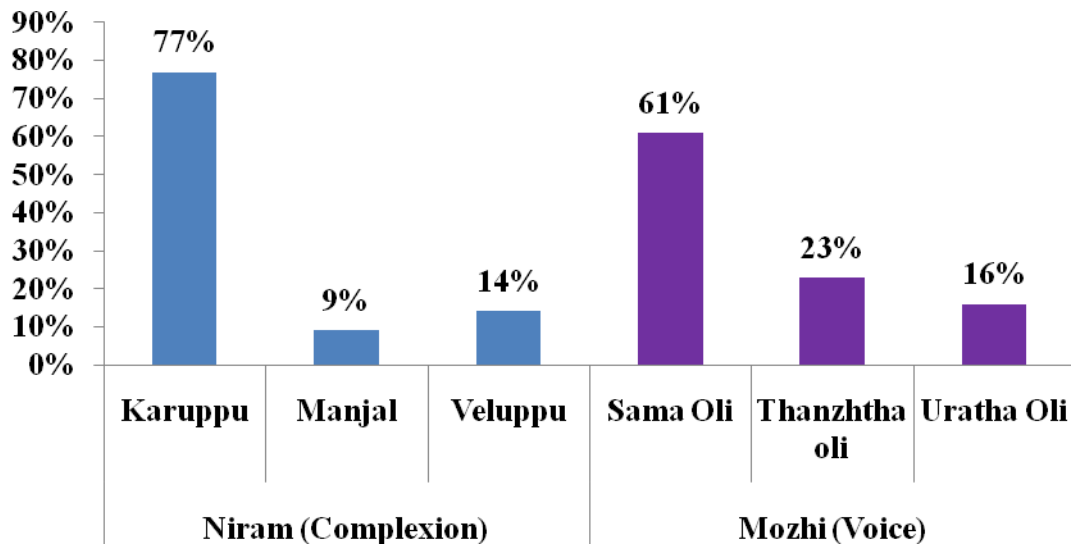


Figure 6.7.17: *Niram and Mozhi*

Complexion (Niram)

- Among 70 cases, 77% cases had black (Karuppu), 14% cases had Fair (Velluppu) and 9% cases had yellowish (Manjal)

Voice (mozhi)

- Among 70 cases, 61% cases had medium pitched voice (Sama oli), 23% cases had low pitched voice (Thazhatha oli) and 16% cases had high pitched voice (Urattha oli)

Mei kuri (Physical signs)

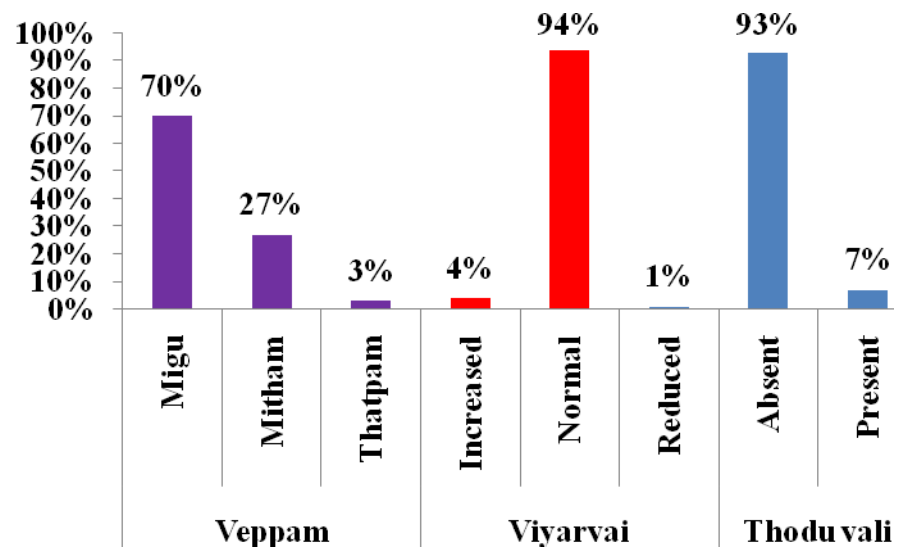


Figure 6.7.18: Mei kuri (Physical signs)

Warmth (Veppam)

- Among 70 cases, 70% cases had moderate (migu) warmth, 27% cases had mild warmth (mitham) and 3% cases had decreased (thatpam) warmth

Sweat (Viyarvai)

- Among 70 cases, 93% cases had no tenderness and 7% cases had tenderness

Tenderness (Thodu vali)

- Among 70 cases, 94% cases had normal sweat, 4% cases had increased sweat and 1% cases had reduced sweat

Vizhi (Eyes)

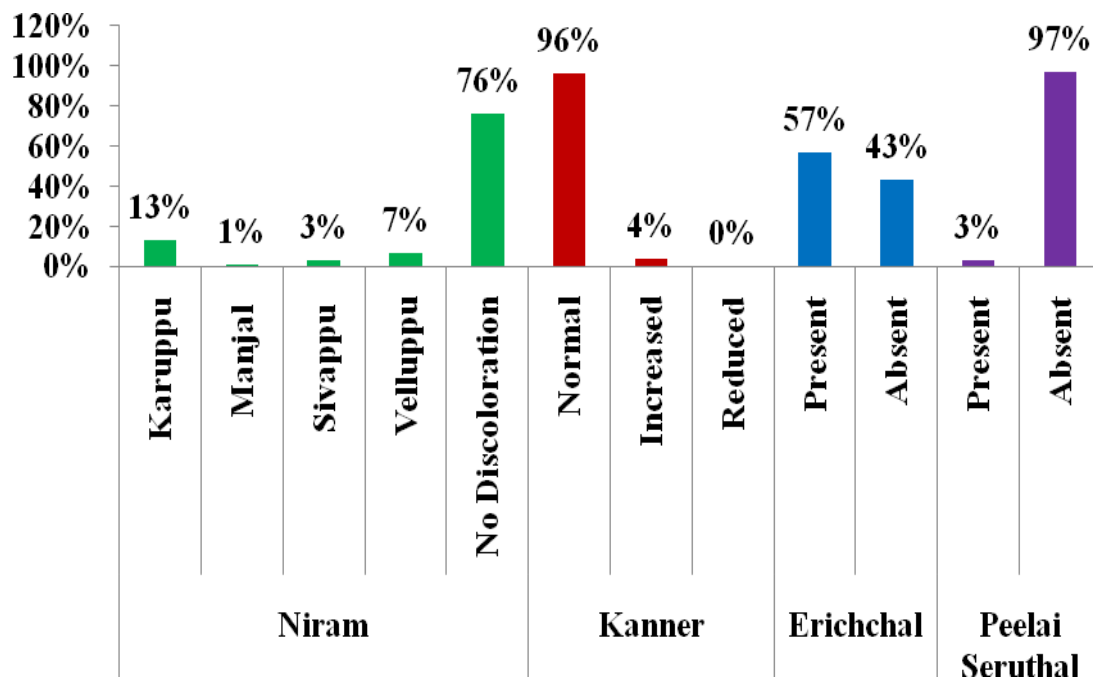


Figure 6.7.19: Vizhi (Eyes)

Discolouration of eye

- Among 70 cases, 76% cases had normal eye colour, 13% cases had Dark (Karuppu) eye colour, 7% cases had White (Velluppu) eye colour, 3% cases had Red (Sivappu) eye colour and 1% cases had Yellow (Manjal) eye colour

Tears (Kanneer) of eye

- Among 70 cases, 96% cases had normal eye and 4% cases had tears

Burning sensation (Erichchal) of eye

- Among 70 cases, 57% cases had burning sensation of eye and 43% cases had normal eye

Mucus excrements (Peelai seruthal) of eye

- Among 70 cases, 97% cases had normal eye and 3% cases had Mucus excrements of eye

Malam (Stools)

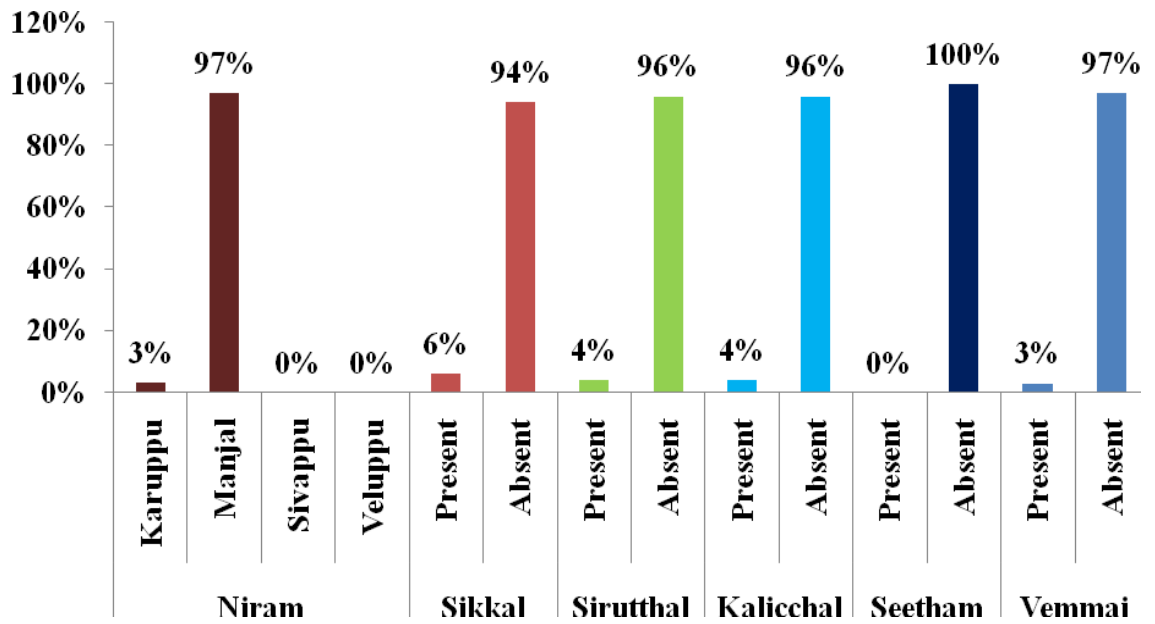


Figure 6.7.20: Malam (Stools)

Colour of Stool

- Among 70 cases, 97% cases had Yellow (Manjal) colour stool and 3% cases had Black (Karuppu) colour stool

Constipation (Sikkal) of Stool

- Among 70 cases, 94 % cases normal bowel habits and 6% cases had constipation

Poorly formed Stool (Sirutthal)

- Among 70 cases, 96 % cases normal bowel habits and 4% cases had poorly formed stool

Loose watery stool (Kalichchal)

- Among 70 cases, 96 % cases normal bowel habits and 4% cases had loose watery stool

Watery and mucoid excrements stool (Seetham)

- Among 70 cases, 100 % cases normal bowel habits

Warmth stool (Venmai)

- Among 70 cases, 97 % cases normal bowel habits and 3% cases had warmth stool

Neerkuri (Urine-Physical characteristics)

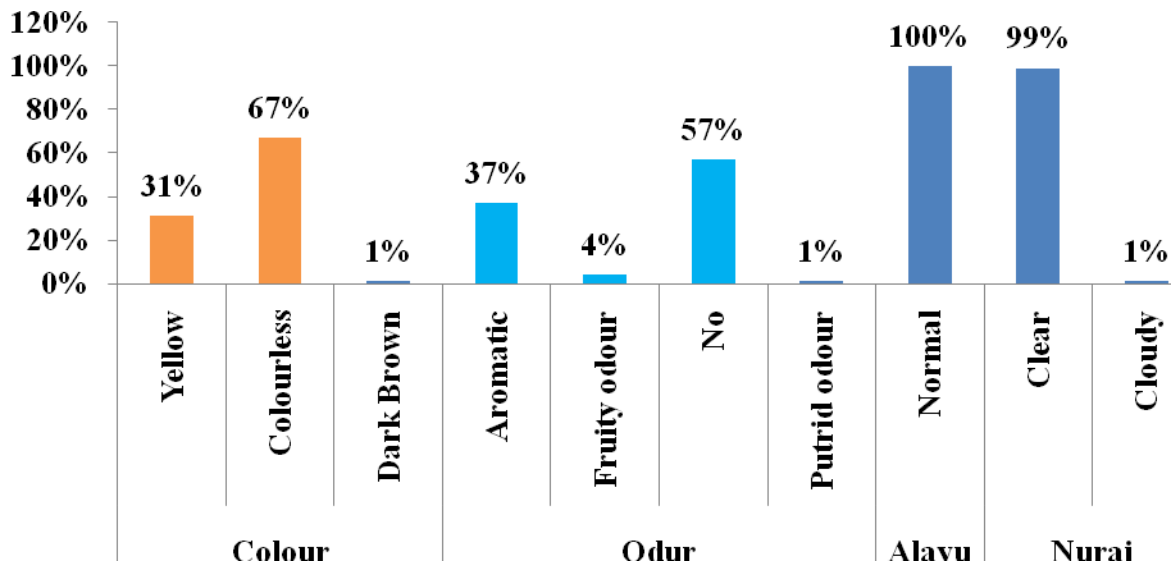


Figure 6.7.21: *Neerkuri (Urine-Physical characteristics)*

Colour of urine

- Among 70 cases, 67% cases had colourless urine, 31% cases had yellow colour urine and 1 % case had dark brown colour urine.

Odour of urine

- Among 70 cases, 57% cases had no odour, 37 % cases had urine Aromatic odour, 4 % cases had urine fruity odour and 1 % case had urine putrid odour.

Volume (Alavu) of urine

- Among 70 cases, 100% cases normal quantity of urine

Turbidity (Nurai) of urine

- Among 70 cases, 99% cases clear urine and 1 % case had cloudy urine

Neerkuri

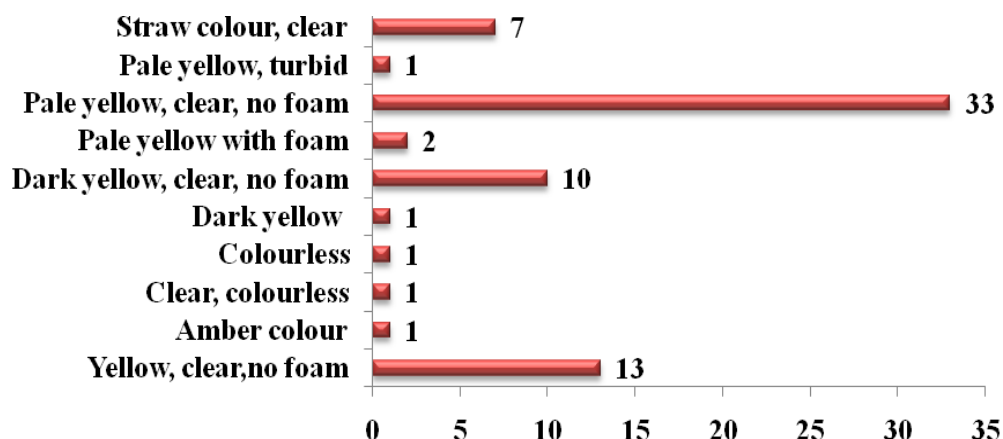


Figure 6.7.22: Neerkuri

Among 70 cases, 33 cases had pale yellow colour clear urine no foam, 13 cases had yellow colour clear urine no foam, 10 cases had dark yellow colour clear urine no foam, 7 cases had Straw colour clear urine, 2 cases had pale yellow colour urine with foam, 1 case had pale yellow, turbid, 1 case had Dark yellow, 1 case had colourless clear and 1 case had Amber colour

Neikkuri (Oil on Urine sign)

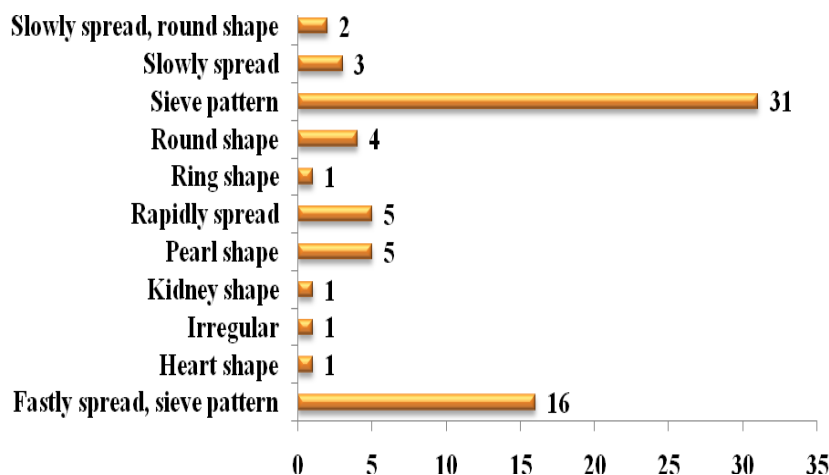


Figure 6.7.23: Neikkuri

Among 70 cases, 31 cases had Sieve pattern, 16 cases had Fastly spread, Sieve pattern, 5 cases had rapidly spread and Pearl shape, 4 cases had round shape, 3 cases had slowly spread, 3 cases had slowly spread, round shape and 1 case had ring shape, Kidney shape, Heart shape and irregular shape

6.7.15. Temperature distribution

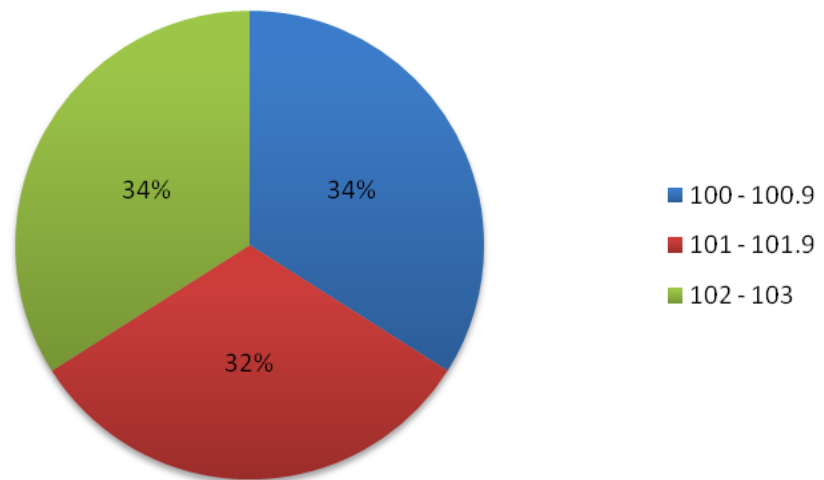


Figure 6.7.24: Temperature distribution

Among 70 cases, 34% cases had temperature between 100-100.9 and 101-101.9 and 32% cases had temperature between 102-103

6.7.16. Reduction of fever after treatment

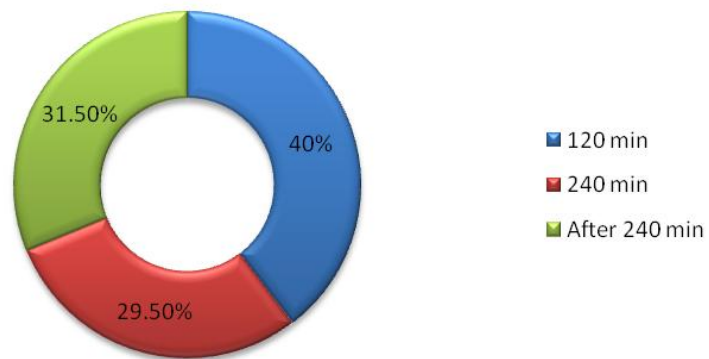


Figure 6.7.25: Temperature distribution

Among 70 cases, raised body temperature came to normal in 28 cases with in 120 min and in 20 cases with in 240 min after single administration dose of LC

6.7.17. Effect of LC on Reduction of Temperature within 120 min among 28 patients

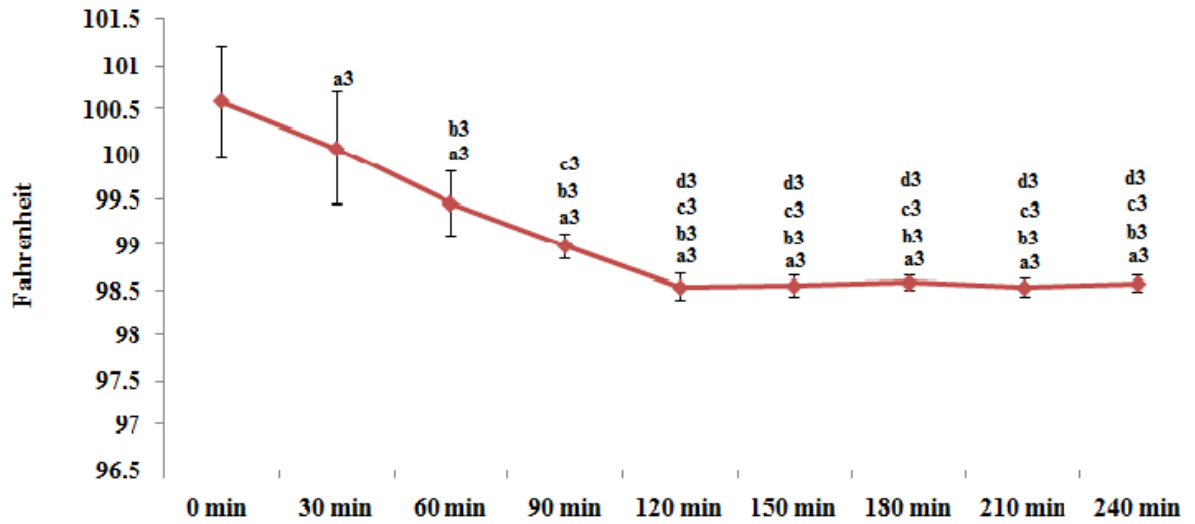


Figure 6.7.26: Effect of LC on Reduction of Temperature within 120 min among 28 patients

Data were expressed as mean \pm S.D for 28 patients; One way ANOVA: Tukey's multiple comparison tests.

^{a3}p<0.001 significantly different compared with all test group

^{b3}p<0.001 significantly different compared all test group

^{c3}p<0.001 significantly different compared with all test group

6.7.18. Effect of LC on temperature pattern of 28 patients

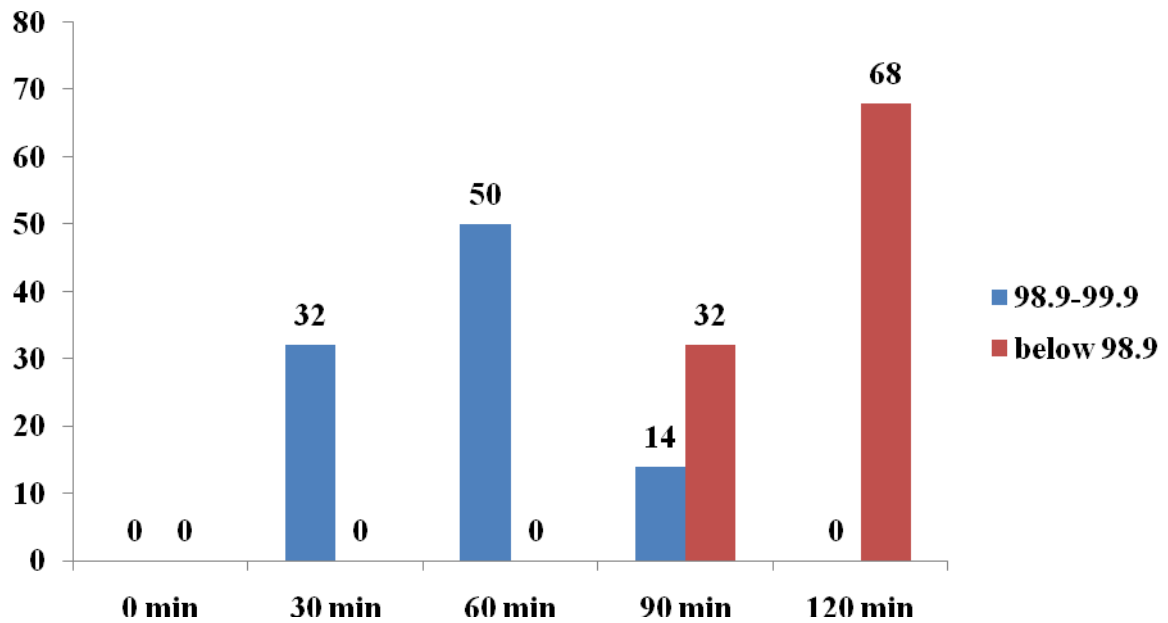


Figure 6.7.27:

Among the 28 cases,

Raised body temperature reduced between 98.9-99.9, 32% cases had 30% min, 50% cases had 60 min and 14 % cases had 90 min

Raised body temperature reduced below 98.9, 32% cases had 90% min and 68% cases had 120 min

6.7.19. Effect of LC on Reduction of Temperature within 240 min among 20 patients

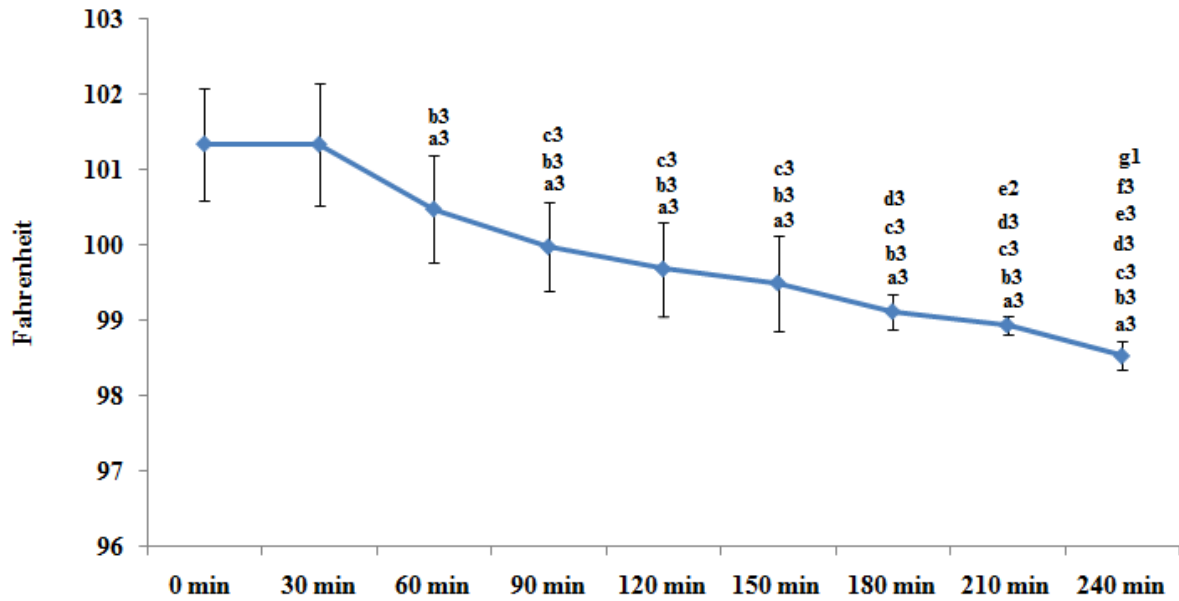


Figure 6.7.28: Effect of LC on Reduction of Temperature within 240 min among 20 patients

Data were expressed as mean \pm S.D for 28 patients; One way ANOVA: Tukey's multiple comparison tests.

^{a3} $p < 0.001$ significantly different compared with all test group

^{b3} $p < 0.001$ significantly different compared all test group

^{c3} $p < 0.001$ significantly different compared with all test group

6.7.20. Effect of LC on temperature pattern of 20 patients

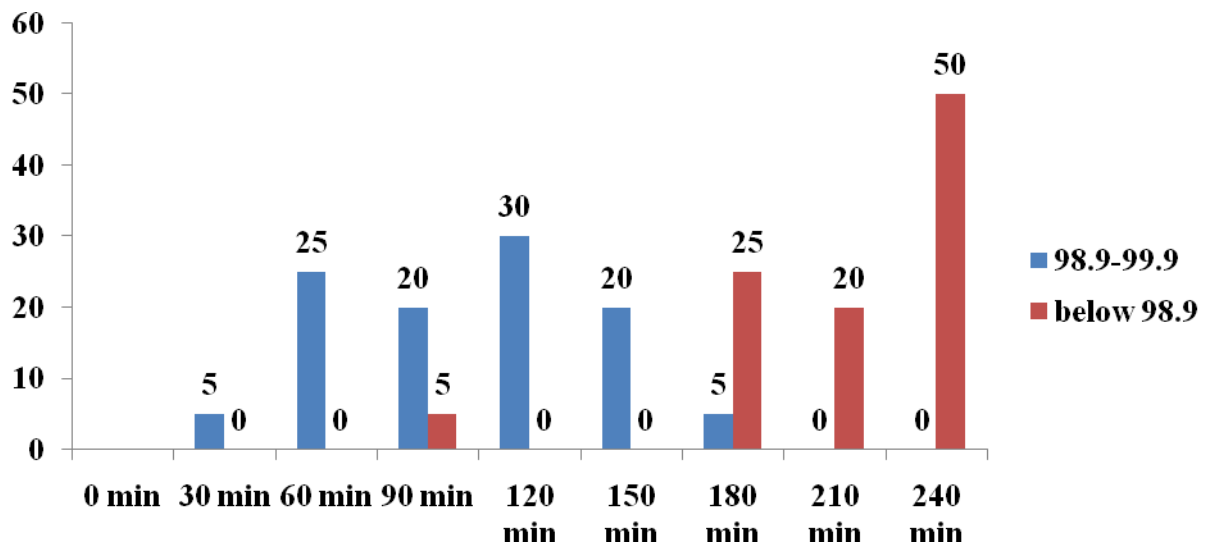


Figure 6.7.29:

Among the 20 cases,

Raised body temperature reduced between 98.9-99.9 were 5% cases had 30% min, 25% cases had 60 min, 20% cases had 90 min, 30% cases had 120 min, 20% cases had 150 min and 5% cases had 180 min

Raised body temperature reduced below 98.9, 25% cases had 180% min, 20% cases had 210 min and 50% cases had 240 min,

6.7.21. Effect of LC on Reduction of Temperature after 240 min among 22 patients

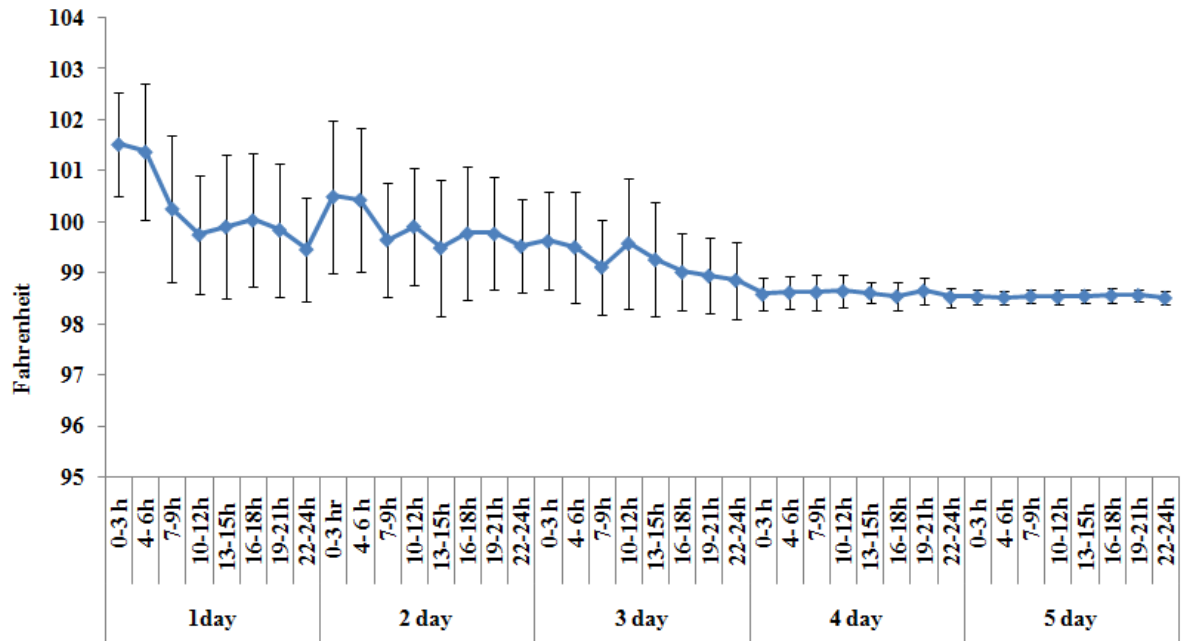


Figure 6.7.30: Effect of LC on Reduction of Temperature after 240 min among 22 patients

6.7.22. Pain scale of 70 patients

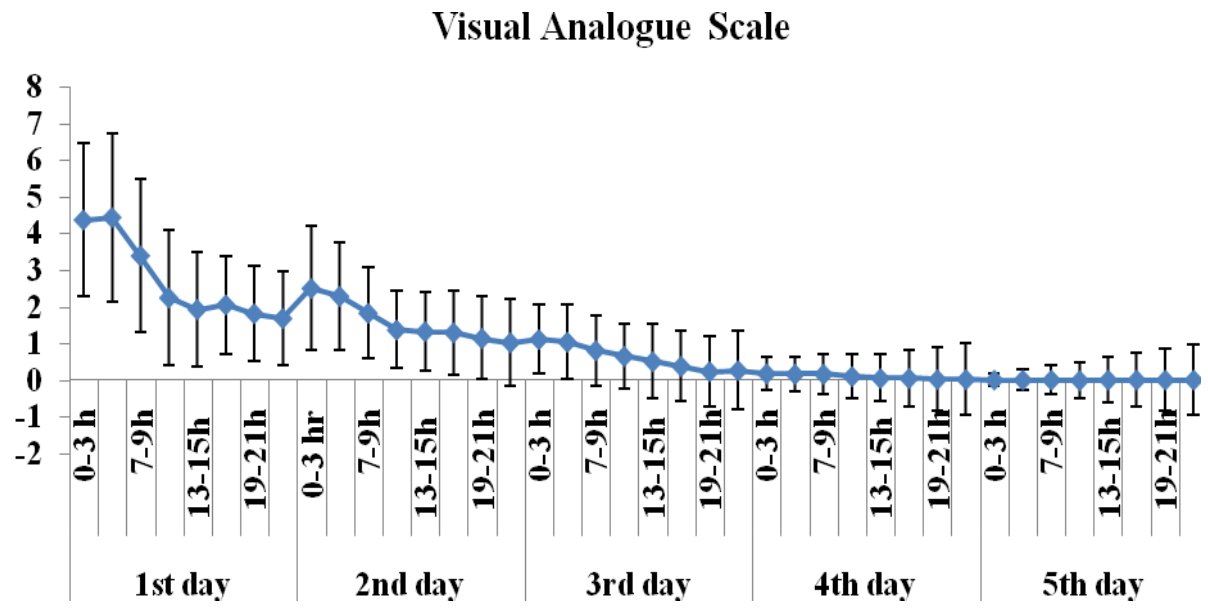


Figure 6.7.31: Effect of LC on pain scale treated on 70 patients

6.7.23. Pain severity of 70 patients

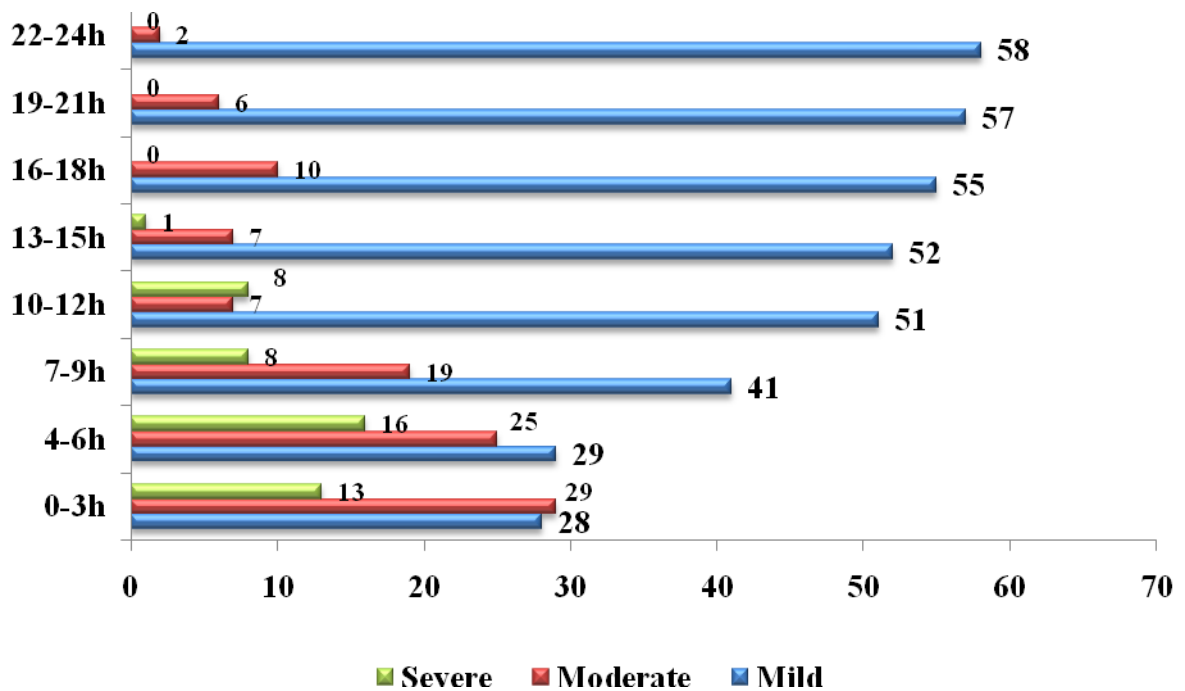


Figure 6.7.32: Effect of LC in the reduction of Pain on 70 patients - first day treatment

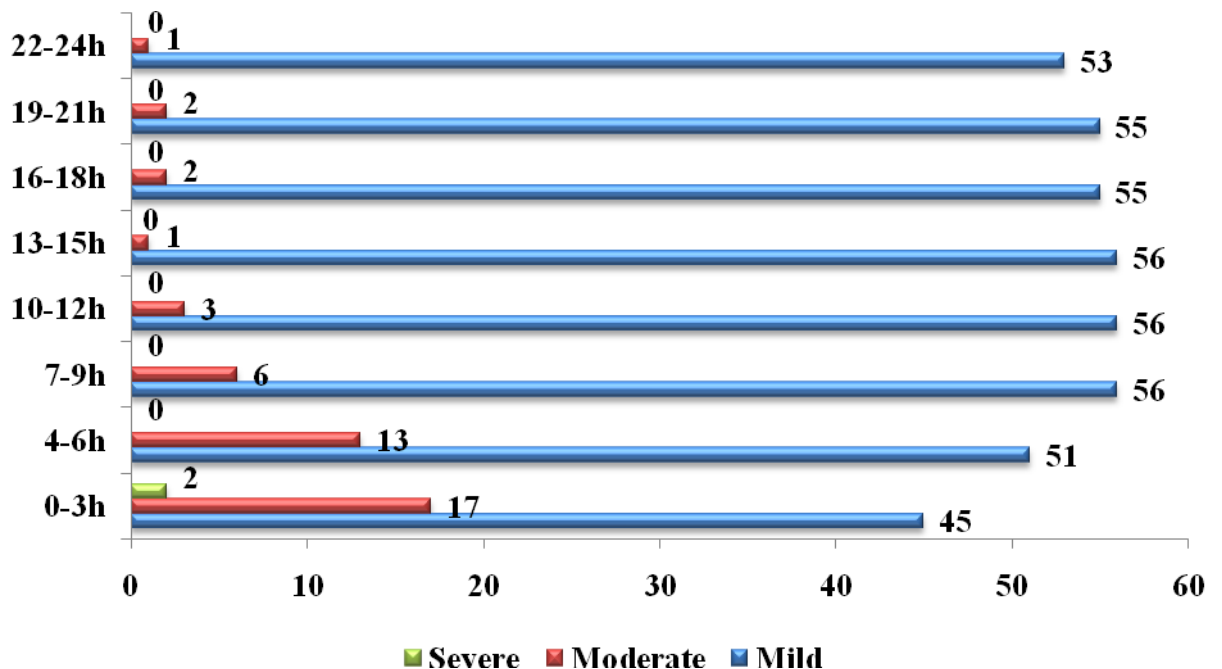


Figure 6.7.33: Effect of LC in the reduction of Pain on 70 patients – 2nd day treatment

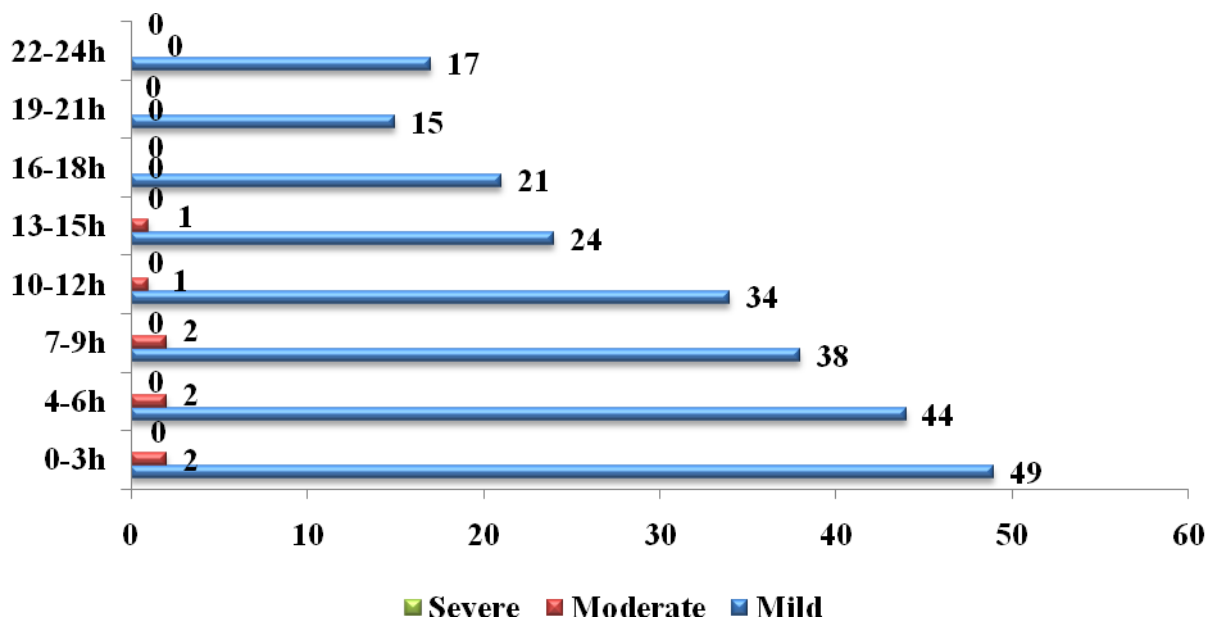


Figure 6.7.34: Effect of LC in the reduction of Pain on 70 patients – 3rd day treatment

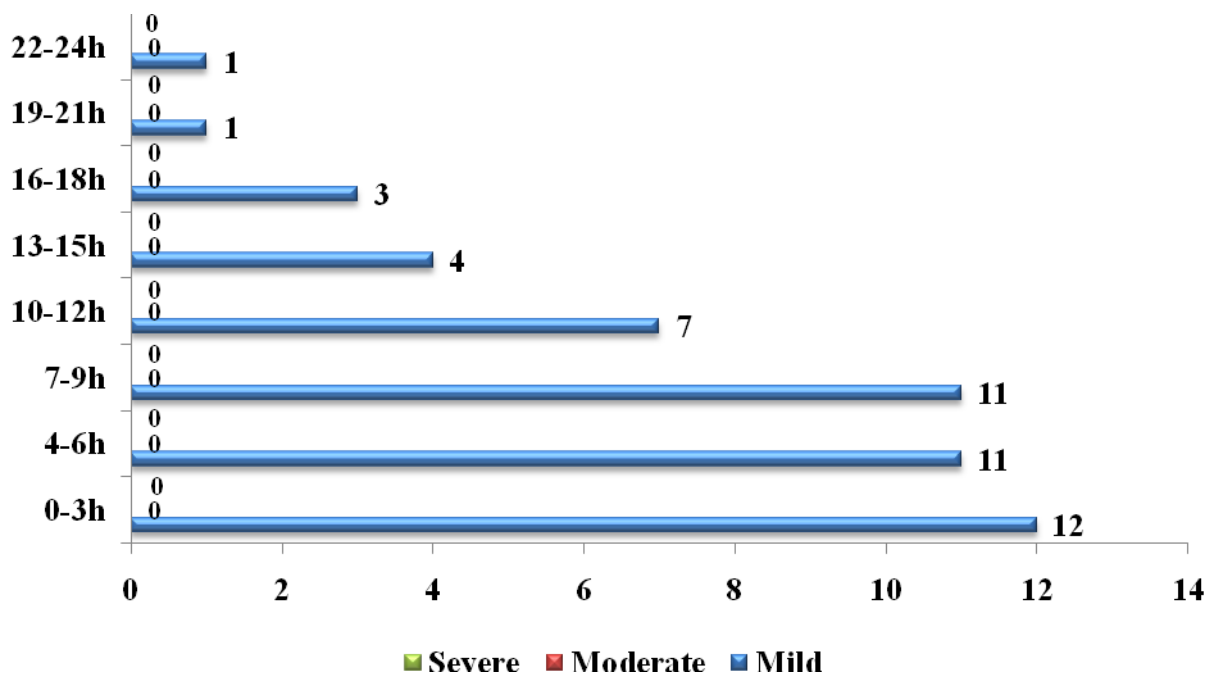


Figure 6.7.35: Effect of LC in the reduction of Pain on 70 patients – 4th day treatment

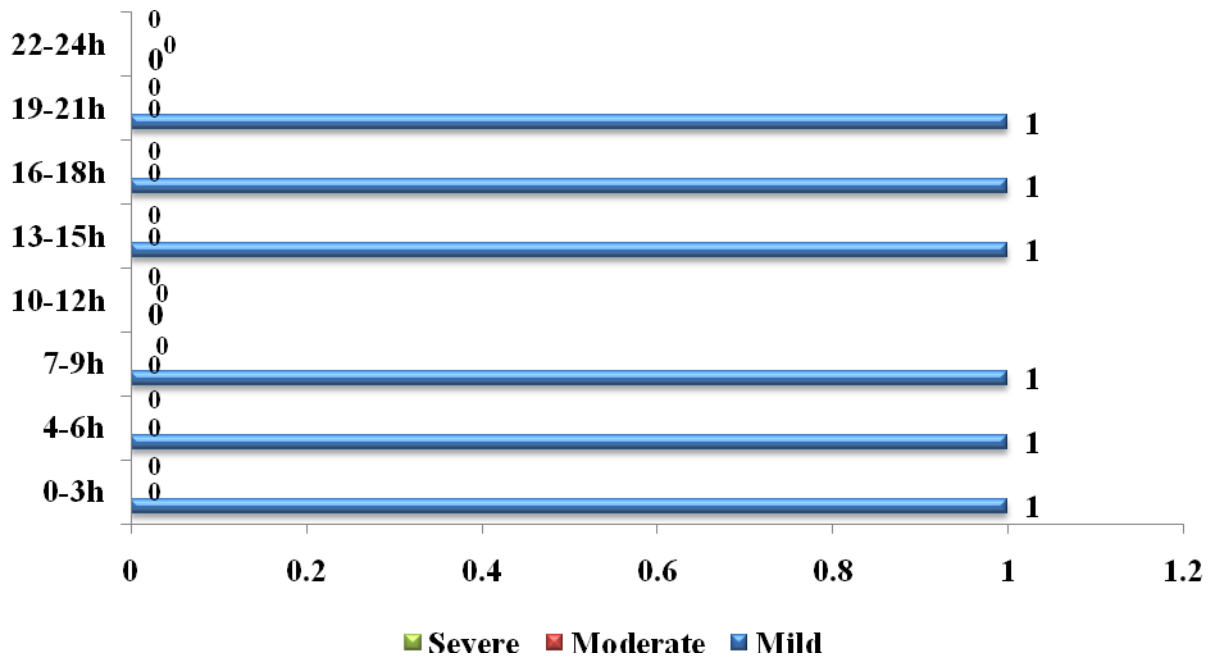


Figure 6.7.36: Effect of LC in the reduction of Pain on 70 patients – 5th day treatment

6.7.24. Effect of LC on 70 *Suram* patients to assess the primary and secondary outcomes

Symptoms	Before treatment	After treatment		
		120 min	240 min	After 240 min
Fever	70	28	20	22
Body pain	70	52	41	39
Burning sensation of eye	23	5	7	11
Nausea	15	5	2	8
Vomiting	6	3	2	1
Cough	49	3	9	37
Headache	30	6	12	12
Shivering	15	7	4	4
Tiredness	10	1	2	7

Table.6.7.1. Effect of LC on 70 *Suram* patients to assess the primary and secondary outcomes

- Among the 70 cases, the raised body temperature came to normal in 28 patients with in 120 min and in 20 patients with in 240 min and remains 22 patients after 240 min with administration of LC
- Among the 70 cases , 23 cases had burning sensation of eye, for that 23cases, 5 cases was burning sensation of eye reduced in 120 min, 7 cases was burning sensation of eye reduced in 240 min and 11 cases was burning sensation of eye reduced in after 240 min
- Among the 70 cases , 15 cases had nausea, for that 15 cases, 5 cases was nausea reduced in 120 min, 2 cases was nausea reduced in 240 min and 8 cases nausea reduced in after 240 min
- Among the 70 cases , 6 cases had vomiting , for that 6 cases, 3 cases was vomiting stopped in 120 min, 2 cases was vomiting stopped in 240 min and 1 cases vomiting stopped in after 240 min

- Among the 70 cases , 49 cases had cough, for that 49 cases, 3 cases was cough stopped in 120 min, 9 cases was cough stopped in 240 min and 37 cases cough stopped in after 240 min
- Among the 70 cases , 30 cases had headache, for that 30 cases, 6 cases was headache reduced in 120 min, 12 cases was headache reduced in 240 min and 12 cases headache reduced in after 240 min
- Among the 70 cases , 15 cases had Shivering, for that 15 cases, 7 cases was Shivering reduced in 120 min, 4 cases was Shivering reduced in 240 min and 4 cases Shivering reduced in after 240 min
- Among the 70 cases , 10 cases had Tiredness, for that 10 cases, 1 cases was Tiredness reduced in 120 min, 2 cases was Tiredness reduced in 240 min and 7 cases Tiredness reduced in after 240 min

6.7.25. Incidence no of patients had relapse of fever after 120min & 240 min

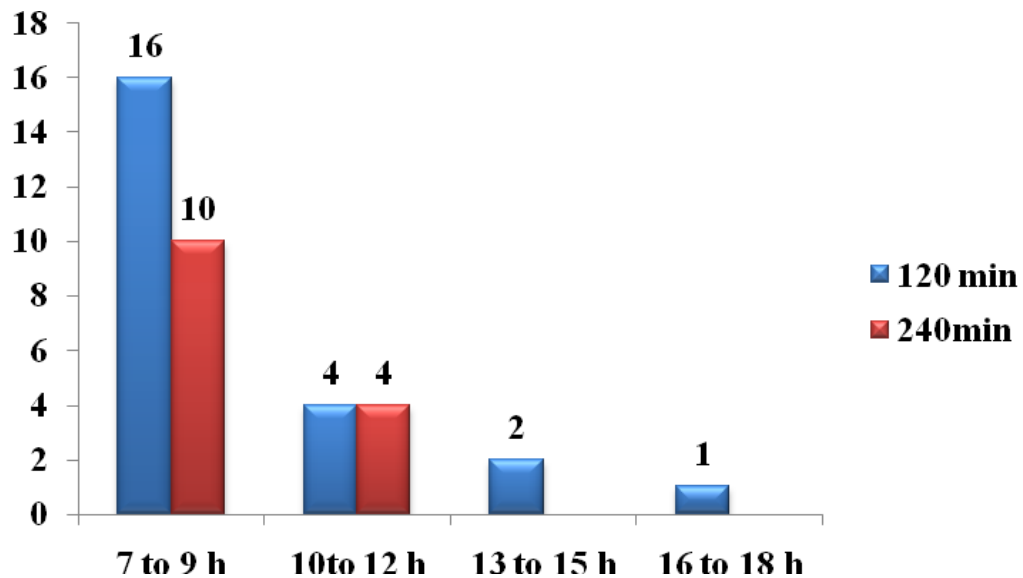


Figure 6.7.37: Incidence no of patients had relapse of fever after 120min & 240 min

In *Siddha* concepts, Pyrexia (*Suram*) is a disease, indicates the presence of infection and inflammation in our body. According to the concept of *Siddha*, the taste of food which is consumed in improper proportion plays a key role for diseases. Theran denotes Pyrexia (*Suram*) is a disease, which is caused by the accumulation of aggravated Seetham in the alimentary tract which makes increase of body temperature. Pyrexia (*Suram*) can be defined as increased body temperature above its normal range, burning sensation in eyes, pain in the body, nausea and vomiting (5).

Siddha system of medicine has more number of anti pyretic herbal drug and their formulation, metal and mineral formulations. In the study, the investigator selected mineral formulation of Linga chendhuram. Because Arun sudha et al (2009) reported that the particle size of LC analysed by DLS and TEM showed the presence of LC in nanoparticles i.e., below 100nm and that may enhance the bioabsorption and efficacy (56). Jiunn-Jye Chuu et al (2001) report that 0.1 g/kg/day of HgS for 7 day with mice did not produce toxic effect and disappeared completely in 5 weeks on cessation of administration (57). Long term use of mercurial preparation without proper indication may lead to the accumulation of mercury in liver and kidneys. The classical preparation methods, appropriate dosage with adjuvant, disease condition and age greatly influence mercurial toxicity. “*Linga chendhuram* (LC)” a herbometallic Sastric formulation has been practiced a long time for treating Pyrexia (*Suram*) and Arthritis. LC has been chosen for the study to prove it is a safer and efficacious drug in the management of Pyrexia (*Suram*) in both animal and human model. LC has literature evidence for the treatment of fever (9). The ingredients of LC were purified *Lingam* (Cinnabar) and *Atruthumatti* (Entire part of *Citrullus colocynthis*).

The raw material of *Lingam* and *Citrullus colocynthis* were identified collected and authenticated. The LC was prepared as per *Siddha* literature, under supervision at the *Gunapadam* laboratory of National Institute of *Siddha*, Chennai-47. Any drug may be prescribed to human before which we should validate the quality, safety and efficacy of the drug.

LC satisfies all the quality parameters of a chendhooram such as having brick red colour without shininess, taste and odour. On subjected to heat, LC lose its luster. On sprinkled over water, LC floats denoting the absence of heavy metals. Impingement in the

papillary ridges on rubbing LC in between Index finger and Thumb denotes the fineness and nano size of the particles present in it (47).

LC was subjected to Qualitative and Quantitative analysis with the help of instrument like XRD, SEM, AAS and ICP-OES .During the process of LC preparation, the added metallic ingredient is converted into the compound oxide forms which are proved in the study WDXRF studies. The elements found in the test drug except mercury, all were in compound oxide forms. In this quantitative study, the presence of Sulphur trioxide 27%, Arsenic trioxide 4% and some traces of other elemental oxides in LC are observed. During the preparation, the added herbal juice had blended well with hot Lingam and has made it into a consolidated state. The raw cinnabar contains 96% mercury sulphide and 4% of other elements as impurities (58-59).

On giving high temperature with juice, the sulphur present in the *Lingam* is oxidized into sulphur trioxide. The other trace elements found in the Lingam along with the juice are also converted into non toxic forms. Moreover LC is in brick fine red colour that supports that the elements are found in the oxide forms. LC is present in crystalline form which is evident from the characteristics peaks near to 26°, 31°, 37°, 43°, 45°, 53° and 56° 2-Theta. XRD finger print of LC was found to be similar to the previous work of Sudha et al., 2009, micro particle size existence of LC. (56). The peak value in between 26° - 27° and 31°-32° corresponds to the standard peaks for HgS. The remaining peaks indicate the presence of other compound formed due to the herbal juice processing.

Scanning electroscopic microscope revealed the stability of the particles having crystalline nature and presence of micron level particles. The presence of micron level sized particles influenced the LC for the better absorption in the intestines when suspended with the adjuvant honey. The size of this particle makes the LC as efficacious in its lower dosage form. XRD pattern of LC also makes a evidence that the particles size are in micron levels and crystalline nature. Arun sudha et al (2009) reported that the particle size of LC analysed by DLS and TEM showed the presence of nano particles below 100nm and that may enhance the bio absorption and efficacy which is mentioned earlier (56).

ICP-OES study revealed that LC has lower concentration of mercury, and cadmium, arsenic, lead, nickel and vanadium were below the deduction limit. Sulphur, cadmium and nickel were in reduced concentration. Mercury (3.02 ppm) and Suphur (72 ppm) were found more than the WHO permissible limit. The concentration of these

elements present at the initial stage of LC process was high since *Lingam* contains 96% of Mercury, but on continuous titration with *Citrullus colocynthis* juice causes reduced concentration of mercury and sulphide added in the finished product of LC and might be responsible for the safety and potent therapeutic value of this drug. WHO prescribed the limits for heavy metals in herbal raw material and food substances not in traditional mineral formulations. So, the therapeutic safety of LC is validated through the biological samples.

LC was orally administered at higher dose 2 gm/kg to the Wistar Albino rats in acute toxicity study and during 28 days of repeated (sub acute) toxicity study, at daily doses of 18, 90 & 180 mg/kg of body weight to the Wistar Albino rats. The acute toxicity study showed no mortality of rats up to the dosage of 2000mg/kg. No behavioural changes or abnormal clinical signs of toxicity were observed up to the above dosage throughout the end of 14 day study period. No gross pathological abnormality in the organs was found even at this high dose. LD₅₀ value was found to be more than 2000 mg/kg body weight and therefore this test drug *Linga chendhuram* falls under (Unclassified) category V with reference to Globally Harmonized classification System. Jiunn-Jye Chuu et al (2001) report that 0.1 g kg/day of HgS for 7 day with mice did not produce toxic effect and disappeared completely in 5 weeks to the cessation of administration (57). In this study, the dose of test drug administered in rat was 100 times more than that of the human therapeutic dose. It was clearly proved that the human therapeutic dose was absolutely free from acute toxicity.

For a period of 28 consecutive days of oral treatment of LC at 18, 90 & 180mg/kg/day in both sexes of rat, no treatment related toxicity signs or mortality were observed. Feed and water consumption of treated groups were found not to be significantly affected or changed in both sexes compared to the distilled water treated rats. Consumption of toxic substances effects at least a minimal reduction in body weight gain and internal organs weight (60). But no significant change in the body weight gained or lost in the treated test groups were observed compared with control group during the study. The absolute and relative organs weight was also not altered by LC treatments. If LC is a toxic substance, there will be a minimal reduction in body weight gain and internal organs weight. The changes observed in blood parameters analyzed in animal laboratory animals provide the evidence of risk of toxic effects on haematological system (61).

Haematological parameters such as Total WBC, Lymphocytes, Monocytes, Granulocytes, Hemoglobin, Total RBC, RDW, Hematocrit, Platelet, Platelet crit, PDW and MPV in male rats showed no significant differences in relation to the control group. MCH and MCHC value was decreased significantly in all three doses (18, 90 & 180mg/kg) levels on compared with control group. Haematological parameters such as Total WBC, Lymphocytes, Granulocytes, MCV, PDW and MPV in female rats showed no significant differences in relation to the control group. LC at 18 mg/kg (Low dose) induced to increase the values of Hb, RBC count, RDW, Hematocrit and platelet and decrease the values of platelet crit in relation to the control group. LC at 90 mg/kg (Mid dose) induced to increase the values of RBC count, Platelet and Platelet crit and decrease the values of monocyte in relation to the control group. LC at 180 mg/kg (High dose) induced to increase the values of RBC count and Platelet and decrease the values of MCH in relation to the control group. Haematopoiesis is the process of formation of blood cellular components which includes leukopoiesis stage i.e. the formation of white blood cells in bone marrow (adults) and haematopoietic organs (foetus) (62). However, the significant differences noted in the parameters at low, mid and high groups lies within normal physiological limits indicated that LC did not affect haematopoiesis or leukopoiesis in rats and that suggested LC did not produce any toxicity in the blood forming organs affecting the haematopoietic indices.

Estimation of SGOT, SGPT, Bilirubin and ALP levels are the useful indicators of hepatic function and Protein, Urea, Creatinine and electrolytes such as Sodium, Potassium and Chlorides are the useful indicators of renal function. Parameters such as glucose, cholesterol, SGOT, sodium, potassium and chloride in male rats showed no significant differences in relation to the control group. LC at 18 mg/kg (Low dose) induced to increase the values of triglycerides and bilirubin and decrease the values of urea, creatinine and ALP in relation to the control group. LC at 90 mg/kg (mid dose) induced to decrease the values of bilirubin and ALP in relation to the control group. LC at 180 mg/kg (Low dose) induced to increase the values of triglycerides and decrease the values of SGPT in relation to the control group. But in female rats, treated with LC at three doses (18, 90 & 180mg/kg) compared with control group, no statistically significant difference was recorded in any of the biochemical parameters examined. Liver and Kidney play a significant role in eliminating toxins or foreign materials. If these organs are damaged, it will result in increased excretion of hepatic enzymes and protein, urea,

and creatinine in the blood. All biochemical parameters investigated were within the normal limits in the test group's favors LC did not cause damage to the heart, liver, kidney and other organs.

This was further confirmed by the gross necropsy studies that had been on the organs which revealed no abnormal pathological morphology. H&E stained Kidney slices of female rats of high dose group showed changes in their architecture by the presence of minimal infiltration of mononuclear cells that indicated slight inflammation of renal parenchyma. But, in the kidney of male rats, no evidence of such lesions was observed. Generally, mononuclear cell infiltrations of kidney had been seen in one third of the normal laboratory rats (63) and this infiltration is not expected to be originated from bone marrow (64). No abnormal changes were observed in other Haematological and Eosin stained slices of organs and so their images were not presented here. From the above studies, it is inferred that LC at high dose (180 mg/kg) produced statistically significant variations in relation to the control group and lowest observed adverse effect level (LOAEL) were seen at high dose 180 mg/kg/day. The high dose of LC employed in this study is ten times more than the dose employed in humans for therapy. Moreover, the oral intermittent dose 90mg/kg/day (Similar to therapeutic dose in human) of LC administered for 28 consecutive days does not induce any biochemical, haematological, anatomical and histopathological signs of toxicity and the above dose produces no observed adverse effect level (NOAEL) for both the gender under the experimental conditions used.

The pharmacological studies such as Analgesic activity (hot plate, writhing study), anti inflammatory activity (Carrageenan induced paw edema and cotton pellet) and Anti pyretic activity was carried out on LC at the dose of 18, 90 & 180 mg/kg b.wt. The stimulation by thermal induced hot plate is a specific model for central analgesic activity (65). The results of analgesic activity by hot plate method in mice of LC observed at the three different dose levels showed significant response in prolongation of time when compared to control. At the higher dose 180 g/kg b.wt of LC prolonged the hot plate latency at 1 h comparable with the standard analgesic drug Aspirin.

Analgesic activity of LC done in mice shows significant activity at the dose of 90 and 180 mg/kg against peripherally induced acetic acid induced abdominal writhing. On administration of acetic acid in the peritoneum, it is believed to act indirectly by inducing the release of pain mediators such as prostaglandins and lipooxygenase into the peritoneum and nociceptive neurons are stimulated (66). These neurons are sensitive to

the NSAID's and hence this model is used for the screening of anti-analgesic drugs. The result of the study suggests that LC might inhibit the pain mediators in the peripheral tissues.

The potency of test drug against acute inflammation is commonly tested in Carrageenan induced paw edema model. On injection of Carrageenan leads to formation of paw edema in the rat as biphasic event. The first phase occurs due to release of histamine and serotonin by increased vascular permeability and this phase lasts for one hour. The second phase is by the leucocytes infiltration and there is the release of prostaglandins, proteases and lysosomes (67 -68).

Carrageenan causes the release of bradykinin which leads to the formation of prostaglandins. These prostaglandins are responsible for the formation and infiltration of inflammatory exudates (69). In our present study, LC at the dose of 90 and 180 mg/kg significantly reduced the paw oedema within one hour of treatment when compared with control. The underlying mechanism of anti-inflammatory activity in acute condition for LC might be involved in the inhibition of mediators responsible for first phase of carrageenan induced inflammation.

Against chronic inflammation, cotton pellet granuloma method is a validated method for screening the drug activity (70). This method employed to access the transudative and proliferative components of chronic inflammation. The proliferative components such as macrophages, neutrophils and fibroblasts invaginated in the granuloma formed. The fluid exudates from the lesion absorbed by the pellet strongly increase the wet weight of the granuloma. The amount of granulomatous tissue formed correlate with the dry weight of the pellet (71). The decrease in dry granuloma weight in the study done on rats indicated that LC suppressed effectively on the proliferative phase of inflammation.

Elevation of body temperature is caused by various endogenous pyrogenic substances such as interleukins, macrophages and prostaglandins . The synthesis of Prostaglandin E2 is activated by tumor necrosis factor- α (72) and phospholipase A2. Formation of Prostaglandin E2 is the main cause for the elevation of body temperature by triggering the hypothalamus (70). For the screening of a test drug against pyrexia, Brewer's yeast induced pyrexia model is a validated one in rat (73).

Brewer's yeast is a lipopolysaccharide component in cell wall gram -ve bacteria. On injection of Brewer's yeast, the lipopolysaccharides is linked with an immunological

protein i.e., Lipopolysaccharide-Binding Protein. This linkage involves in endogenous pyrogens generation which leads to the synthesis of prostaglandins and its releases. Generally, NSAIDS drugs reduced the increased body temperature by the inhibition of Prostaglandin E2 biosynthesis in hypothalamus. The anti pyretic activity of *Linga Chendhuram* might be due to the inhibition of Prostaglandin E2 biosynthesis. In this study, the results showed that the *Linga Chendhuram* at 18, 90, 180 mg/kg possesses a significant ($p < 0.001$) anti pyretic effect in Brewer's yeast induced pyrexia in Wistar rats. This anti pyretic effect was maintained up to 5 hours and it justifies the validity of efficacy of *Linga Chendhuram* at the therapeutic dose of 65 mg/kg/dose.

The clinical study was carried out to validate the efficacy of *Linga Chendhuram*. In this study, totally 145 patients were screened for fever between the age group 21-60. Out of 145 patients, 100 patients were fulfilled major inclusion criteria and eligible for the study. Among 100, 75 patients gave willingness to participate in the study and 75 patients were enrolled. Finally 70 patients completed the study.

Among the study population, the incidence of fever in both gender are more less equally affected. According to Siddha, majority (89%) of patients were belonging to the land tract of *Neithal* (Coastal region). This might be due to the proximity of study centre. National institute of Siddha is situated in sub urban area of Chennai near to coastal region. Naadi nadai (Pulse readings) among the study population was found to be *valiazhal* and *azhavali* which are 36% and 33% respectively. As per Siddha text (4) naadi nadai mentioned for fever are *valiazhal* and *azhavali*. Thus the findings of the study are in line with Siddha concept. In urine examination (Neerkuri), it was observed that majority of the patients had pale yellow and yellow colour urine. Thus the results of neerkuri (4) shows increased body temperature under the pathological characteristics of yellow colour urine (*Manjal neer vigarppam*). Neikuri was observed among the study population. 31 patients had sieve pattern and 16 patients had the mode of quickly spread along with sieve pattern.

70 patients were treated with LC at 65 mg/kg/dose, thrice a time daily with the adjuvant honey for five days. The primary outcome measure in this study was reduction in body temperature after treatment. Nearly, 70% of patients were responded well (Reduction of temperature) to the first dose of treatment. The level of fever decreased rapidly and substantially within 2 h of the administration of the first drug dose. The subjects had practically no fever until very close to the time of the second dose and this

trend continued for the subsequent doses as well. The periodic peaking of fever levels just before the drug administration also showed a clear trend of sharp decrease as compared to the initial fever level. One of the secondary outcome measures – pain was reduced in 75% of patients having first dose of treatment.

Evaluation of the anti pyretic action of LC during the clinical trial indicates that this herbomineral preparation exhibits significant antipyretic efficacy that is a substantial and sustained nature as some of the existing chemical anti pyretic such as paracetamol. The use of LC also led to a substantial reduction in the incidence of associated secondary physical parameters of fever.

SUMMARY AND CONCLUSION

Fever (Pyrexia) is a symptom of elevation in body temperature associated in various disease conditions particularly in infectious and autoimmune diseases. Because of the hypothalamic thermoregulatory control normal body temperature is maintained instead of maintaining environmental temperature due to the the excessive heat production derived from metabolic activity in liver and the muscle with heat dissipation from the lungs and skin. In *Siddha* literature, fever is considered as a disease under the terminology -*Suram*. Sage *Theran* denotes *Suram* is caused by an accumulation of aggravated *Seetham* in the alimentary tract which makes increase in body temperature. *Suram* is manifested as increased body temperature above its normal range, burning sensation in eyes, pain in the body, nausea and vomiting. For treating pyrexia, numerous medications were illustrated in the *Siddha* literatures. Among them, the herbometallic Sastric formulation “*Linga Chendhuram (LC)*” has been practiced frequently for treating fever, arthritis and venereal diseases. LC has been chosen for the study to prove as a safe and efficacious state of the drug in the management of fever in both animal model and human subjects.

LC was prepared in Gunapadam laboratory of National Institute of Siddha, Chennai as per the method cited in the “1940 drug and cosmetic act” authenticated literature “*Siddha Vaithiya Thirattu*”. LC was analyzed for both qualitative and quantitative estimations. Preliminary physical parameters such as total ash, moisture content and extractive values were analyzed. The crystalline nature of LC and main contents were analyzed using X ray diffraction study. The content of lead and cadmium were analyzed using Atomic Absorption Spectroscopic study. The concentration of elements in oxide form was analyzed through Wavelength dispersive X-Ray Fluorescence. The concentration of trace and heavy metals were analyzed using Inductively Coupled Plasma – Optical Emission Spectrometer. From the result of above studies, we inferred that LC was feasible to conduct the study on animal model and human subjects. Mercuric and Sulphur trioxides were found as major constituents in LC.

To evaluate the safety of LC, Wistar albino rats were used for performing acute and 28 days repeated oral toxicity studies following OECD guidelines. 2000 mg/kg of LC was tested on six rats and observed nil mortality and morbidity. Median lethal dose was

estimated as more than 2000 mg/kg for the test drug. The sub-acute toxicity was observed on Wistar rats by giving LC at three dose levels (18, 90 & 180 mg/kg) for 28 days along with its vehicle (Diluted honey). The test drug dose was fixed from the human conversion dose (195 mg/day) to rat. No mortality and abnormal clinical signs were observed during 28 days. All test dose treated animals gave comparable body weight and organ weight gain with that of control. Haematological, biochemical parameters and urinalysis were within the normal limit. No significant abnormality was detected in gross necropsy study on organs and in H&E sliced organs.

The efficacy of LC at the dose of 18, 90 and 180 mg/kg was evaluated on BALB/C mice and Wistar rats. In the evaluation of analgesic activity by hot plate method, the pain reaction time was prolonged in test groups on 60 min post treatment when compared with control. Acetic acid induced writhing test was performed on mice and observed LC has significant analgesic activity by inhibiting the incidence of writhing as compared to control. LC at high dose 180 mg/kg has better central and peripheral analgesic activity. In the model of Carrageenan induced acute inflammation of paw, significant reduction in paw volume was observed in all the test groups when compared to positive control group. The percentage of inhibition of maximum oedema inhibition was high in LC group treated at 180 mg/kg and observed that the inflammatory action achieved by LC in dose dependent manner. Cotton pellet granuloma method was performed on rat and observed LC has significant anti-inflammatory activity by reducing the granuloma formation. LC at three dosage levels was studied for antipyretic activity in rats using Brewer's yeast-induced pyrexia models. LC at all tested dose levels produced a significant dose dependent inhibition of temperature elevation compared with the normal rat. After 2 h treatment, LC significantly decreased yeast induced pyrexia in rats. These results indicate that LC has potent antipyretic activity which and pharmacologically justifies its use in the management of fever.

The safety and efficacy of LC to humans was determined by giving LC at its therapeutic dose 65 mg/dose suspended with vehicle honey for three times daily for the duration of 5 days to the clinical subjects was done on human. The study was designed as non- randomized open labeled without control single centric study. The age group between 20 to 60 years and both gender of 75 patients were recruited after analyzing the

inclusion and exclusion criteria. The patients were recruited having temperature between 100 to 103°F. Among 75, 70 patients (36 male and 34 female) completed the study of 5 days treatment. The temperature was measured by digital thermometer every 30 min for first 4 h and three hours once for further period. The intensity of pain was observed using Visual Analogue Scale (VAS) three hours once during the course of treatment. 28 patients show better reduction of elevated body temperature with in 120 min after treatment and 20 patients showed better reduction of elevated body temperature with in 240 min after treatment.

From the above studies, it can be concluded as follows

1. LC has long shelf life period and free from toxins.
2. Median lethal dose for LC was calculated as more than 2000 mg/kg body weight.
3. No observed effect level (NOEL) of LC administered by us to Wistar Rats through oral route over a period of 28 days was found to be 180 mg/kg body weight for both male and female rats.
4. LC at 18, 90 & 180 mg/kg demonstrated anti pyretic, analgesic and anti-inflammatory properties in animal model.
5. The clinical study proved that LC at its intended human therapeutic dosage 65 mg/kg along with honey is a safe and efficacious drug in treating *Suram* (Pyrexia).

RECOMMENDATIONS

The future studies have to be carried out on LC on the following aspects

1. Solubility studies on LC as per OECD guideline 105
2. Creating the standard physiochemical finger print for LC by comparing the qualitative and quantitative analyzes of different batch preparations.
3. Mechanism of anti-pyretic activity in the field of molecular biology.
4. Double blind randomized controlled clinical trial for global acceptance on LC against pyrexia and also for pain management in arthritis.

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