PRECLINICAL STUDY OF SIDDHA DRUG LINGA CHENDURAM 'S APHRODISIAC, SPERMATOGENESIS AND ANTI-OXIDANT ACTIVITIES

Dissertation submitted to

THE TAMILNADU DR. MGR MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfilment of the requirements

for the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II-GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

THE GOVERNMENT SIDDHA MEDICAL COLLEGE

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OCTOBER 2019

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PALAYAMKOTTAI
DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Pre clinical study of herbo mineral drug *LINGA CHENDURAM* for its aphrodisiac, spermatogenesis and anti-oxidant activities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. R. Antony Duraichi M.D(s)., Lecturer Gr-II,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Palayamkottai and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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ABBREVIATIONS

LC	-	LINGA CHENDURAM
CPCSEA	-	Committee for the purpose of control and supervision of experimental animals.
DC	-	Differential Count
ESR	-	Erythrocyte Sedimentation Rate
FTIR	-	Fourier transform infrared spectroscopy
Hb	-	Haemoglobin
IAEC	-	Institutional Animal Ethical Committee.
ICP-OES	-	Inductively coupled plasma optical emission spectrometry
Ig E	-	Immunoglobulin E
LDH	-	Lactate Dehydrogenase
MCV	-	Mean Corpuscular Volume
OECD	-	Organisation for Economic Co-operation and Development
PCV	-	Packed Cell Volume.
PGE	-	Prostaglandin E
RBC	-	Red Blood Corpuscles
SEM	-	Scanning electron microscope
CCD_S	-	Charge coupled devices.
SPME	-	Solid phase micro extraction
TCD	-	Thermal conductivity detector
FID	-	Flame Ionization detector
CCD	-	Catalytic combustion detector
LD	-	Low dose
Mg		Milligram
Kg		Kilogram
LD_{50}		Lethal Dose $_{50}$
p.o		peros
ML		Milliliter
%		percentage
R&D		Research and Development

EDTA	Ethylene Diamine Tetra Acetic Acid
M	Male
g%	Gram percentage
g	Gram
NOAEL	No-Observed-Adverse-Effect-Level
MLD	Minimum Lethal Dose
MTD	Maximum Tolerated Dose

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CERTIFICATE

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Name of the principle investigator : Dr. A.Suganya

Title of the Project : *Linga Chenduram* for its Aphrodiasiac , Spermatogenisis & anti oxidant activities

Proposal Number : AKCP/IAEC/15 ,43 / 2018-19

Date of received after modification (if any) : Nil

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Animals : Mice & Rats

Expiry Date : Nil

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CERTIFICATE OF GUNAPADAM AUTHENTICITY

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TAMIL NAME	ENGLISH NAME	CHEMICAL NAME
LINGAM	CINNABAR	RED SULPHIDE OF MERCURY

Date : 4.7.18.

Station: Palayamkottai

4/7/18.

Authorised Signature.

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TAMIL NAME	BOTANICAL NAME	FAMILY	PART USED
THAMARAI	*Nelumbo nucifera,Gaertn.*	**Nelumbonaceae**	**Tuber**

Date : 4.7.18.

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1. INTRODUCTION

Siddha system of medicine is one of the oldest medicinal systems of India, existed separately in early times.

"Siddha" derives from the word of "Siddhars" who formulated this therapy. Siddha system is based on balancing of equilibrium of Pancha bhoothas *(Doshas)* as "LAND (*Prithivi),* WATER (*Appu),* FIRE (*Theyu),* AIR *(Vayu),* SPACE *(Agayam).*

The human body in also made up of seven physical constituents, three vital humours. In normal state the vital humours the body occur in healthy condition any imbalance (or) derangement leads to disease.

It focus on physical, mental and spiritual wellbeing thus giving a total perfection in life. As herbs, metals, minerals, animals and its derivatives are the core sources for siddha medicines; this system endorses the safest medical care. Siddha is the only system comprising 64 varieties of medicine, i.e 32 types of internal medicines and 32 types of external medicines.

According to siddha changes in diet and lifestyle modification, environmental factors, congenital, genetical inheritance are the key factors for the investigation of each disease via disturbing the equilibrium status of the vital humorus namely vali, azhal and iyyam.

"வெல்லும் புவியில் விளங்கிய தாபரம்
மல்லிடுஞ் சங்கமம் பொறியிடும் விந்தே
விந்தினாலல்லரோ மேதினி யாச்சுது (மேதினி – உலகம்)
செந்துக்களொல்லாஞ் சிவமய மாச்சுது
வந்திடும் நாதம் மெலாம்கலந்திது
சிந்தையிலுள்ளத் தெளிவாகச் செப்பு"

\- தேரையர்

According to siddhar therayar, it is understood that the vital thing that is essential for the origin of life is "vindhu" from the fusion of vindhu(sperm) with nadham (ovum), living organisms get generated, which all symbolises Lord siva (Sivamayam), thus the reproductive process is so important for the origin and existence of life in this world.

In human beings, sexual health is a state of physical, emotional, mental and social well-being in relation to sexuality along with the absence of disease, dysfuntion

or infirmity. Sexual health requires a positive and respectful approach to sexuality as well as possibility of having pleasurable sexual experience.

Reproduction is initiated by the mating of a male with a female in sexual intercourse, which facilitates the coming together of sperm and ovum for fertilisation. For that there to be a normal sexual intercourse and sexual fulfillment.

In males, the male sexual organs (copulatory organ, penis) and the factor relating to erection must function normally. Inability to perform this function effetively is a major problem affecting the reproductive process.

In our siddha system the medicine, premature ejaculation may be compared to thuritha vinthozhuku (Sukkila vatham).

சுக்கிலவாதம் (Premature Ejaculation)

"வாயுவாதம் காற்றினிடை வந்தால் அவயவங்கள்
பாயுங்கால் வலிக்கும் பண்ணுகுணம் - காயத்தின்
சுக்கிலக் காலந்திரத்திற் துன்னு துரிதமின்னும்
புக்கிநிறத் தாது கெட்டுப்போகும்."

அகத்தியர் வைத்திய சிந்தாமணி 4000 பாடல்

Ejaculating of semen immediately during coitus, weakness of physical constituents.

Premature ejaculation (PE) is likely the most common sexual dysfunction in men with a worldwide prevalence of approximately 30%. The prevalence of PE appears to vary across socio-cultural and geographic populations. Premature ejaculation is defined as ejaculation of semen during sexual intercourse before (or) immediately after penetration premature ejaculation, the most common form of sexual dysfunction in men, often is due to performance anxiety during sex. Intercourse after long intervals may also result in premature ejaculation. In some cases, premature and inhibited ejaculations are caused by psychological factors, including a strict religious back ground that causes the person to view sex as sinful, a lack of attraction for a partner and past traumatic events. In addition, certain medication, particularly those used to treat mood disorders, may cause problems with ejaculation. Aphrodisiac can be categorized according to their mode of action into three groups. Substances that increase libido (i.e sexual desire arousal), substances that increase sexual potency (i.e effectiveness of erection), substances that increase sexual pleasure.

In ancient indian system of medicine, a number of drugs, from indigenous plant source have been described for PE, sexual dysfunction. In our siddha system the medicine, premature ejaculation may be compared to thuritha vinthozhuku (Sukila vatham). In that aspect there is a preparation in the literature of "Anuboga vaidhiya Navaneetham", "Linga chenduram" which is indicated for premature ejaculation. Linga chenduram is one of the Herbo-mineral medicine having aphrodisiac, spermatogenesis anti oxidant.

2. AIM AND OBJECTIVES

AIM

The aim of this study is to do a scientific review to validate the safety and efficacy of *'LINGA CHENDURAM '* for "*sukilavatham" (*pre mature ejaculation) by pre-clinical studies.

OBJECTIVES

The following methodology was adopted to evaluate the safety and efficacy of the test drug.

1. To collect the relevant Classical *Siddha* literature as well as Modern Sciences that supports the study.
2. To standardize the preparation of the drug according to classical *siddha* literature.
3. To subject the drug into Physico chemical & Phyto chemical analysis.
4. To analyze the drug biochemically for the detection of acidic and basic radicals.
5. To Estimate the percentage of elements present in the drug by Instrumental analysis.
6. To evaluate the acute and sub-acute toxicity studies profile of *LINGA CHENDURAM* according to OECD guidelines.
7. To Establish the following Pharmacological activities:
    - Aphrodisiac,
    - Spermatogenisis,
    - Antioxidant.

3. REVIEW LITERATURE

3.1. CINNABAR *(Mercury sulphide)*

3.1.1 GUNAPADAM ASPECT

TAMIL NAME :

Lingam

SYNONYMS

Aankuri, Inkuligam, Irraasam, Kadaivanni, Karppam, Kalikkam, Kaanjanam, Kaaranam, Saaniyam, Sandagam, Samarasam, Chendooram, Maniraagam, Milecham, Vani and Vanni.

NAME REASON:

Vannikarpam : It indicates colour of fire, potency of heat.

-Gunapadam Thadhu – Jeeva vaguppu pg.no-200

-Thatchanayanar vaidhya attavanai pg.no.45-46

-Anuboga vaidhya navanitham part 4 pg.no -2

VERNACULAR NAMES:

Tamil	:	*Lingam*
Eng	:	*Cinnabar*
Tel	:	*Ingileekam*
Arab & Persi	:	*Hind*
Guj	:	*Hingalo*
Canarose	:	*Inglika*
Malayal	:	*Chayilyam*
Urudu	:	*Singraff*

In Sanskrit:

Hingula, Tharadha, Milecha, Maniraga, Adhiraktha, Karkada, Seershona, Paaraja, Sarmaranjana, Maniragakara, Ranga, Indirakobaruna, Aruna.

In Arabic:

Janjbar, Jankbar, Shanjrab, Shanjarab, Gunbare nanari, Gunbaresiyus, Asreegun, Yavuth, Surk, Kaha, Hajruththeen, Hingool, Shankaap, Eengoor.

PROPERTIES:

Colour	:	Bright red colour, Cochineal red
Appearance	:	Red crystal, Rhombohedral to tabular,Granular to massive and as incrustations
Cleavage	:	Prismatic, Perfect
Fracture	:	Uneven to subconchoidal
Tenacity	:	Slightly sectile
Hardness (Mohs scale):		2.0 – 2.5
Luster	:	Adamantine to dull
Streak	:	Scarlet
Specific gravity	:	8.176
Potency	:	Hot
Taste	:	No taste

ACTION:

- Antipyretic
- Tonic
- Antidiarrhoeal
- Antivaadha
- Astringent
- Alterative
- Stimulant

GENERAL CHARACTER

"பேதிசுரஞ்சந்தி பெருவிரண நீரொடுத
காதகடி காசங் கரப்பான்புண் -ணோத
வுருவிலிங்க சங்கதமா யூறுகட்டி யும்போங்
குருவிலிங்க சங்கமத்தைக் கொள்".

"ஆதி பிரதவுக் காதலாற் சாதிலிங்க
மோதி லிரதகுண முற்றூடலிற் -றீதுபுரி
குட்டங் கிரந்தி கொடுஞ்சூலை வாதமுத
ட்டங் நோய்களையோட் டும்."

- குணபாடம் தாது சீவ வகுப்பு

This preparation is effective in the treatment of diarrhorea, pyrexia, delirium, ticaria, dieresis, 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.

In Ayurvedha:

Heat potency.

It cures *Nanju, kuttam, Sivandha noi, Thol noi.*

In Unani:

It cures *Varattu sori, Kuttam* , burns.

It is used for leprosy, skin diseases, Venereal diseases also.

OCCURRENCE OF LINGA PADANAM:

The ancient history says, that when the Lord Shiva burnt the Tripura a molecule containing Rasam & Gendhi fell from the third eye of Lord Shiva fall on the universe under the mountain of Bengal at the east side of Meru.

Rasam is otherwise called Siva and Gandaga is otherwise called Sakthi. So lingam is called Sivasakthi. Rasam is related to the sun and Gandaga is re;ated to the moon. So Indian system concluded that all the drugs have been formed from Rasam and Gandagam. They are also being used to cure almost all the diseases.

Nowadays, the red sulphide of mercury used by us, is called as *jathi linga* paadana, grouped under *vaippu paadanam.*

Speciality of Lingam:

Lingam is the basic material for Rasavadham and Vaithyam. Since it is the combination of mercury and sulphur.

If it is properly purified and used, it will be very powerful and better than any other drugs.

METHOD OF PREPARATION (VAIPPU):

Purified mercury	:	280gm
Sulphur	:	70gm
Pottasium Nitrate	:	70gm

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 not potency. It has properties of a tonic.

PREPARATION OF LINGARASAM FROM LINGAM:

One part of cinnabar powder is added with four parts of the root bark powder of Ceylon leadword (Plumbago zeylanica) and placed in sublimation appliances and burnt. The mercury Rasa will stick to the upper portion of the apparatus. This is collected carefully to get linga rasam.

Another Method:

Lingam - one part

Turmeric powder (curcuma longa) – Four part

The above ingredients are mixed together and rolled in a cloth in the form of wick and the wick is placed in a mud plate. The mud plate is covered by another big mud pot and the wick is burnt. The mercury which is sediments in the upper mud pot is collected out.

METHOD OF PURIFICATION:

Alangium bark (Alangium saulifolium) – 1400gm is powdered and added with vinegar is powdered and added with vinegar 5.2litres and placed indews in the night. The next day it is rubbed and mixed well 35gm of cinnabar is tied well in a cloth and put into the above liquid. The pot is covered with another pot sealed with mud pasted cloth, 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 (pulikarunai) and Indian sarasaparilla (Nannari) root as stressed in the following Tamil verses.

சொல்லப் கேள் புலத்தியனே மகனேயிந்தந்
துறையான சாதிலிங்க சுத்திதானே
வெல்லக்கே ளழிஞ்சில் புளிங் கருணைபோடு
மேலான நன்னாரிக் காடித் தண்ணீர்

புல்லன் கேளதிலோர் முன்று வைகல்

புகையாமல் விளக்கிலெரி யமுக்கு நீங்கும்

வெல்லக்கே ளளவுதொடி கொன்றே விசை

வெறுந் தண்ணீர் நாலுபடி வீதமாமே"

"மேகப் பிணி முதல் போல வென்றால்

மேலாம லிங்கம் சுத்தி செய்ய

மாகத் தமிஞ்சி கருணை நாரி

வாய்த்த புளிநீ ரொன்பா நாள்

பாகத் தழலெரி தீவி கைபோற்

பாம்புப் பீடகர் கைப்பண் பிதென

வேகமதை நீக்கென் றாடாய் பாம்பே

மேலாஞ் சரக்கிதென் நாடாய் பாம்பே"

(OR)

1. Lime 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

(OR)

2. 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.

"முன்னுசாதி லிங்கந் தன்னை

முலைப்பாலி லாறவைத்தெ"

"பின்னரு நற் சம்பீரத்தின்

பெருங்கனிச் சாற்றித் சுத்தீ'

"இங்குலிகச் சரக்கொன்றே சரக்குக்கெல்லா மிறை யாகும்"

சுரக்குகளுக்கொல்லம் இலிங்கம் இறையெனவும், மேக நோய்களுக்கு நமன்போன்றதெனவும், புகன்றிருப்பதை,

"மேகவகை வினைக்கு நமனான லிங்கம்"

தேரன் மருத்துப் பாரதத்தில் அலறு சந்நிக்கு இலிங்கத்தின் ஆட்சி கூறப்பட்டுள்ளது.

Lingam is the chief dominant among the all drugs as well as its effective in sexually transmitted diseases.

(OR)

3. 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

(pulikarunai) and Indian sarsaparilla (Nannari) root as stressed in the following tamil verses.

(OR)

4. Lime 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.

(OR)

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

(OR)

6. Cinnabar is soaked in mother's milk for 30 naazhigai (72 mins). It is removed and again fresh milk is added and the process is repeated above for 2 time.

VARIETIES OF LINGAM :

Generally there are two types of lingam namely.

i. Natural (or) red lingam and
ii. Artificial Lingam

Natural lingam is obtained along with the ones of gold and copper.

Artificial lingam is classified as

i. Ullantha lingam
ii. Rubi lingam
iii. Mathulai lingam
iv . Misitri (or) bambi lingam and
v. Cheena (or) nattu lingam

In general *ullantha lingam* is available. *Rumi lingam* is available in Punjab, Kashmir etc.

ULLANTHA LINGAM :

320 gms . of mercury and 106 ¼ grams of sulphur are burnt in reduced fire cautiously in an earthern pan until effervescence . then fire is cut off immediately and cooled with care. After cooling, the contents are transferred in vallugha apparatus and burnt as above . thus ullantha lingam is obtained.

Though various varietiesare dealt in the literature,lingam is artificially prepared according to the method described in yakhobhu vaidhyam 300.

RUMI LINGAM :

Purified mercury 12 parts, sulphur 8 parts and manosilai (yellow orbiment) 5 parts ground well and subjected to as above in vallugha apparatus. Thus Rumi lingam is obtained.

MATHULAI LINGAM :

Equal parts of mercury and thotti padanum are ground well and subjected for burning in vallugha apparatus as above. Thus mathulai lingam is obtained.

BAMBAL LINGAM:

Purified rasam (mercury) 7 parts and 2 parts of purified kanthakam (sulphur) is ground well, called kajalli. This is subjected to kuppi – erippu method in vallugha apparatus for not less than 16 hours. After cooling the bottle is broken and the lingam is preserved. Thus bambal lingam is prepared.

CHEENA LINGAM :

Equal parts of purified mercury and sulphur is subjected to as above in vallugha apparatus. Thus cheena lingam is prepared

தானென்ற சாதிலிங்கம் வைத்துக்கேளு
சாற்றரிய சூதமது பலந்தா நூறு
தேனென்ற கெந்தகந்நான் இருபத்தஞ்சு
சேரப்பா கெந்தகத்தை வேட்டிலிட்டு
வானென்ற தனலில் வைத்து உருக்கிக் கொண்டு
வரிசையாய் சூதத்தை யதிலே கொண்டு
கானென்ற அயத்துடுப்பால் கிண்டிக்கிண்ட
கருப்பாகத் தூள் போல வாகும் பாரே''.

ஆகுமப்பா கெந்தகத்தில் நாகமொன்
அறையாதே வெடியுப்பும்
வாகுமப்போ செம்பாலே விட்டுப்புப்போல
வகையாக அதுக்குள்ளே தூளைப் போட்டு
ஏகுமப்போ அரைவாசி மட்டும் போடு
ஏளிதான வாலுகையின் மேலே வைத்து

போகுமப்போ தீயெரிப்பா யெட்டுநாள் தான்
புகழாகப் பதங்கிச் சங்கிலியுமாமே.
ஆகுமப்பா பதங்கிச்சு பவழம் போல

ஆரைப்பாதே போலரின் றதுதான் லிங்கம்
போமப்பா பாண்டமது செம்புக் கொட்டி
புகழாக அடியில் நின்ற தீயுமுகலாச்சு
காமப்பா நடுவில் நின்ற கார் முசியுமாச்சு
கட்டியிதை மெழுகு செய்யில் வேதையாச்சு
சேமப்பா பாசாணம் முப்பத்திரண்டு
சிறப்பான வைப்புக்கு நாவுமாச்சே''

OTHER MEDICINAL PREPARATIONS

1. ***Seena Linga Chenduram***

Dosage : *1 – 1 ½ kundriedai*

Indication : *Suranoi ,maradaippu,vayu noi , keel vayu , thathu valimai undagum*

- Anupoga vaithiya navaneetham part – 4

2. Linga Kattu

Dosage : *½ - 1 kundriedai*

Indication : *Uthiravayu , pakanovu ,thathu ilappu*

- Anupoga vaithiya navaneetham part –4

3. Linga Ilagam

Dosage : *Sundaikkaialavu*

Indication : *Thathu viruthi , Megasoodu*

4. Linga Chunnam

Dosage : *½- 1 arisiedai*

Indication : *Thathu kuraivu, thathu valimai udal valimai Undagum, soolai noi mega noi therum*

- Anupoga vaithiya navaneetham part - 4

5. Linga Chenduram

Dosage : *½ - 1 kundri edai*

Indication : *Thathu viruthi , thega sakthi udangum , soolai noi theerum*

- Anupoga Vaithiya Navaneetham Part – 4

6. Lagu Suvarna Linga Chenduram

Dosage : *1 – 1 ½ kundri edai*

Indication : *Thathu valimai thathu viruthi undagum,*

- Anupoga vaithiya navaneetham part – 4

7. Linga Chenduram

Dosage : *½ - 1 kundri edai*

Indication : *Thathu viruthi udal sakthi thampana sakthi, narumpugaluku palam undagam*

- Anupoga vaithiya navaneetham part – 4

8. Linga Chenduram

Dosage : *1 - 3 arisiedai*

Indication : *Thathu kuraivu therntthu ,thathu viruthi undagum.*

- Anupoga vaithiya navaneetham part - 4

9. Linga Kattu

Dosage : *½ - 1 kundri edai*

Indication : *Thathu ilappu, narampu thalarchi, pakavatham theerum*

- Anupoga vaithiya navaneetham part - 4

10. ***Linga Chenduram***

Dosage : *¼ - ½ kundri edai*

Indication : *Thathu viruthi , thampana sakthi undagum, keel vayu mudaku vayu theerum*

- Anupoga vaithiya navaneetham part – 4

11. ***Madhana Kameswara Kuligai***

Dosage : *Thetran vidhai alavu*

Indication : *Veeriya viruthi*

- Kosayi anuboga vaithiya brahma ragasiyam

12. ***Sorna Pushparasa Chenduram***

Dosage : *Kundri edai*

Indication : *Thathu viruthi*

-Kannusamy parambarai vaithiyam

13. ***Thanga Linga Chenduram***

Dosage : *Thuvaramparuppu alavu*

Indication : *Thathu viruthi*

-Kannusamy parambarai vaithiyam

14. ***Thambana Mathirai***

Dosage : *1-2 mathirai*

Indication : *Vindhu thambanamagum*

-Theraiyar segarappa

15.*Gaarudap brayogam*

Dosage : *Payaru alavu*

Indication : *Aanmai perugum*

-Theraiyar segarappa

OTHER MEDICINAL PREPARATIONS WHICH CONTAIN LINGAM:

1. Saathilinga mathirai

Dosage : *Payaralavu*

Indication : *Vadham 84, Gunmam, Karapan*

- anupoga vaithiya Murai volume 3.

2. Linga Kattu Chenduram

Dosage : *Ulunthedai*

Indication : *Vadharogam, Sannipadha suram*

- Sikitcharathnadeepam

3. Linga Bhoopathy

Dosage : *Ulunthedai*

Indication : *Suram, Vadharogam, Sanni*

- Siddha vaidhya thirattu

4. Saathilinga Chenduram

Dosage : 35mg

Indication : *Megavayvu, Kaichaludan kudiya nadukkam, vayvu*

- Siddha vaidhya thirattu

5. *Linga Melugu*

Dosage : 65- 130 mg

Indication : *Murai suram, suram, dhegavali,vayvu*

- Parambarai vaidhyam

6. Thanga linga mezhugu

Dosage : 30mg

Indication : *Vadha sambanthamana pala pinigal*

- Siddha treatment of diseases

7. Saathilinga Chenduram

Dosage : *Panavedai*

Indication : *Vayvu, soolai, sanni*

- Therayar karisal.

8. Padigalinga Chenduram

Dosage : *3 – 5 kunri*

Indication : *Perumbadu, seedhabethy, rathabethy, Oozhi surathudan kudiya bethy*

- Gunapadam Thadhu Jeeva vaguppu.

9. Thangalinga Kattu Chenduram

Dosage : 65 – 130 mg

Indication : *Suram, Sanni, Isivu, Soolai, Keelvayu*

- Pulipani 500

10. Linga Vellai

Dosage : 30 – 65mg

Indication *:* *All vadharogangal, vadhakudaichalgal*

- Noigalukku siddha parigaram volume 3

11. Kanagasundara rasam

Dosage : *Kadalai alavu*

Indication : *Agnimandham, suram, adhisaram, kiragani, moolam*

-Kosayi anuboga vaithiya brahma ragasiyam part 2.

TOXIC SYMPTOMS OF LINGAM:

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

ANTIDOTE:

Nutmeg (Myristica fragrans), cubeb peper (Piper cubeba), root bark of a red cotton tree (Gossypium arboreum) and sugar candy each 4.2gm are made into a decoction and administered twice a day for 48 days.

3.1.2. GEOLOGICAL ASPECT

CINNABAR

This is a combination of mercury and sulphur. It is pinkish scarlet red in colour and on its broken surface white lines of mercury lines are founded. Naturally available in the mines. Artificially prepared lingam available in the market and are used in medicines.

Chemical Name

Natural	-	Cinnabar
Synthetic	-	Vermilion

Scientific Name

Mercuric sulphide (or) Mercuric II sulphide

Colour	-	Cochineal-red toward brownish red and lead gray
Symbol	-	HgS

Molecular formula

Molecular weight	-	232.68
Hardness	-	2.5
Specific gravity	-	8.176
Sublimes at	-	446 °C
Crystal systems	-	Hexagonal (or) Rhombohedral

Character

Cinnabar is practically insoluble in water, not allowed by HNO_3 or cold HCl, but decomposed by Con.H_2SO_4 soluble in aquaregia with separation of sulphur and in warm HCl with evaluation of Hydrogen sulphate.

Preparation of cinnabar at Laboratory

One part of the mercury and four parts of the sulphur and to be placed parts of the sulphur and to be placed in an iron pot and heated for sometimes. The amalgam is then to be broken into pieces and put into a glass bottle, previously coated all round with mud and rag one inch deep and dried in shade. The bottle is to be heated for five days continuously by means of the five increasing gradually intensity at a uniform rate. The heating is then to be discontinued and the contents of the glass bottle taken out on the seventh day. The product will be found to be cinnabar.

History

Cinnabar commonly known as Lingam, Hingul, Shingara is fine red colour powder. Alchemy is an art of refurination with using mercurial Indian alchemy probably being about 500 A.D. Mercury and cinnabar was known and used in Europe, China 100 years before the first mention is Indian medicinal works. Chinese could properly select cinnabar as the best substitute of blood in colour. This made cinnabar soul and its components sulphur and mercury sub souls. Nothing better than cinnabar was found as equal to red colour. Cinnabar is a heavy native one mercury. Extracted all over the world found in all countries, except Antartica.

Occurance:

It is a mineral and important chief of mercury it occurs in minerable deposits in a very few localities, commonly found in veins and impregnation deposited near the surface of recent volcanic rocks and hot springs and most important deposists are Almaden and spine and it has been mined for more than 2500 years from these places.

Other localities are Idria, Italy/Kweichow, China and New Almaden, New Idria, California of USA. It is also mined in Navadautah, Olegon Arkananas and Texas.

Properties

Cinnabar exist in 2 modifications black and red. Both occur in native, artificially prepared cinnabar, however a vivid scarlet substances and is used as an artist pigment called 'vermilion'.The scarlet red variety occurs as lumps and in Hexagona α – form crystals)

Colour	-	Vermilion red
Hardness	-	2.5
Specific gravity	-	8.10

The shortest distance HgS. is 2.52 A and the binding between mercury and sulphur probably ionic in character.Black merury sulphide (meta Cinnabar) (black Kubic β – form)

Colour	-	grayish black
Hardness	-	3
Specific gravity	-	7.6

The zinc blends structure with 5.82 A the shortest HgS distance in the same as in cinnabar. Black one is found in nature in small amount.The meta cinnabar is the

natural of mercury II sulphide black variety. But it also be synthesized artificially by following methods.Passing hydrogen sulphide (H2S) gas into mercurial salt solution.

Hg++ + H2S HgS+2H+

Conversion of mercury II sulphide (black) to mercury sulphide (red)In black variety is heated up to 500 C. It changes to red.When the black powder of mercury II sulphide is sublimated to 446C.It yields red form of HgS.SIn ancient period it was prepared by vaidya using Hg and S in 5:1 ratio Cinnabar on heating in a test tube. It sublimes and fumes of SO4 and black mercury sulphide are obtained. Cinnabar by heating either in a current of air or with the addition of iron or quick lime giving or yielding mercury. When treated with an a current air.

$HgS + O_2 \rightarrow Hg + O_2$

When Erected with Iron

$HgS + 2\ Fe \rightarrow Hg + Fe_2S$

With quick lime

$HgS + CaO \rightarrow Hg + CaS + CaSo4$

According to report of Dr.Chatergy, Cinnabar contains 86.22% mercury and 13.78% of sulphur It is black in acid medium while in renstral medium. Change of red coloured cinnabar powder to back in presence of 3 myrobalans of fruit juice is due It change in pH value. Neutral form of mercuric sulphide is obtained by reacting sublimated mercury and sulphur in kupi pak vs.apparatus.

Conversion of mercury II sulphide (black) to mercury sulphide(red)

1. In black variety is heated upto 500◦C it changes to red
2. When the black powder of mercury sulphide is sublimitated to 446◦C. It yields from HgS.
3. In ancient period it was prepared by Vaidya using Hg and sin 5;1 ratio.
4. Cinnabar on heating in a test tube it sublimes and fumes of SO_4 and black mercury sulphide are obtained.
5. Cinnabar by heating either in a current of air or with the addition of iron or quick lime, giving or yielding mercury.

3.1.3. LATERAL RESEARCH:

Toxicological Studies of Lingam

Lingam widely used Siddha drug was evaluate for acute,subacute and chronic toxicity in rats with reference to Histopathological,Haematological, parameters and detections of Mercury from the tissues of liver and kidney. The drug showed acute toxicity from 100mg onwards . But it produced subacute and chronic toxic efforts from 50mg onwards and elevation in blood urea level.Feed and water intake failed to reveal any marked changes in subacute and chronic toxicity studies. Mercury was detected from 75mg with concurrent increase in dose on both subacute and chronic toxicity studies. The Histopathological lesions were non specific when compared to microscopic changes along with the drug dosages. In the present study the drug possesed no toxic effect below 20mg. Anoop, M.Jegadeesan, S.Subramanium 18 October 2001

3.2.*THAMARAI KIZHANKGU* - தாமரைக் கிழங்கு

(Nelumbo nucifera)

3.2.1. GUNAPADAM ASPECT

Synonyms:

Aravindham, Sooriyanatpu, Pundarigam, Kamalam, Nalinam, Mundagam, Kanjam, Appusam, Vindham, Ellimanai, Ponmanai, Salasam, Ambooragam, Bangerugam, Vanasam, Mulari, Maalundhi, Vaarisam, Bangasam, Indai.

Vernacular Names:

Eng	–	*The sacred lotus*
Tel	–	*Tamara*
Mal	–	*Aravindam*
Sans	–	*Pankaja*
Arab & Pers	–	*Nifure*
Assam	–	*Podum*
Beng	–	*Padma Phool*
Guj	-	*Kamal*
Punj	–	*Kanwal*
Hind	–	*Kamal*
Ori	–	*Padma*

Parts Used :

Underground (Rhizome), Flowers, Seeds, Pollens

Organoleptic Character:

Taste	–	Sweet *(Inippu)*, Astringent *(Thuvarppu)*
Potency	–	Cold *(Seetham)*
Biotransformation	–	Sweet *(Inippu)*

Actions:

Rhizomes:

Demulcent

General Character:

கண்ணுக் கொளிகொடுக்குங் காசபித்தம்போக்கும்
ஏண்ணுங் குளிர்ச்சிதரும் ஏந்திழையே! – புண்ணுகளில்
தூமரைப்புண்ணும் போக்குந் தொந்திக்க டுப்பகற்றுந்
தூமரைக் கந்தமது தான்.

-(அகத்தியர் குணவாகடம்)

It gives vision and cooling to eyes.

It cures cough, Frog's skin rashes, dysentery.

Leaf

தாமரைப்பன் னத்திலுண்டால் தாங்கரிய உட்டிணமாம்
நாமவர் நஞ்சினந்து நண்ணுங்கான் - தூமமுறா
அக்கினிமர் தங்களுண்டாம் அன்றே மலர்த்திரவத்
திக்கினிலி ராளெனவே தேர்.

- அகத்தியர் குணவாகடம்

When we eat on Lotus Leaf it promotes body heat, vadha diseases, affect hunger, make poorness.

Flower

Synonyms:

Raseevam, Irumbu, Maraippu

பரத்தநற் றாமரைப்பூ பல்வாந்தி நோயைத்
துரத்திவிடும் இன்னுஞ் சொல்வோ- கரத்தில்
எடுத்தணைக்கக் கண்குளிரம் ஏகுஞ்சுரமும்
எடுத்தவி தாகமும்பொம் எண்.

- அகத்தியர் குணவாகடம்

It treats eye irritation due to heat, *Suram, Neervetkai.*

Actions:

Cooling

Astringent

Expectorant

Sedative

Flower Pollens:

"சண்டளையுஞ் சுண்டனையுந் தள்ளமல நுள்ளுறையுண்
சண்டளையுஞ் சண்டளையுஞ் சார்"

- தேரையர் யமகவெண்பா

For hermaphorodism, loss of libido lotus pollen grains powder with sugar and honey in morning.

White water Lotus:

It is used in the treatment of liver disorders and gastritis due to medications.

ஈரலைப் பற்றிமிக ஏறுகின்ற வெப்பமும்போங்
கோர மருந்தின் கொடுமையறும் - பாருலகில்
தண்டா மணத்தையுள்ள தாழ்குழலே! காந்தல்விடும்
வெண்டாமரைப் பூவால் விள்.

Seeds:

It treated *Alasam, Suvayinmai and have aphrodisiac activity.*

மந்தத் தவகமற்றும் வல்லருசி நீக்கிவிடும்
மந்தத் துறைதாது விற்கடலே – தந்திடம்பின்
னுன்னிலின்னுந் தோடகுணம் யாவுமோட் டுஞ்சலசந்
தன்னிலண் ணுக்கிரவுஞ் சம்.

தேரையர் குணவாகடம்

Actions:

Tonic

Nutrient

Lotus pond's water:

It increase vadha diseases, chronic fever, *neervetkai.*

தண்டா மரைக்குளத்திற் றங்கு புனலதனால்
உண்டாகும் வாதபித்த மண்மையே - பண்டான
வெக்கைநொய் மாறாது வீறுந் தவனமுமாம்
மைக்கருங்கண் மாதே வழுத்து.

- அகத்தியர் குணவாகடம்

VARIETIES OF LOTUS:

1. *Vellai thamarai*, White lotus – Nelumbium speciosum
2. *Sivappu thamarai*, Senthamarai, Red lotus – Nelumbium speciosum
3. *Neelathamarai*, Blue lotus – Pontederia vaginalis
4. *Oorilai thamarai*, One leaf lotus – Lonidium suffruticosum
5. *Malaithamarai,* Hill lotus or hill nelumbo or will sarasaparilla – Smilax ovalifolia
6. *Aakasa thamarai*, Sky lotus – Pistia straliolus alias Tala indica
7. *Vatta thamarai*, Round leaved lotus – Macuranga indigo
8. *Katramarai,* Stone lotus – Elacocarpus monocera
9. *Nilathamarai,* the rose shrub

10. Kadarthamarai

11. Kulirthamarai

12. Alli thamarai

13. *Mettu thamarai*,dry ground lotus

14. *Muzhugu thamarai*, Auray – Marsilea quadrifolia so called from the plant the leaf of which resembles lotus being submerged under water.

15. *Krishnathamarai, Kalvazhai* – Stony plantain – Canna indica

16. Anthara thamari

17. *Alli thamarai*, Red Indian waterlily – Nymphea lotus alias Nymphea esculenta

18. Manjal thamarai

19. Eerilai thamarai

20. Moovilai thamarai

Treaditional uses:

1. Flower juice given for diarrhoea. It acts as cardiac tonic.
2. Flower decoction used for fever due to azhal kutram.
3. Flower's honey cures eye diseases.
4. 17 – 35gm Fragrent lingtus gives for cough, bleeding piles, dyscentry.
5. 1 – 2 gm of seed powder gives body strength.
6. Seeds grind with honey and applied on tongue for stop vomiting and hiccups.

OTHER MEDICINE PREPARATION:

1.Naagaravindhathi Maha Kameswara Ilagam:

Dose : *1-1 ½ varaganedai*

Indications : *Vindhu viruthi , Narai Thirai , Kanavu Olukku , Vindhu velipaadu.*

- Anuboga vaidhya navaneetham, part 8

2. Sandhanasavam:

Dose : *½ - 1 Palam*

Indications : *Vindhoorum , Kanpugaichal , Sori , Venmegam , Aseeranam.*

- Parambarai vaidhyam

3. Mrungai poo Legiyum:

Dose : *Punnaikai size*

Indications : *Vindhoorum.*

-Vaidhya anugoola jeevaratchani, part 1

4. Amitha Sanjeevi Legiyum:

Dose : 1-5gm

Indications : *Thadhu pushti , Narambuthalarchi , Kaangai, Kamalai , Asthi suram, Paandu.*

5. Ashevu bhagavayu sikitchai:

Indication : *Thadhu viruthi , Vikkal , Adhimandham , Gunmam, Irumal.*

-Anuboga vaidhya deva ragasiyam

6.Sathaveri Rasayanam:

Indication : *Thadhu pushti, megam 20, pitham*

-Anuboga vaidhya deva ragasiyam, part 3

OTHER MEDICINAL PREPARATIONS WHICH INCLUDE THAMARAI:

1.Indiriya pushti thambanadhi legiyum:

Dose : *Kechakkai alavu*

Indications : *Thadhuviruthi*

- Kannusamy parambarai vaidhyam

2. Abbirega sanjeevi chooranam:

Dose : *Kalarchikkai alavu*

Indications : *Pitham, megam, Irumal*

-Thanjai vaidhya raja sinthamani

3. Pooshanuga chooranam:

Dose : *2 ½ - 5 varagan*

Indications : *Moolarogam, yonirogam,brahmiyam*

-Thanjai vaidhya raja sinthamani

4. Maladivandhiya sikitcha kalyana kirudham:

Dose : *2 – 4 varagan*

Indications : *Maladu, yonisoolai*

-Thanjai vaidhya raja sinthamani part 2

5. Elathy chooranam:

Dose : *1 varagan*

Indication : *Paandu, kamalai, vikkal, unmantham*

-Anuboga vaidhya deva ragasiyam

6.Kamadeva girudham:

Indications : *Paandu, soolai, kamalai*

- Anuboga vaidhya deva ragasiyam part 3

7. Arugampul kirutham:

Dose : *2 ½ - 5 Varagan*

Indications : *Rathapitham*

-Thanjai vaidhya raja sinthamani part 3

8. Kana ennai:

Dose : *½ karandi*

Indications *:* *18 types of kanam*

-Anuboga vaidhya navaneetham part 2

9*. Kudutchiyadhi thailam:*

Indications : *Soothaga noi neengi karbavathy avargal*

-Anuboga vaidhya deva ragasiyam

10. Thoorva girutham:

Indication : *Ratha vandhi*

-Anuboga vaidhya deva ragasiyam part 3

11. Satyadhi kashayam:

Indication : *Vadha suram*

-Anuboga vaidhya brahma ragasiyam

THAMARAI KIZHANGU **(UNDERGROUND RHIZOME)**

3.2.2. BOTANICAL ASPECT

Nelumbo Nucifera

Taxonomical Classification:

Kingdom	:	Plantae
Division	:	Tracheophyta
Subdivision	:	Spermatophytina
Class	:	Magnoliopsida
Family	:	Nymphaeaceae
Genus	:	Nelumbo
Species	:	N.nucifera

Distribution:

A very small genus of aquatic herbs distributed in Asia, Australia and America. One species occurs in India , Srilanka. It is commonly found in tanks and ponds throughout India. White flower variety of Nelumbo nucifera is very rare.

Botanical Description:

It is an underground rhizome. It has a stout yellowish - white or white - brown coloured rhizomes. Rhizomes from the market are usually from the plant with red flowers. Rhizomes are long and slender (60 – 140cm). Their diameter is 0.5cm to 2.5cm. The rhizome have nodes and internodes. Mucilaginous juice is exuded when the are freshly cut.

Habit :

A common perennial acquatic herb Root-Stock , cylindrical embedded in the mud. It has floating leaves and flowers. The plant normally grows upto a height of about 150cm and a horizontal spread of upto 3 meters.

Leaves :

Leaves are peltate, orbicular and glaucous. The leave are harvested in autumn.Its petioles are long, smooth and with prickles. The venation of the plant is palmate. The leaves may be as large as 60cm in diameter.

Flowers:

Flowers are large, solitary, handsome and fragrant. Flowers may be white are red and accordingly the plant is kown as Pundarika with white flowers kokanad with red flowers. Carpels numerous , ovoid, fleshy, sunk separately in cavities of receptacles, maturing into nut-like achenes. Skin hard and blackish brown when ripe. The flowers are harvested in summer

Seeds:

The seeds are harvested with fully ripe. Seeds consist of nuts twice the size of peas and when ripe, the are hard as to require a hemmer to break them and if shaken make a noise like a rattle.

Tubers:

The tubers are not cold resistant but can resist temperature below 0◦C (32◦F). If the are covered with an insulating cover of water or soil.

Stems:

Stems petioles and flower stems are raised above the water.

Roots:

The root occurs in the bazaar in small pieces of varied sizes. It is odourless and slightly mucilaginous in taste.

Medicinal uses:

- The flowers, seeds and roots are medicinally similar to Indian waterlily, filaments are astringent and cooling.
- Seeds are diuretic and refrigerant, large leaves from a cool bed in fever.
- The milky viscid juice of the flower stalks is a remedy in diarrhea.
- Petals are slightly astringent, syrup of the plant is used as a refrigerant.
- Root is used as a paste in cutaneous affection.
- Seeds are eaten either raw roasted or boiled, roots after being cut into slices, are dried and fried in oil or ghee and eaten.
- Stalk and leaf are used like greens in eating and as a dish on which offering placed.

Chemical composition:

Analysis of fresh rhizomes have the following values:

Water 83.80, crude protein 2.70 , fat 0.11, reducing sugar 1.56, sucrose o.41, starch 9.25, fibre o.80, ash 1.10 and calcium 0.06%. The vitamins reported to be present are as follows (in mg/100g):Thiamine 0.22.m.,riboflavin 0.06., niacin 2.1 and ascorbic acid 15%. The rhizome contain asparagine 2%. Sterol, reducing sugar and alkaloids are found in rhizomes.

And Raffinose and stachyose from rhizome. Nelumboside, nuciferine, roemerine, anonaine, Linalool, palmitic acid, Gallic acid , 1,4-dimethoxy benzene , myristic acid , terpinen-4-ol , α-humalene, naphthalene, methyl palmitate , methyl stearate, ethyl stearate are present.

Therapeutic uses

1. The leaf juice is used in the treatment of diarrhoea and is decocted with liquorice for the treatment of sunstroke.
2. The flower dicoction is used in the treatment of pre-mature ejaculation.
3. A decoction of the floral receptade is used in the treatment of abdominal cramps, bloody discharges...etc.
4. The root is tonic, the root starch is used in the treatment of diarrhoea, dysentry etc, a paste is applied to ringworm and other skin oilments.

3.2.3. LATERAL RESEARCH WORK :

Ethno-medicinal uses and pharmacological activities of lotus (Nelumbo nucifera)

Many pharmacological studies on lotus have proven its antioxidant, aphrodisiac activities. Lotus rhizome and its extracts have shown antioxidant activities. The antioxidant property of rhizome knot extracts has been reported to be higher than those from the whole rhizome.

Ethno-medicinal and pharmacological activities of lotus rhizome

Traditional knowledge reveals many medicinal uses of lotus plant. Rhizomes are used for spermatorrhoea. The methanol and acetone extract of lotus rhizome showed free-radical scavenging activity(Anti – Oxidant activity), at 66.7 and 133.3 mg/l, respectively; The rhizome knot also exhibited radical scavenging activity, measured spectrophotometrically and by electron spin resonance. The pharmacological investigations demonstrated that it's various organic and aqueous extracts possess an array of multidimensional pharmacological activities such as antioxidant, aphrodisiac, activities. The plant is also reported to contain a wide range of chemical constituents.

***Nelumbo Nucifera* (Lotus): A Review on Ethanobotany, Phytochemistry and Pharmacology**

in-vitro studies of the antioxidant activity of methanol and acetone extracts of the *N. nucifera* rhizome using the DPPH assay.[71] The methanol and acetone extract showed highest DPPH scavenging activity, at 66.7 and 133.3 mg/l, respectively; the methanol extract exhibited a higher antioxidant activity coefficient than ascorbic acid. The rhizome knot also exhibited radical scavenging activity, measured spectrophotometrically and by electron spin resonance. Stamens of *N. nucifera* white variety uniformly suspended in 2% Carboxy Methyl Cellulose (CMC) in water (Test drug) to obtain 100mg/ml concentration as stock solution has definite positive effect on male sexual behaviour and increased in hormone profile. Sexual behavioural parameters were observed on wister albino rats. Blood samples were collected from control and experimental rats to measure hormone testosterone. Testosterone levels showed significant increase in experimental animal compared with control. The test drug may be effective as aphrodisiac through mechanisms such as vasodilation,

generation of nitric oxide, elevation of androgens and gonadotropins. Test drug at the dose of 500mg/kg body weight could be used as a stimulator of sexual behaviour in male rats and also indicates the profound increase in improvement of sperm health and possesses aphrodisiac activity.

3. 3. DISEASE REVIEW

3.3.1. SIDDHA ASPECT

Formations of Semen

"சுக்கில நாடியில் தோன்றிய வெள்ளியும்
அக்கிர மத்தே தோன்றுமவ் வியோனியும்
புக்கிடும் எண்விரல் புறப்பிட்டு நால்விரல்
அக்கரம் எட்டும் எண் சாணது வாகுமே."

- திருமந்திரம் - திருமூலர்

அழிகின்ற விந்து அளவை யறியாற்
கழிகின்ற தன்னையுட் காக்கலுந் தேரார்
அழிகின்ற காயத் தழிந்தயற் வுற்றோர்
அழிகின்ற தன்மை யறிந்தொழி யாரே".

As per the Thirumoolar Thirumandiram it has been described that 6400 drops of Blood Cells make one drop of Vindhu (Example: 80 drops of red cell make one drop of white corpuscle and 80 drops of white corpuscle make one drop of vindhu) Thus 80 x 80 = 6400 drops of blood cells makes one drop of vindhu. If extensive loss of vindhu occurs in one human body naturally it will reflect on blood cells.

சுக்கிலவாதம் (Premature Ejaculation)

'வாயுவாதம் காற்றினிடை வந்தால் அவயவங்கள்
பாயுங்கால் வலிக்கும் பண்ணுகுணம் - காயத்தின்
சுக்கிலக் காலந்திரத்திற் துன்னு துரிதமின்னும்
புக்கிநிறத் தாது கெட்டுப்போகும்".

- அகத்தியர் வைத்திய சிந்தாமணி 4000 பாடல்

Ejaculating of semen immediately during coitus, weakness of physical constituents.

சம்பேகவாதம் (Erectile Dysfunction)

சம்பேக வாதமது தையலார் சங்கமத்தின்
வம்போக வீழ்வதில் பிறக்கும் -அம்புந்
தளருமூர்ச் சிக்கும்நீர்த் தாகம் பலம்போய்
உளரும்பின் நோயாம் உரை".

- அகத்தியர் வைத்திய சிந்தாமணி 4000 பாடல்

Decrease erection, Thirst, Asthenia, Syncope.

மேட்ஷரிய வாதம் (Matsaria Vatham)

Decreased penile function, Burning sensation, Pain & Congestion, Swelling of Testis.Formation of Semen

கனவிற் கழிதல்

"காயத்திலே மூன்று நாளிற் கலந்திட்டுக்
காயத்துட் டன்மன மாகுங் கலாவிந்து
நேயத்தே நின்றோர்க்கு நீங்கா விடாமையின்
மாயத்தே செல்வோர் மனத்தோடழியுமே"

- **திருமூலர் திருமந்திரம்**

Vatham Vazhumidam

Spermatic cord, Infra Umblical, Ganglion, Muscle.

Vatha Iyargai panpu

Energetic, Energises five Sense organs, Controls 14 Vital Reflex.

Vatha Seithoyil

Asthenia, Hypotonia.

Vatha Migukunam

Weakness of five Sense organs, Body weakness.

Vatha Kuraikunam

Symtopms of increase in the Kabam.

Nadinadai

VathaPitham, PithaVatham

சுக்கில குணம்

"உண்மையான சுக்கில முபாயமா மிருந்ததும்
வெண்மையாகி நீரிலே விரைந்துனீர் தானதும்
தண்மையான காயமே தரித்துருவமானதும்
தெண்மையான ஞானிக தெளிந்துரைக்க வேணுமே"

- சிவவாக்கியர்

At the time of copulation, the semen is ejaculated. The prostatic fluid gives the semen a milky appearance. In the early minutes after ejaculation, the sperm remains immotile, possibly because of the coagulum. As the coagulam dissolves the sperm becomes highly motile.

"உன்னிய கர்ப்பக் குழியாம் வெளியிலே
பன்னிய நாதம் பகர்ந்த பிருத்திவி
வன்னியும் வாயுவும் மாயுருஞ் சுக்கிலம்

மன்னைய சமனாய் வளடர்க்கு முதகமே
விழுந்தது இலிங்கம் விரிந்தது யோனி
ஒழிந்த முதல் ஐந்தும் ஈரைந்தோடு ஏறிப்
பொழிந்த புனல்பூதம் போற்றும் கரணம்
ஒழிந்த நுதல் உச்சி உள்ளே உளித்ததே"

- திருமூலர்

The ovum consists of element earth whereas the sperm consists of elements free and air. The uterine wall which nourishes it has water and uterine cavity is of the elemental space. Therefore all the five elements combine and create the foetus.

"வேர்க்கவே வேலிபோல் வளைந்து காக்கும்
விந்துவுடன் பிராணவாயு விளக்களாமே"

- யூகிமுனி

Abanan stays outside and the pranan goes long with spermatozoa and biseas the size of the zygote. Udhanan helps in the growth of an embryo.

According to theraiyar,

- ❖ Food nourishes saaram or essence on the first day.
- ❖ It then nourishes blood on the second day.
- ❖ It then nourishes muscle on the third day.
- ❖ It then nourishes adipose tissue on the fourth day.
- ❖ It the nourishes bone on the fifth day.
- ❖ It the nourishes bone marrow on the sixth day
- ❖ It the nourishes semen on the seventh day
- ❖ Concepts about formation of embroyo by semen.

சுக்கிலத்தனையடக்கினால்

'சுக்கிந் தனையடக்கின் சுரமுடனீர்க்கட்டாகும்.
பக்கமாங் கைகால் சந்து பாரநோய் வழியிறங்கும்
மிக்கமார் நோயுண்டாகும் மிகுந்திடும் பிரமேகந்தான்
தக்கதோர் போதுமாகின் தரித்திடும் வாயக் கூறே".

- **உடல் தத்துவம்**

If we controls ejaculation voluntarily it leads to Fever, Oliguria, Arthralgia, Involuntary ejaculation of semen, Chest pain, Palpitation.

Effect of Premature Ejaculation

"ஆண்மிகில் ஆணாம் பெண்மிகில் பெண்ணாம்
பூணிரண் டொத்துப் பொருந்தில் அலியாகுந்
தாண்மிகு மாகில் தரணி முழுதாளும்
பாவை மிக்கிடில் பாயந்ததும் இல்லையே"

- திருமூலர் திருமந்திரம் பாகம் 2

During sexual intercourse, the sexual activity is increased for a long duration, the child born due to that act has power to rule the world. If the act is for a short duration the child born due to that act has a inferior complex.

3.3.2. MODERN ASPECT

Premature Ejaculation

Premature Ejaculation (PE) is persistent or recurrent ejaculation with minimal sexual stimulation before, upon or shortly after penetration and before whishes it and is associated with marked distress or interpersonal difficulty.

PE has historically been considered a psychological disorder. One theroy is that males are conditioned by societal pressures to reach climax in a short time due fear of discovery when masturbating as teenagers or during early sexual experiences. This pattern of rapid attainment of sexual release is difficult to change in marital or long term relationship.

Prevalence of PE

Prevalence is also part of the PE debate and the following quotes have been made this topic.

- There is limited information concerning the extent of PE in the general population (Roblin, 2000).
- High prevalence across all socioecnomic groups (Butcher, 1993).
- Highly prevalent through under reported (Lane, 1997).
- Common problem (Athanasiadis, 1998, Gupta, 1999).
- Commenest form of male sexual dysfunction (Vale, 1999).
- 21% (Laumann et.al., 1999).
- 22% - 38% (Roblin, 2000).
- 35% (Nathan, in Lane, 1997).
- 36% (Frank et.al., 1978).
- Almost 40% on a recurring basis (Riley, 2000).
- 40% ongoing problem (Nettlebldt nd Uddenberrg, 1979).
- 1% - 75% (Metz and Pryor, 2000).
- 75% (Kinsey et.al.,1948, Athansiadis, 1998).It is apparent that PE is a significant problem for many men at some point in their lives, but the figures are inconsistent and may not reflect the true incidence of the problem.

Psychologiacal factors, Dpression, concern about permance, emotional conflicts and ignorance - all these factors frequently contribute to diffffículties with intercourse.

DMS IV Criteria: American Psychiatric Association 1994

- There is an inability to delay ejaculation sufficiently to enjoy love making manifesting ad either of the following:
- Occurrence of ejaculation before or very soon after the beginning of intercourse (if a time limit is required: before or within 15 seconds of the beginning of intercourse).
- Ejaculation occurs in the absence of sufficient erection to make intercourse possible. The problem is not the result of prolonged abstinence from sexual activity. (ICDIO, WHO, 1992 - 1994).

Clinicians use the distinction between primary (lifelong) and secondary (acquired) to determine the focus of the treatment. If a man has never achieved control, clinicians presume that this is a developmental problems suggest that something relatively recent has happened and clinicians tend to focus on the recent past. Terms such as psychogenic and organic although suitable as descriptors for erectile dysfunction (ED) remain hypothetical for PE (Rowland & Slob, 197). Most men presenting with PE readily recognize their problem and there is no lack of self diagnose.

Lantency time

There is no clear definition of the intra vaginal ejaculatory latency time (IELT) that qualifies for the diagnosis of PE. Waldinger Hengeved, Zwinderman and Olivier (1998) argue that the standard be set at an IELT of less than one minute. Others would recommend that PE be defined to occur prior to or within 1 to 2 minutes following vaginal intromission. Men with latencies above 3 minutes are thought to overlap with sexually functioning individuals, who do not view themselves as having a problem. Men with PE also report little or no control over ejaculation, whereas sexually functional men do perceive relatively high degree of control. Unfortunately there is no well controlled study of ejaculatory latency in normal men over the lifespan. We do not know if IELT stays the same, increases or decreases with age.

Three Big Causes

- First is the all too well - known culture role of males in their programing as men. This programing could have involved prostitute experience, where money is time. If the young man is involved with his 'girl friend', he is

conccerned with 'getting caught' by a parent or society. And if the women is a virgin there is even more encouragement to 'get in and get out'.

- A secount mechanism is interactional. The love partner, likely out of a sence of frustration, puts the burden of performance on the male and he reacts with anxiety in an effort to perform. His hurt is tuned to anger and his anger to revenge. A vicious cycle is set up.
- Thirdly, there are those men, for instance, who are premature ejaculators only when involved with a certain partner but not with others. For example, hostility is expressed by a msn in various ways. If he should feel unable to express his feeling to this particular woman verbally or otherwise because of fear of hurt or rejection or insecurity, he might well wait to get his message across at a time when he feels most able. That time can easily be in the bedroom. Thus that vicious circle starts again from a different point.

Alcohol seems stimulate sexual desire. In many women and men, it frequently reduces the ability to perform. However, small amounts of alcohol may reduce anxiety or stress responses to the extent that coitus is somewhat prolonged.

Circumcision and PE

In their investigation, Masters and Johnson failed to find any clinically significant difference between the men with circumcised and uncircumcised glans in terms of duration of intercourse or ability to perceive sensation.

PE Chaos

Patients with sexual dysfunction are reluctant raise the subject of ejaculatory dysfunction with their physician because they are embarrassed and uncertain if efficacious treatment exit to remedy their problem. Clinician fail to ask about sexual matters because they are more concerned with health conditions with associated mortality and morbidity risks, are under intense time pressure and may be uncomfortable asking patient about their sexual lives. Perhaps these phenomena account for Indian doctors reporting that they found a rate of sexual dysfunction of only 0.2 - 3% in their patients.

Moreover in the cause of PE, this is coupled with the lack of recognition of PE as causing patients and partners significant distress and the limited choice and lack of awareness of the available therapeutic option. It could also be that some men do not

care about their ejaculatory dysfunction or perhaps, they are either selfish lovers or are unconcerned with how long they last. Finally they may deny, minimize or not recognize PE as a significant problem and it is only when their partner complains, that men then seek treatment.

PE (Impact on a man's life)

T.Symonds, D.Robin and K.Hart of Pfizer Global R & D, U.K., conducted a series of qualitative individual in- person interviews with self-reported PE suffers. Their study revealed the following facts

The overriding concern for men with PE was the erosion of their sexual self confidence. To a lesser extent, they also were concerned with the impact of the sexual dysfunction on their relationship, embarrassment about having the condition and feelings of depression.

The quarters (68%) of men mentioned that 'confidence' generally or in a sexual encounter was affected by their PE.

Relationship issues were second most widely mentioned issue reported by the sample (50%). Specifically, men focused on their reluctance to establish new relationships and for men in existing relationship, on their distress regarding not satisfying their partner. Those already with a partner had found understanding partners and/ or had found ways around the problem. Starting and maintaining a relationship may arguably be a larger issue for PE patients because a large proportion of PE suffers are in the younger age range and therefore probably more likely to still be dating.

Anxiety often is mentioned as either being a reason for PE or a consequence of PE but more often it is a combination of the two. Less widely mentioned effects of PE were embarrassment about the condition and depression.

Eighty nine percent of interviewers (PE men) have tried some form of treatment for their PE, regardless of whether or not they have consulted a health care professional. Treatment options were stop and start technique (54%), Distraction/focus technique (36%), Creams/lotions/thicker condoms (21%), Masturbation prior to intercourse (21%), Herbal treatment (21%).

It interesting to note that of those men who had sought treatment from a physician 38% had tried some form of pharmaceutical drug even though no drugs are

licensed for use in this condition. Overall this study has provided a first insight into how men with PE feel and talk about their condition.

Drugs in Vogue

No pharmaceutical agent have yet been licensed for the treatment of PE (Robin, 2000). Indications suggest that anti-depressants such as selective serotonin reuptake inhibitors (SSRIs) may be useful in certain cases (Lane, 1997, MacMohan & Touna, 1999), especially where the patient has a severe psychological component to his PE.

SSRIs have been described as the treatment of choice because they are highly effective in the short term (by slightly increasing ejaculatory latency time), but they require further evaluation (Riley, 2000). However, there is some evidence to suggest that the benefit of SSRIs extends beyond the period of active treatment (MacMohan, 1998).

They include fluoxetine hydrochloride, paroxetine hydrochloride and sertraline hydrochloride. Several studies have investigated the use of SSRIs, but there is no concerns with regard to optimal dosing. Some studies suggest that treatment should be given as required 3-4 hours before intercourse (Roblin, 2000), and others recommend treatment to be given on a chronic long term basis (MacMohan & Tourma, 1999). These studies also recommended varying doses. On withdrawal of the drug, ejaculatory latency time(ELT) is almost the same as in those subjects on placebo (Lane, 1997, Robin, 2000) and ELT returns almost to baseline.

Nine types of PE

Evidence in the research and clinical literature identifies nine types of PE based upon etiology; four physiological, four psychological, as well as PE with another sexual dysfunction 'Mixed PE' (Metz & Pryor, 2000).

The four physiological types of PE are Neurological system, Physical illness, Physical injury and Drug side effect. The four Psychological types are Psychological system, Psychological distress, Relationship distress and Psychosexual skills. Outstanding clinical psychologists and marital therapists pair Michael Metz and Barry McCarthy (2004) have developed a new approach to PE. These nine types of PE are path breaking in the field of human sexuality.

The Physiologic types

Neurological system PE is due to an innate biological 'reflex' or a hypersensitive physiological predisposition to ejaculate quickly. This type of PE is lifelong, generalized to all sexual situations (partner sex and masturbation), and very common in clinical practice.

Neurological system PE

Often responds well to detailed psychosexual skills training to compensate for the physiologic predisposition and/or pharmacologic agents. Some medications (e.g. SSRIs like sertaline) must be taken daily. Others (e.g. clomipramine) can be taken 4-6 hours before sex and have been found to significantly delay orgasm in about 30 percent of cases (Slthof, Levine, Corty, Risen & Stem, 1995).

Physical illness PE

It can be caused by a number of acute diseases, is acquired and occurs in all sexual situations. The most common cause is prostatitis, which can fester in mid form for years without clear symptoms expect for PE. Other Possible causes are diabetes, sexually transmitted diseases, virtually any urologic pathology such as prostatic hypertrophy, epilepsy, endocrine irregularities, atherosclerosis, cardiovascular disease, generalized neurological disease localized sensory impairment, spurious polycythemia, cerebral tumors and polyneuritis.

Physical illness is an occasional cause of PE in the clinical setting. Even when the illness is adequately treated the negative effect on the man and couple often require sex therapy to facilitate the recovery process.

Physical injury PE

It is rare. It may be caused by spinal cord injury. trauma to the sympathetic nervous systems from abdominal aortic aneurysm surgery, pelvic fractures and other torso traumas or localized sensory impairment. When injury results in permanent psychological damage pharmacological treatment with agents that inhibit ejaculation may offer effective management. Sexual therapy designed to facilitate adaptation to permanent impairment (e.g. modified sensate focus exercise) may also be helpful.

Drug side effect PE

It is related to withdrawal from specific drugs such as trifluoperazine hydrochloride, the opiates (e.g. morphine), or even use of sympathomimetics such as over the counter cold medication like pseudoephidrine hydrochloride. Other reports have noted rare dosage related spontaneous orgasm and ejaculation with serotoninergic anti depressants such as clomipramine hydrochloride, fluoxetine or desipramine hydrochlorife in low dosage. This type of PE is acquired and generalized and is rare in clinical practice.

However, some men attempt to slow ejaculation with alcohol or drugs, which in addition to being a worrisome and dangerous strategy, may actually exacerbate PE.

The Psychological Types

Psychological system PE

It is caused by a personality characteristic that speeds ejaculation due to a psychological disorder, such as dysthymia, bipolar mood disorder, generalized anxiety disorder, obsessive compulsive disorder or one of the personality disorder. While classical psychological theory and clinical observations claimed common psychological traits among men with PE, empirical studies do not yield evidence of a common personality profile. This type of PE is rare.

Treatment options include long-term psychotherapy and the use of psychotropic medications. A number of medications for managing psychopathology (such as SSIRs and dopominergic neuroleptics) may also have ejaculation inhibiting side-effects. Cognitive behavioral couple sex therapy often is a component of the comprehensive treatment plan.

Psychological distress PE is clinical,- common, caused by temporary psychology as difficulties, such as an adjustment disorder, acute stress disorder or an acute depressive episode. Psychological stresses (e.g. occupational stress, sexual performance pressure, powerful negative events, extended bereavement, financial difficulties or acculturation problems) may precipitate PE. The critical difference between Psychological System PE and Psychological distress PE is its onset and prognosis. Chronic Psychological system PE is more difficult to treat than **acquired** acute psychological distress PE. Treatment of this common type of PE targets the specific psychological cognitive behavioral and emotional features with psychological and / or pharmacological therapies. In some cases, resolution of the

psychological problem will bring a return to normal sexual function, while in others, sex therapy may be needed to overcome the secondary features of PE such as anticipatory or performance anxiety Lack of sexual confidence can maintain the PE although the psychological cause has been ameliorated.

Relationship Distress PE is rooted in interpersonal dynamics such as unresolved relationship conflicts, hypersensitivity to the partner, or other distress such as reactivity to infidelity

There are three basic relationship scenarios

- Interpersonal issues may be the cause of the PE.
- Interpersonal issues may maintain the PE caused by another source (e.g. prostatitis) or
- **PE** caused by another source may have a detrimental effect on relationship quality.

Relationship distress can involve 'Relationship Identity' (e.g. issues such as autonomy and cohesion), co-operation (behavioral interactions) and emotional intimacy (empathy and emotional connection).

When interpersonal issues are a cause of or are maintaining the PE, couple therapy is required, specifically focusing upon the dysfunctional feature(s), such as balancing autonomy and cohesion, unresolved conflict, dysfunctional communication or inadequate empathy. Couple sex therapy may also be required to ameliorate detrimental sexual effects.

Whatever the etiology of PE, sexuality is best understood and treated as a couple issue. For men who do not have a partner it is still important that he understands his PE as a relationship issue. Even without a current sex partner his mind will imagine a partner, react to a partner from the past or anticipate meeting that special person in the future. For example he will cognitively relate to this Virtual partner' during masturbation. This 'relationship' can serve him well as he focuses on the sexual relationship as well as his performance with realistic expectations and learns the essential psychosexual skills to manage his ejaculation.

Psychosexual skills PE results from the man's lack of sensual skills to manage his body during sexual arousal and is very common in clinic. Frequently these men are lacking in dating or interpersonal skills as well as deficient **in** adequate knowledge of sexual physiology and specific sensual skills.

The man has difficulty focusing on his own sensations, thoughts of anticipatory failure, difficulty relaxing his body while sexually aroused, a lack of awareness of body management techniques (such as relaxation of the pelvic muscle), over focus on the partner's body and response and a restricted uneasy or anxious sensuality.

PE concomitant with another sexual dysfunction (Mixed PE)

Finally PE that co- exists with another sexual dysfunction, whether lifelong or acquired, generalized or situational warrants careful attention. This type of PE occurs in as much as one third of cases (Loudon, 1988). For men whose PE is the symptom of and compensation for a fear of erectile dysfunction (ED), treating the ED can in turn, resolve the PE. PE may also be reactive to his partner's sexual dysfunction (e.g.dyspareunia). Sex therapy may be helpful to remedy detrimental effects on the individual and relationship from the ED or another sexual dysfunction induced PE.

WAYS TO CHECK PE

PC muscle exercise for men

How to locate the PC muscle?

To locate your PC muscle, place two fingers behind your testicles. Now imagine that you are urinating and want to stop the flow. The muscle that you squeeze internally to stop the flow is the PC muscle. Practice stopping and starting your urination a couple of times so that you know where this muscle group is located.

How to do?

Every day, three **times a day, flex and relax** this muscle group twenty times. Now that you know where the muscle is, you won't have to touch it to exercise it. Make sure that you have isolated the muscle and are not tightening your abdomen, buttock, or your facial muscles during this exercise. Keep every other muscle in your body as relaxed as you can and remember to breathe evenly as you squeeze and relax. It is easy to do this muscle exercise.

Tightening and relaxing this muscle should feel good: you may even feel slightly arouse because when you exercise this muscle you are increasing blood flow into the genital are. It may take you a while to work up to twenty repetitions; if you are older or overweight you may want to start with **only five or ten** repetitions, then work your way up. Don't do more than twenty repetitions at a lime or your PC muscle

may get sore. It will take about three weeks for your PC muscle to get in shape and then you really need to do this exercise for the rest of your life.

It is an important part of maintaining your sexual fitness. Men whose PC muscle is in good shape can have more enjoyable **erections, more** sensation in the genital area, better ejaculation control, stronger orgasms and even multiple orgasms.

Tantric vajroli

Among the various anti-ejaculatory methods vajroli is the most successful and has turned many premature ejaculators into experts in the control of ejaculation.

Of all the numerous esoteric Tantric techniques, Vajroli remained a well kept secret for thousands of years. The precise and detailed instructions as how to achieve this practice was described by Andre Van Lysebeth, author of “Tantric The Cult of the Feminine".

Vajroli is a very easy, very effective method to have a full control over ejaculation. In short to perform vajroli one must insert a catheter into the urinary tract, as far as the bladder. This technique sounds terrible, but infect it is neither difficult nor really painful.

Modern tantrists use a rubber catheter or plastic catheter, the kind used to dram off urine One should buy three catheters, each with a different diameter (medium sized is always better).

Procedure :

First put some gynaecological golly bought at **the drug store onto the** catheter to lubricate and at the same time keep it sterile. With one hand doctor should hold the penis vertically (never when an erection or semi-erection is present) and with the other, slowly glide the catheter into the aperture at the tip of the penis. For the first half inch there is no pain, absolutely no problem! Further on since the delicate mucous lining of the urinary tract (which is also the ejaculatory tract) is very sensitive, doctor has to be very careful and proceed very slowly. On the first day, one half an inch is enough. **Tomorrow go** a little further. This should be really painful. While the catheter is inserted, make a few short movements backwards and forwards inside the penis to gradually desensitize the lining of the urethra. The whole procedure takes only about one minute and as soon as the catheter is **out,** immediately pass water to rinse the urethra. The whole procedure takes only about

one minute and as soon as the catheter is out, immediately pass water, to rinse the urethra. Before starting the process, one should **urinate** without **totally emptying the** bladder; some urine should remain in the bladder. Intact the least pleasant part of the whole process is passing urine, because this is a bit painful. After a short time this pain will subside. Now the process is over.

The next day proceed a little further inside the urethra. The aim of the whole thing is to desensitize the urethra. You may take two or three weeks before you get the catheter into the bladder. In a hospital, if the nurse has to drain a patient's bladder, because it is urgent, she has to insert the whole catheter at once!

One day the catheter will reach the bladder. How shall we know this? Since we keep some urine in bladder, as soon as the catheter is in the bladder this urine flows. Immediately, withdraw the catheter a bit, in order not to empty the bladder, make some backward and forward motions with the catheter and pull it out. Now totally empty the bladder. Proceed very slowly and cautiously throughout.

One must reach the bladder and then retreat a few millimeters, because this is the strategic zone where the sperm duct from the prostate gland runs into the urethra and it is from here that ejaculation really starts. With practice after some time, the catheter will easily glide into the urinary tract without causing any irritation or even discomfort.

The action of vajroli :

The aim of vajroli is the inhibition of ejaculation During ejaculation, spasmodic, uncontrollable waves of contractions go all along the ejaculatory tract to eject the seminal fluid out of the body. By desensitizing the nerves of the ejaculatory tract, the ejaculatory reflexes will be dulled without making the sensual bliss less intense. Once we get to this point, it is no **longer** necessary to perform vajroli daily One can do it only twice a week and later on only once a week or once a month.

Vajroli has another advantage. It makes us aware of the entire ejaculatory tract from the aperture at the tip of the penis up to the prostate gland. Lastly, vajroli invigorates the scrotum as well as the testicles, increasing the strength, vigor and vitality while the increased secretion of gonadal hormones rejuvenates the body.

Stop and squeeze method

Also called pause and squeeze method to learn ejaculatory control c**hoose** a time when you are absolutely free for half an hour without an interruption. Remove

all your clothes and lie on the bed. Relax for 5 minutes Take deer relaxing breaths. After S minutes relaxing, stroke your penis while concentrating on the sensations in the organ. Hold the organ in the way you like best and stimulate it slowly. As you feel the tension is building up, concentrate on the erotic sensations in the penis until you feel ejaculation is inevitable. Stop masturbating and squeeze the glans penis between the thumb, the index and **middle lingers. Do not** worry if you trigger off (ejaculate) at first while attempting this exercise. You will soon learn when to stop and start squeezing.

When the feeling of ejaculatory inevitability has gone, restart stimulating and enjoy the pleasurable sensations within. Stop once again when you get the signal that it is going to come (ejaculate). Repeat the procedure a third **and a fourth time. At** the fourth attempt masturbate till you ejaculate and enjoy the feeling. This is called dry stimulation.

After four or five days of dry stimulation lubricate the penis with K Y. **Jelly or** Vaseline and repeat the procedure while fantasizing a film star or a model. This is more exciting than the dry stimulation as it stimulates the type of sensations you feel in the vagina Repeat the same stop squeeze procedure as before and discharge at the fourth time.

This practice will increase your ejaculatory control gradually. You are now ready to start **exercise after a week or ten** days with your partner. Practice relaxation for a few days before starting stimulation

Female on top position

To prevent premature ejaculation, the female sits astride the male who is lying on his back. With her figures, she **spreads labia a** puts her vulva against his penis, taking care touch the glans. In doing so s! establishes a genital contact with out, penetration. While the male relaxes (his buttocks and his abdomen above all) female lets her moist vagina glide alongside. his penis

The thrusts from the lower back follow **one** after another becoming stronger and faster, at each thrust the pubic bones meet and spread wider each time the penis retreats The unavoidable result is well known; after twenty or more long deep movements of the penis inside the vagina, the semen burst forth, leaving the men exhausted. Calm, relaxed without any haste, both **per-** and vagina will be able to stay united eventually for longer duration at a stretch without any difficulty.

Magic mantra

Psychiatrist David Ruben suggests this following tips, **based** on a very effective psychoanalytic formula that has helped many people. The next time you are going to have intercourse and you are worried about ejaculating prematurely, repeat this word: Silently **to** yourself over and over in an emphatic and determined way. If it sound-, strange to **you** don't **worry** about it.

Just say it this way to yourself

'I will not spill my milk!

I don't want to spill my milk!

I am not going to spill my milk!'

It's effective about 70 percent of the time.

Dolly breathing

This it simple breathing **exercises you should** do everyday. Lie comfortably on a bed, on **your** hack **Loosen (if any) tight clothing. Place** one, hand on your abdomen and the other on your **heart. Now take a deep slow** breath that you can feel all the way down to your abdomen. Breathe as **if you are** drawing breath down through your body, into your legs **and** toes. This type **of** breath will cause **your** abdomen to expand and rise; **when you** exhale it will **contract.**

As you breathe, inhaling and exhaling should be one continuous process. Don't hold your breath alter you inhale. Feel the air flowing all the way into your lungs and all the way out. Visualize that air as a white light flowing in and out of you not only relaxing you but also energizing you. If you want to, rest after each exhale. To 'belly breathe' take two deep belly breaths and then breathe normally for about a minute. Take two belly breaths again and breathe normally for another minute. Repeat this pattern for about ten minutes.

This lime tested exercise will help to allay anxiety, increase the ability to relax and activate the benevolent energies of the parasympathetic nervous system.

Rubber to decrease sensitivity

The easiest and cheapest method to decrease the sensitivity of the penis is to wear two or more condoms. The extra layer of rubber may just dull his sensation enough to retard orgasm. They may also give him the unmistakable feeling he is making love to a rubber glove.

Local anesthetic ointments

It's simply an ointment containing benzocaine, which numbs the sensory receptors **of the** penis and sometimes delays orgasm. It may also prevent orgasm completely and convert premature ejaculation to no ejaculation at least for the night. The other disadvantage **is that some men are allergic** to this chemical.

The real problem with anesthetic ointments is that in the long run they tend to make premature ejaculation worse. The penis becomes negatively conditioned and gradually less and less stimulation is required to trigger orgasm. After a few months of using the ointment, just the touch a woman's hand may be enough to jettison a full lead of semen then and there.

Medieval approach

The most primitive approach is simply to repeat intercourse until the sexual reflexes become sufficiently fatigued to delay ejaculation. This is a tedious business at best and requires sending the lady to the corner to get a pack of potato chips while the man is waiting for the next erection to arrive. Some men even masturbate in advance so that what appears to be their first orgasm of the evening is really number two. With this approach, masturbation can become the **main event and** intercourse is anticlimax.

Mental diversion

One technique men try to delay orgasm is by 'thinking of other things' during intercourse. This is the technique recommended by some experts in the field of marriage counseling.

Another variation is to think of something 'disgusting' to delay orgasm. Psychiatrists argue 'If the patient can think of something disgusting enough he may solve his problems by losing his erection completely and throwing up. The whole concept is self defeating anyhow. They continue to comment how much fun can it be to have sex **while you** are thinking of dead rats?

Lying around

Another method calls for the couple to merely' lie next to each other in bed naked and think of other things They are to avoid any sex stimulation at all. According to the experts this calms down over excitable men who tend towards prematurity. For the man who ejaculates with him lying around thinking of other things instead of having intercourse is not much of a thrill.

Stop start technique

Dr. Semans and Dr. Donald Hastings were the pioneers in this technique of premature ejaculation. In this technique the female partner stimulates the erect penis without indulging in intercourse. As soon as the male feels the sensation to ejaculate he tells her and she stops the stimulation until the ejaculatory feeling has disappeared. She then starts stimulating him and continues till the male can hold himself indefinitely. Dr. Semans reported a hundred percent disappearance of the symptom within a month **with this** technique.

3.4. PHARMACEUTICAL REVIEW

3.4.1. SIDDHA ASPECT OF THE FORMULATION

Chenduram

It is a process of medicinal preparation intended for internal use. Generally majority of the *Chenduram* are microfine powder, red in colour. A few *Chenduram* are not red in colour . eg.*Gowri Chinthamani*- (black in colour).

The raw drugs, metals, minerals, salts and animal products are used in this process. Single and compound *Chenduram* are used daily now a days.

The above drugs are collected and cleaned properly by wiping, washing and drying.

Pretreatment of metals and minerals as follows :

1. Keeping the drugs soaked in leaf juice, fruit juice, rhizome juice, bark juice, cow's milk, goat's milk, human urine, cow's urine, cow dung mixture for a particular period.
2. Cover the drug by the paste of particular leaf paste, bark paste, stem paste, whole plant paste, flower paste, rhizome paste, calcium hydroxide paste as mentioned in the text.
3. After the above process, if necessary, calcination has to be done as per the heat process. *Pudam(* calcination) is done by a particular wood or by the use of specific number of cow dung cakes as stated. The calcination process must be maintained as scheduled 12, 24, 48 or more hours as stated.
4. Some of the drugs kept soaked in lime water, alkaline, acid mixture, calcium hydroxide water, decoction for a particular time as stated.
5. Few drugs are soaked in mother's milk, cow's milk, goat's milk for a particular period as stated.
6. few drugs are boiled in water, tender coconut water, leaf juice, rhizome juice for a particular time as stated.
7. Some drugs must be exposed or kept in full moon day and no moon day; waxing and waning of moon; sunlight and shade as the case may be.

Methods of preparation of *Chenduram* are of different processes.

1. Calcination methods - direct heat, indirect heat.
2. Frying method
3. Sublimation method
4. Direct grinding method
5. Direct flame method

***Chendurams* are prepared by the following different methods**

1. *Candamaarutha Chenduram* (Grinding process)
2. *Arumuga Chenduram* (Burning process)
3. *Suyamakkini Chenduram*(keeping under sunlight)
4. *Ayaveera Chenduram* (frying process)
5. *Gowri chinthamani Chenduram*(Indirect calcinations in sand bath)

STORAGE :

The *Chendurams* are stored in glass bottles when they are to be stored in bulk. For small retail packings, vials are convenient. Capsulation could be tried if contrivances are available.

SHELF LIFE :

Chenduram keep their potency for 75 years.

4. MATERIALS AND METHODS

4.1.PREPARATION OF THE DRUG

DRUG SELECTION

The trial drug " LINGA CHENDURAM " was selected from the text Anuboga vaithiya navaneetham part IV for the validation of safety, efficacy of its aphrodisiac , spermatogenic, and antioxidant activity.

ABOUT THE DRUG

1.Ingredients

a. *Lingam* (Mercuric sulphide)- 50gm

b.*Thamarai kilangu(Nelumbo nucifera.L) – 7 kilangu*

Collection of Drug

Lingam were collected from Gobala aasan shop Nagerkovil, Kanyakumari District.

The Thamarai Kizhangu were collected from Sri lanka.

Purification of Drugs

Lingam (cinnabar)

Lingam was taken as a single mass ; equal parts of lemon juice , cow's milk and acalypha juice were mixed together and was kept in a beaker . Nearly 300ml juice for 75 gram lingam .

Lingam was put in a clay plate and heated. The mixture of juice was added by drop on the surface of lingam and it was turned side to side to wet all the parts by the juice. When the juice were evaporated completely, again drops of the mixture was poured into plate. It was continued till all the juice was used up. This is described as a churruku in siddha medicine.

Thamarai kilangu (the sacred lotus)

Wash the tuber scrap the outer skin and remove inner nerves.

Preparation Process:

Nelumbo tuber is taken and pierce the center portion of it. Cinnabar is kept in the center of tuber and closed it with the tuber and covered with three layer of clay cloth and subjected into incineration process by using cow dung cakes. Cow dung cakes should be taken three times then that of kavasam. After cooling remove the

coverings and again it is placed in the center tuber of the sealed with three layer clay cloth. Again it is subjected into incineration process by using dried cow dung cakes. This process is repeated for 5 times. Then it is place in a stone mortar and made into a fine powder. Finally LINGA CHENDURAM is obtained and store in a glass container.

Dosage

1 – 1 ½ kundri alavu (130 – 195 mg)

Shelf Life:

75 years.

Adjuvant

Poppy seed decoction, Poppy seed paste, Butter.

Indication:

Thathu valivu, Udal valivu undagum. Peenisa noi, Neer peenisam, Adikadi undagum neerkovai theerum.

LINGAM

Before Purification

After Purification

THAMARAI KIZHANGHU

KAVASAMIDUTHAL

PUDAMIDUTHAL

PREPARATION

PREPARED MEDICINE

LINGA CHENDURAM

4.2. STANDARDIZATION OF THE DRUG

4.2.1. AS PER *SIDDHA* CLASSICAL LITERATURE:

Standardization of drug means confirmation of it's quality and purity and detection of the nature of adulterant of various parameters like morphological, microscopic, physical, chemical and biological observations.

1. Colour:

The finished form of *chenduram* is red in colour.

2. Odour:

The finished form of *chenduram* is odourless ie, without any odour related to it's ingredients.

3. Taste:

A small amount of *chenduram* was kept in the tip of the tongue, which is tasteless.Properly prepared *chenduram* should be completely tasteless. If any taste present in *chenduram,* it indicates the preparation was not well prepared. It needs another *pudam* (incineration) process.

4. Finger Print Test:

Well prepared *chenduram* should be very fine. A pinch of *chenduram* was taken and rubbed in between the thumb and index finger. It entered into the depressions and furrows of the fingers.It confirmes the fineness of *chenduram.*

5. Floating on Water:

A pinch of *chenduram* was sprinkled over the water in a glass container. The *chenduram* particles did not sink but floated on the water surface. It indicates the lightness of *chenduram*.

6. Lustre:

If any glowing particles seen in the *chenduram* , it shows that the drug is not prepared properly and possess unchanged substances like metals and other toxic substances. So, there should be no glowing particles present in the properly prepared *chenduram*. The *chenduram* was taken in a Petri dish and observed for any lustre in daylight via magnifying glass.

4.2.2. STANDARDIZATION OF TEST DRUG BY USING MODERN TECHNIQUES:

Standardization of drug helps to authenticate and determine its quality and efficiency. Thus, the process involves qualitative and quantitative analysis by means of physico – chemical properties and instrumental analysis.

The physico – chemical analysis of *LINGA CHENDURAM* has been done in iit Lab- chennai

The chemical finger prints are engaged by using modern analytical technique Fourier Transform Infra –Red Spectroscopy (FTIR) and Powder X-ray diffraction methods.

The particle size and qualitative analysis of chemical elements of *LINGA CHENDURAM* are also assessed by Scanning Electron Microscope (SEM) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

4.2.3. PHYSICO – CHEMICAL ANALYSIS:

1. LOSS ON DRYING (INDIAN PHARMOCOEPIA, 1996)

Loss on drying is the loss in percentage w/w resulting from water and volatile matter of any kind that can be driven off under a specified condition. A glass stopper, shallow weighing bottle was weighed accurately and the quantity of the sample as specified was transferred to the bottle was weighed accurately and the quantity of the sample as specified was transferred to the bottle covered and weighed. The sample was distributed evenly and the bottle was placed in the drying chamber. The sample was then dried for a specific period of time, and the bottle was removed from the chamber and allowed to cool at room temperature in a desiccators before weighing.

2. TOTAL ASH:

Two grams of ground air dried powder of *LINGA CHENDURAM* was accurately weighed in a previously ignited and tared silica crucible. The drug was gradually ignited by raising the temperature to 450^{o}C until it was white. The sample was cooled in a desiccators and weighed. The percentage of total ash was calculated with reference to air-dried drug.

a)Acid insoluble ash

The ash was boiled with 25ml of 2M hydrochloric acid for 5 minutes, the insoluble matter was collected on an ash less filter paper, washed with hot water, ignited cooled in a desiccators, and weighed. The percentage of acid insoluble ash calculated with reference to the air-dried drug.

b)Water soluble extractive

Proceed as directed for the determination of Alcohol-soluble extractive , using chloroform water instead of ethanol.

c)Alcohol soluble extractive

Macerate 5g of the air dried drug, coarsely powdered, with 100 ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{o} to constant weight. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

MICROBIOLOGICAL EXAMINATION OF LINGA CHENDURAM

Evaluation of Total Aerobic Bacterial Count

1.1. Preparation of Sample for Experimental Work

Weighed 10 gm of the homogenized drug sample aseptically and dissolved in 10 ml of sterile water and made up to 100 ml with the sterile water. The insoluble drug product was suspended in 100 ml of buffered sodium chloride-peptone solution (pH 7.0).

1.2. Serial dilution of Sample

A serial dilution is the dilution of a sample, in 10-fold dilutions. From the sample, 1 ml of the sample was added to 9 ml of sterile distilled water and mixed it well. This dilution was denoted as 10^{-1} dilution. From this dilution, 1ml was taken from that mixture is added to 9 ml, and designated as 10^{-2} dilution. The same procedure was repeated up to 10^{-4}.

1.3. Isolation of Total Viable Aerobic Microbial Count

1.3.1. Isolation of Bacteria by Plate Count Method

In this test, the bacteria in sample were made to grow as colonies, by inoculating a known volume of sample into a solidifiable nutrient medium (Casein Soybean Digest agar or Nutrient agar medium) in petridish. The agar plate was prepared by mixing growth medium with agar and then sterilized by autoclaving. Once the agar was cooled to 45^{o}C, approximately 15 to 20 ml of medium was poured into a sterile Petri dish under aseptic condition and left to solidify for 15 minutes. After solidification, each plate was smear with 0.1 ml of sample from the dilution of 10^{-1} and 10^{-2}. After inoculations, all the plates were incubated at 37^{o}C for 24 hours. After incubation, the bacterial colonies were developed as visible to the naked eye and the number of colonies on a plate was counted using Quebec Colony Counter. Plates with an average of from 30 to 300 colonies of the target bacterium were selected for colony count. Because of the statistical problems, plates with lower than 30 colonies greater than 300 colonies were rejected

1.3.1.1. Composition of Nutrient Agar Media

Peptone	: 5.0 gm
Sodium chloride	: 5.0 gm
Beef extract	: 1.5 gm

Yeast extract	: 1.5 gm
Agar	: 15.0 gm
Distilled water	: 1000 ml
pH (at 25°C)	: 7.4±0.2

1.3.2. Isolation of Fungi

From each of the above prepared samples, 0.1 ml of sample was transferred to Sabouraud Dextrose agar (SDA) prepared with Chloramphenicol. The plates were then incubated for 5 days at room temperature (20 to 25°C). After incubation, the fungal colonies were observed and calculated.

1.3.2.1. Composition of SDA

Dextrose	; 40 gm
Peptone	: 10 gm
Agar	: 15 gm
Distilled water	: 1000 ml

1.4. Evaluation of Antimicrobial Activity of Drug

Antimicrobial activity was performed by agar well diffusion method on agar.

1.4.1 Preparation of drug extracts solutions for the experiment

The dried drugs were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations of about 10, 20 and 30µg/ml. They were kept under refrigerated condition unless they were used for the experiment.

1.4.2. Procedure for the Agar Well Diffusion Test

The antibacterial screening of the drugs were carried out by determining the zone of inhibition using agar well diffusion method. All the drug extracts were tested against four pathogenic bacterial strains of gram positive and gram negative organism by agar well diffusion method.

1.4.3. Bacterial Inoculums Preparation

Inoculums of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Bacillus substilis* were prepared in nutrient broth medium and kept for incubation at 37°C for 8 hrs.

1.4.4. Agar well-diffusion method

This method was followed to determine the antimicrobial activity. Muller-Hinton Agar media plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. After inoculation, wells with the size of 10 mm diameter and about 2 cm a part were made in each of these plates using sterile cork borer. Stock solution of each drug extract was prepared at a concentration of 1 mg/ml in water. About 100 µl of different concentrations of drug solvent extracts were added into the wells and allowed to diffuse at room temperature for 2 hrs. The plates were incubated at 37°C for 24 hrs. After incubation, the diameter of the inhibition zone (mm) was measured and the activity index was also calculated.

1.4.4.1. Composition of Muller Hinton Agar Media

Beef Extract	: 02.00 gm
Acid Hydrolysate of Casein	: 17.50 gm
Starch	: 01.50 gm
Agar	: 17.00 gm

1.5. Evaluation of Specified Microorganisms

1.5.1. Isolation & Identification of *Escherichia coli*

One ml of the prepared sample was added in a sterile screw-capped container containing 50 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

1.5.1.2. Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 5 ml of Mac- Conkey broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours.

1.5.1.3. Secondary Test

From the primary test, 1.0 ml of the enrichment culture was taken and transferred aseptically in to 5 ml of peptone water. It was then incubated in a water-bath at 43.5º to 44.5º C for 24 hours and observed the tubes for acid and gas. Then, the culture was subjected to biochemical tests of imvic and the results were observed and correlated.

1.5.1.4. Alternative test

It was done by a loop full of enriched culture in the primary test was streaked on a sterile Mac-Conkey agar medium. Then, the plates were inverted and incubated at 37 ° C for 24 hours. After incubation, the pink or brick red color colonies were examined and transfer them individually into the surface of Eosin Methylene Blue agar medium (EMB), on Petri dishes. Inoculated plates were inverted and incubated at 37 ° C for 24 hours. After incubation, the colonies on medium were checked for their color appearance like green metallic sheen under reflected light. The colonies were subjected to confirmation by further suitable cultural and biochemical tests.

1.5.1.5. Components of Eosin Methylene Blue Agar Media

Pancreatic digest of gelatin	: 10.0 g
Dibasic potassium phosphate	: 2.0 g
Lactose	: 10.0 g
Eosin Y	: 400 mg
Methylene blue	: 65 mg
Agar	: 15.0 g
Distilled water	: 1000 ml

1.5.2. Isolation & Identification of *Salmonella* sp.

One ml of the prepared sample was added in a sterile screw-capped container containing 100 ml of nutrient broth and mixed well. Then, it was allowed to stand for 1 hour and mixed well again. After one hour, the screw caps of the bottle was loosened and incubated at 37º for 18 to 24 hours.

1.5.2.1. Primary Test

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into a tube containing 10 ml of Selenite F broth. Inoculated tubes were incubated in a water-bath at 36º to 38º for 48 hours. After incubation, the culture was subcultured on two of the agar media namely Bismuth sulphate agar and Deoxy cholate citrate agar and incubated the plates at 36º to 38º for 18 to 24 hours. After incubation, colonies were observed on the medium and confirmed the genus *Salmonella* based on guidelines.

1.5.2.2. Secondary test

The suspected colonies of the primary test were subcultured on the slant of triple sugar-iron agar in test tube and in urea broth. Both media were incubated at 37ºC for 24 hours. After incubation, the results were observed according to the development of color change and acid / gas in media. The presence of Salmonella was confirmed by agglutination tests.

1.5.2.3. Composition of *Salmonella Shigella* Agar Media

Beef Extract	: 5.0 gm
Enzymatic Digest of Casein	: 2.5 g
Enzymatic Digest of Animal Tissue	: 2.5 gm
Lactose	: 10 gm
Bile salts	: 8.5 gm
Sodium Citrate	: 8.5 gm
Ferric Citrate	: 1.0 gm
Brilliant Green	: 0.00033 gm
Neutral Red	: 0.025
Agar	: 13.5 gm
Distilled water	: 1000 ml

1.5.3. Isolation and Identification of *Pseudomonas aeruginosa*

From the above prepared enrichment culture, 1.0 ml was taken and transferred aseptically into 100 ml of fluid soyabean-casein digest medium and mixed well. The inoculated tubes were incubated at 37º C for 24 hours. After incubation, the growth of bacteria was checked. From this, a loop full of culture was streaked on the surface of Cetrimide agar medium and Pseudomonas Isolation Agar medium and incubated at incubated at 37º C for 24 hours. After incubation, the colonies from the agar surface of these two media were checked for detection of fluorescein and pyocyanin.

1.5.3.1. Composition of Cetrimide Agar Media

Pancreatic digest of gelatin	: 20.0 g
Magnesium chloride	: 1.4 g
Potassium sulphate	: 10.0 g
Cetrimide	: 0.3 g
Agar	: 13.6 g
Glycerin	: 10.0 g
Distilled Water t	: 1000 ml

1.5.4. Isolation and Identification of *Staphylococcus aureus*

From the above prepared enrichment culture, a loop full of culture was taken and transferred aseptically on Mannitol salt agar and incubated at 37º C for 24 hours.. After incubation, the colonies were subjected to confirmation by hem agglutination test.

1.5.4.1. Composition of Mannitol Salt Agar Media

Pancreatic digest of gelatin	: 5.0 g
Peptic digest of animal tissue	: 5.0 g
Beef extract	: 1.0 g
D-Mannitol	: 10.0 g
Sodium chloride	: 75.0 g
Agar	: 15.0 g
Phenol red	: 25 mg
Distilled Water	: 1000 ml

4.2.4. BIO CHEMICAL ANALYSIS

PROCEDURE:

5gms of the drug was wighted accurately and placed in a 250ml clean beaker. Then 50ml of distilled water is added and dissolved well. Then it is boilded well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is make up to 100ml with distilled water. This fluid is taken for analysis.

QUALITATIVE ANALYSIS FOR BASIC RADICALS:

Test for Calcium:

2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.

Test for Iron (Ferric):

The extract is acidified with glacial acetic acid and potassium ferro cyanide. Formation of blue colour indicates the presence of ferric iron.

Test for Iron (Ferrous):

The extract is treated with concentrated Nitric acid and ammonium thio-cyanate solution. Formation of blood red colour indicates the presence of ferrous iron.

Test for Zinc:

The extract is treated with potassium ferro-cyanide. Formation of white precipitate indicates the presence of zinc.

QUALITATIVE ANALYSIS FOR ACIDIC RADICALS:

Test for Sulphate:

2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.

Test for Chloride:

The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

Test for Phosphate:

The extract is treated with ammonium molybdate and concentrated nitric acid. Formation of yellow precipitate indicates the presence of phosphate.

Test for Carbonate:

On treating the extract with concentrated hydrochloric acid giving brisk effervescence indicates the presence of carbonate.

Test for starch:

The extract is added with weak iodine solution. Formation of blue colour indicates the presence of starch.

Test for albumin:

The extract is treated with Esbach`s reagent. Formation of yellow precipitate indicates the presence of albumin.

Test for tannic acid:

The extract is treated with ferric chloride. Formation of bluish black precipitate indicates the presence of tannic acid.

Test for unsaturation:

The extract is treated with potassium permanganate solution. The dis-colourization of potassium permanganate indicates the presence of unsaturated compounds.

Test for the reducing sugar:

5ml of Benedict`s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes. Any colour change indicates the presence of reducing sugar.

Test for amino acid:

One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

4.2.5. PHYTOCHEMICAL ANALYSIS OF LINGA CHENDURAM

Analysis of the siddha preparation Linga Chenduram

The siddha preparation Linga Chenduram was prepared and used for phytochemical analysis.

Preliminary test, on the siddha preparation Linga Chenduram was carried out for the presence of alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, phenolic compounds, proteins and free amino acids, flavanoids, lignin, fixed oils and fats. The methods adopted for the estimation are as follows:

1.Test for Alkaloids (Evans, 1997)

A small segment of the siddha preparation Linga Chenduram was mixed separately with a few drops of dilute hydrochloric acid and filtered. The filtrates were tested carefully with various alkaloidal reagents as follows:

a) **Mayer's test** (Evans, 1997):

To a few ml of filtrate, a drop of Mayer's reagent is added by the side of the test tube. A white or creamy precipitate indicates that the test as positive.

b) **Hager's test** (Wagner *et al*., 1996):

To a few ml of filtrate, one to 2ml of Hager's reagent is added. A prominent yellow precipitate indicates the test as positive.

c) **Dragendorff's test** (Waldi, 1965):

To a few ml of filtrate, one to 2ml of Dragendorff's reagent is added. A prominent yellow precipitate indicates the test as positive.

2.Test for Carbohydrates (Ramakrishnan *et al*., 1994)

A small quantity of siddha preparation Linga Chenduram was dissolved separately in 5ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates. Filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol solution and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of 2 layers shows the presence of carbohydrates. 37

***3.*Test for Glycosides**

The siddha preparation Linga Chenduram was hydrolyzed with hydrochloric acid for few h on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

(a) Legal's Test:

To the hydrolysate, one ml of pyridine and few drops of sodium nitro prusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red color shows the presence of glycosides and aglycones.

(b) Borntrager's Test:

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammoniacal layer acquires pink color, shows the presence of glycosides (Evans, 1997).

4.Test for Phytosterols (Finar, 1986)

(a) **Liebermann Burchard Test:**

Small amount of the siddha preparation Linga Chenduram was dissolved with 3ml of acetic anhydride, a few drops of glacial acetic acid and followed by the addition of few drops of concentrated sulphuric acid. Appearance of bluish green color shows the presence of phytosterols.

(b) **Salkowski Test:**

Small quantities of the siddha preparation Linga Chenduram were dissolved in chloroform separately. This chloroform solution was added with few drops of concentrated sulphuric acid. The appearance of bluish green color shows the presence of phytosterols.

5.Test for Saponins (Kokate, 1999)

Frothing Test:

The siddha preparation Linga Chenduram was diluted separately with 20ml of distilled water and it was agitated on a graduated cylinder for 15min. Absence of the foam formation shows the devoid of saponins

6.Test for Phenolic Compounds and Tannins (Mace, 1963)

Small quantities of siddha preparation Linga Chenduram was dissolved separately in water and tested for the presence of phenolic compound and tannins. In the process of testing and treating, the following observations were noted:

a) Dilute ferric chloride solution (5%) gives a dark green color. 38

b) 10% aqueous potassium dichromate solution gives yellowish brown precipitate.

c) 10% lead acetate solution gives a white precipitate.

7. Test for Proteins and Free Amino Acids (Fisher, 1968; Ruthmann, 1970)

Small quantities of various siddha preparation Linga Chenduram was dissolved in few ml of water and the following reaction were carried out

(a) Millon's Test :

To 2ml of filtrate, few drops of Millon's reagent were added. A white precipitate indicates the presence of proteins (Rasch and Swift, 1960).

(b) Ninhydrin Test:

To 2ml of filtrate 2 drops of ninhydrin solution was added. A characteristic purple color indicates the presence of amino acids (Yasma and Ichikawa, 1953).

(c) Biuret Test:

An aliquot of 2ml of filtrate was treated with a drop of 2% copper sulphate solution. To this, 1ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets, Pink color in the ethanol layer indicates the presence of protein (Gahan, 1984).

8.Test for Flavanoids

(a) **Shinoda's Test:**

Small quantity of siddha preparation Linga Chenduram was treated with alcohol to that a piece of magnesium was added followed by an addition of concentrated hydrochloric acid drop wise and heated. Appearance of magenta color shows the presence of flavanoids (Harborne, 1984).

(b) **Florescence Test:**

Small quantity of Linga Chenduram was dissolved separately in alcohol and a drop of that extract was placed on Whatman filter paper and observed under UV light. Florescence indicates the presence of flavanoids.

9. **Tests for Lignin**

Small quantities of Linga Chenduram was dissolved separately in few ml of alcoholic solution of hydrochloric acid and phloroglucinol gives red color, which shows lignin is present.

10. Tests for Fixed oils and Fats

(a) **Spot Test:**

A small quantity of siddha preparation Linga Chenduram was placed between 2 filter papers. Oil stains produced with any extract shows the presence of fats and fixed oils in the Linga Chenduram (Harborne, 1984).

(b) **Saponification Test:**

A small quantity of siddha preparation Linga Chenduram was treated with few drops of 0.5N alcoholic potassium hydroxide along with 2 to 3 drops of phenolphthalein. Later the mixture is refluxed for about 2h. Soap formation indicates the presence of fats and fixed oils in the Linga Chenduram.

4.2.6. INSTRUMENTAL ANALYSIS

SCANNING ELECTRON MICROSCOPE (SEM)

Fig. No. 1 Scanning Electron Microscope (SEM)

The microstructure of the powders was examined using a Hitachi S 3000H scanning electron microscope

Introduction:

The scanning Electron Microscope is one of the most versatile instruments available for the examination and analysis of the micro structural characteristics of solid objects. The primary reason for the SEM's usefulness is the high resolution which can be obtained when bulk objects are examined; values of the order of 5nm (50degreeA) are usually quoted for commercial instruments. Advanced research instruments have been described which have achieved resolutions of about 2.5nm (25 degree A). Any solid material can be studied. Sample size is limited to specimens less than about 10μm in diameter

Principle:

The beam is then rastered over the specimen in synchronism with the beam of a cathode ray tube display screen. The eleastically scattered secondary electrons are emitted from the sample surface and collected by a scintillator, the signal from which is used to modulate the brightness of the cathode ray tube. In this way the secondary electron emission from the sample is used to form an image on the CRT display screen. (Goldstein, et. al., 1992)

SEM MECHANISM

Procedure:

An electron beam passing through an evacuated column is focused by electromagnetic lenses onto the specimen surface. Since an electron is a charged particle, it has a strong interaction with the specimen (due to coulomb interaction).So when an electron beam images on a specimen, it is scattered by atomic layers near the surface of the specimen. As a result, the direction of electron motion changes and its energy is partially lost. Once an incident electron (primary electron) enters a substance, its direction of motion is influenced by various obstructions (multiple scattering), and follows a complicated trajectory which is far from a straight line. Also, when electrons with the same energy are incident on the specimen surface, a portion of electrons is reflected in the opposite direction (back scattered) and the remainder is absorbed by the specimen (exciting X- rays or other quanta in the process). If the specimen issufficiently thin, the electron can pass all the way through the specimen (transmitted electrons, scattered or non-scattered).

The depth at which various signals are generated due to electron beam – specimen interaction indicates the diffusion area of the signals in the specimen in addition to the local chemistry of the specimen. Secondary electrons mainly indicate information about the surface of a specimen. Since secondary electrons do not diffuse much inside the specimen, they are most suitable for observing the fine-structures of the specimen surface. That is to say, sharp scanning images with high resolution can be expected from secondary electrons, because of the smaller influence on resolution by their diffusion.

As the incident electron energy increases, the probability of incident electrons Colliding with elemental components of the specimen and releasing secondary electrons also increases. In other words, as the incident energy increases, the emission of electrons from the specimen also increases. However, as the energy increases beyond a certain level, the incident electrons penetrate deeper into the specimen with the result that the specimen derived electrons use up most of their energy to reach the specimen surface. Consequently, the electron emission yield decreases. Therefore, the peak secondary electron emission yield occurs at a specific entry level of the incident electrons.

In order to verify the existence of a substance and recognize its shape, the image contrast must be well defined. In other words, even if a system boasts

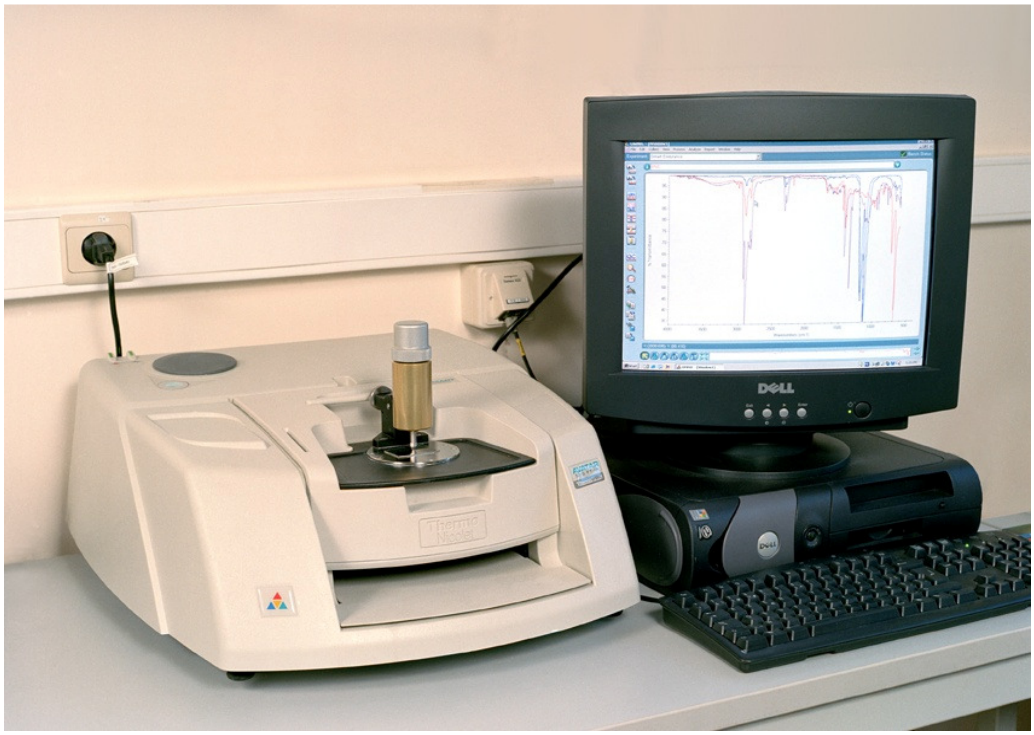
extremely high resolution, if image contrast is poor, it would be extremely difficult to determine the existence of a substance, let alone recognize its shape. Another important feature of the SEM is the three-dimensional appearance of the specimen image, which is a direct result of the large depth of field.

Applications:

The SEM is capable of examining objects at very low magnification. This feature is useful in viewing particle size and shape of any composition at various stages of preparation in *Siddha* system as well as other fields.The large depth of field available in the SEM makes it possible to observe 3-dimensional objects in stereo. Today, a majority of SEM facilities are equipped with X-ray analytical capabilities. Thus topographic crystallographic and compositional information can be obtained rapidly, efficiently and simultaneously from the same area.

The author was chosen this analysis for detecting Particle size of the classical *Siddha* herbo-mineral drug *LINGA CHENDURAM* . SEM results of *LINGA CHENDURAM* were represented in results section.

Fig. No. 2 FOURIER TRANSFORM-INFRA RED SPECTROSCOPY (FT-IR)



Introduction:

Vibrational spectroscopy is an extremely useful tool in the elucidations of molecular structure .The spectral bands can be assigned to different vibrational modes of the molecule. The various functional groups present in the molecule can be assigned by a comparison of the spectra with characteristic functional group frequencies. As the positions of the bands are directly related to the strength of the chemical bond, a large number of investigations including intermolecular interactions, phase transitions and chemical kinetics can be carried out using this branch of spectroscopy. In IR spectroscopy, the resonance absorption is made possible by the change in dipole moment accompanying the vibrational transition. The Infrared spectrum originates from the vibrational motion of the molecule. The vibrational frequencies are a kind of fingerprint of the compounds. This property is used for characterization of organic, inorganic and biological compounds. The band intensities are proportional to the concentration of the compound and hence qualitative estimations are possible. The IR spectroscopy is carried out by using Fourier transform technique.

Principle:

Infra red spectroscopy involves study of the interaction of electromagnetic radiation with matter. Due to this interaction, electromagnetic radiation characteristic of the interacting system may be absorbed (or emitted). The experimental data consist of the nature (frequency of wave length) and the amount (intensity) of the characteristic radiation absorbed or emitted. These data are correlated with the molecular and electronic structure of the substance and with intra- and inter molecular interactions.

Source	:	Nernst Glower
Beam splitter	:	It is made up of a transparent material. Thin films of Silicondepositedon Potassium bromide

(KBr) Bromide (KBr) Detectors: DeutratedTriGlycine Sulphate (DTGS).

Scan Range	:	MIR 450to 4000 cm-1
Resolution	:	4.0 cm-1
Sample required	:	50mg, solid or liquid
Sampling Techniques:		There are a variety of techniques for sample preparation physical form of the sample to be analyzed.
Solid	:	KBr or Nujol mull method.
Liquid	:	Csl / TIBr Cells
Gas	:	Gas cells

Measurements Techniques:

The procedure for recording the %T or %A is as follows:

1. Air is first scanned for the reference and stored. The sample is then recorded and finally the ratio of the sample and reference data is computed to give required % T or % A at various frequencies.
2. Study of substances with strong absorbance bands and weak absorbance bands aswell as possible.
3. Small amount of samples are sufficient
4. High resolution is obtained.

Procedure:

Typically,1.5 mg of protein, dissolved in the buffer used for its purification, were centrifuged in a 30 K Centric on micro concentrator (Amicon) at 3000_g at 4oC until a volume of approximately 40 Al.

1. Then, 300 Al of 20 mM buffer, prepared in H_20 or $2H_20$, pH or p2H 7.2, were added and the sample concentrated again. The p2H value corresponds to the pH meter reading + 0.4. The concentration and dilution procedure was repeated several times in order to completely replace the original buffer with the This buffer.
2. The washings took 24 h, which is the time of contact of the protein with the 2H20medium prior FT-IR analysis. In the last washing, the protein was concentrated to fine a volume of approximately 40 Al and used for the infrared measurements.The concentrated protein sample was placed in CaF2 windows and a 6 Am tin spacer or a 25 Am Teflon spacer for the experiments in H20 or 2H20, respectively. FT-IR spectra were recorded by means of a Perkin-Elmer -Spectrum-1 FT-IR spectrometer using a deuteratedtriglycinesulfate detector.
3. At least 24 h before, and during data acquisition, the spectrometer were continuously purged with dry air at a dew point of 40^oC. Spectra of buffers and samples were acquired at 2 cm_1 resolution under the same scanning and temperature conditions. In the thermal denaturation experiments, the temperature was raised in 5^oC steps from 20 to 95^oC.
4. Before spectrum acquisition, samples were maintained at the desired temperature for the time necessary for the stabilization of temperature inside the cell (6 min).Spectra were collected and processed using the SPECTRUM software from Perkin-Elmer. Correct subtraction of H_20 was judged to yield an approximately flat baseline at 1900-1400 cm_ 1, and subtraction of $2H_20$ was adjusted to the removal of the$2H_20$ bending absorption close to 1220 cm_ 1

KBr Method

1. The sample is grounded using an agate mortar and pestle to give a very fine powder.
2. The finely powder sample is then mixed with about 100mg dried KBr salt.
3. The mixture is then pressed under hydraulic press using a die to yield a transparent disc and measure about 13mm diameter and 0.3mm in thickness.

Nujol Mull Method:

1. The sample is ground using an agate mortar and pestle to give a very fine powder.
2. A small amount is then mixed with nujol oil to give a paste and this paste is thenapplied between two sodium chloride plates.
3. The plates are then placed in the instrument sample holder ready for scanning.

Liquids:

1. Viscous liquids can be smeared in the cell and directly measured.
2. For dilute solutions, liquid cells and variable path length cells are employed.

Applications:

Infrared spectrum is useful in identifying the functional groups like -OH, -CN, -CO, -CH, -NH2, etc. Also quantitative estimation is possible in certain cases for chemicals, pharmaceuticals, petroleum products, etc. Resins from industries, water and rubber samples can be analyzed.

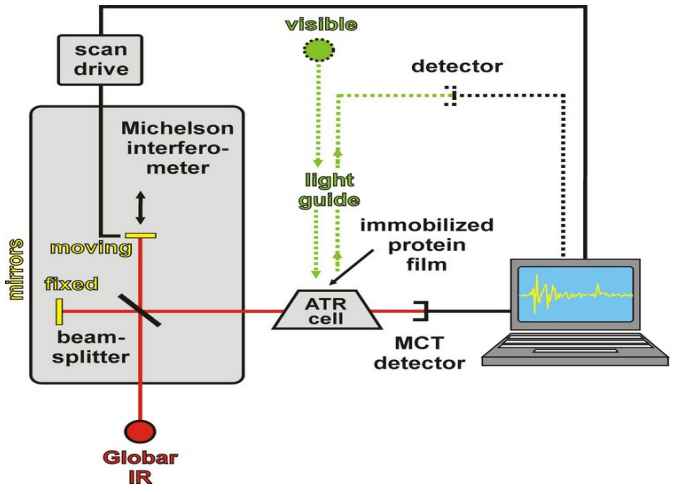


Fig. No. 3 Mechanism of FTIR analyzer

Analytical Capabilities:

1. Identifies chemical bond functional groups by the absorption of infrared radiation which excites vibrational modes in the bond.
2. Especially capable of identifying the chemical bonds of organic materials
3. Detects and identifies organic contaminants.
4. Identifies water, phosphates, sulphates, nitrates, nitrites, and ammonium ions
5. Detection limits vary greatly, but are sometimes $<10^{13}$ bonds/cm^3 or sometimes sub monolayer .Useful with solids, liquids, or gases.

Fig. No. 4 INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY(ICP-OES):

Introduction:

Inductively coupled plasma optical emission spectrometry (ICP-OES) is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. The intensity of this emission is indicative of the concentration of the element within the sample.

Mechanism:

The ICP-OES is composed of two parts: ICP and the optical spectrometer. The ICP torch consists of 3 concentric quartz glass tubes. The output or "work" coil of the radiofrequency (RF) generator surrounds part of this quartz torch. Argon gas is typically used to create the plasma.

When the torch is turned on, an intense electromagnetic field is created within the coil by the high power radio frequency signal flowing in the coil. This RF signal is created by the RF generator which is, effectively, a high power radio transmitter driving the "workcoil" the same way a typical radio transmitter drives a transmitting antenna. The argon gas flowing through the torch is ignited with a Tesla unit that

creates a brief discharge are through the argon flow to initiate the ionization process. Once the plasma is "ignited", the Tesla unit is turned off.

The argon gas is ionized in the intense electromagnetic field and flows in a particular rotationally symmetrical pattern towards the magnetic field of the RF coil. Stable, high temperature plasma of about 7000 K is then generated as the result of the inelastic collisions created between the neutral argon atoms and the charged particles. A peristaltic pump delivers an aqueous or organic sample into a nebulizer where it is changed into mist and introduced directly inside the plasma flame. The sample immediately collides with the electrons and charged ions in the plasma and is itself broken down into charged ions. The various molecules break up into their respective atoms which then lose electrons and recombine repeatedly in the plasma, giving off radiation at the characteristic wavelengths of the elements involved.

Within the optical chamber(s), after the light is separated into its different wavelengths (colours), the light intensity is measured with a photomultiplier tube or tubes physically positioned to "view" the specific wavelength(s) for each element line involved, or, in more modern units, the separated colours fall upon an array of semiconductor photo detectors such as charge coupled devices (CCDs). In units using these detector arrays, the intensities of all wavelengths (within the system's range) can be measured simultaneously, allowing the instrument to analyse for every element to which the unit is sensitive all at once. Thus, samples can be analysed very quickly. The intensity of each line is then compared to previously measured intensities ofknown concentrations of the elements and their concentrations are then computed by interpolation along the calibration lines. In addition, special software generally corrects for interferences caused by the presence of different elements within a given sample matrix.

Applications :

ICP-OES is used in the determination of metals, arsenic present in Traditional medicines, and trace elements bound to proteins. ICP-OES is widely used in minerals processing to provide the data on grades of various streams, for the construction of mass balances.

The author used it for elemental identification and quantitative compositional information of the *LINGA CHENDURAM* **.**

X-RAY POWDER DIFFRACTION
(XRD) INSTRUMENTATION

X-ray diffractometers consist of three basic elements: an X-ray tube, a sample holder and an X-ray detector.

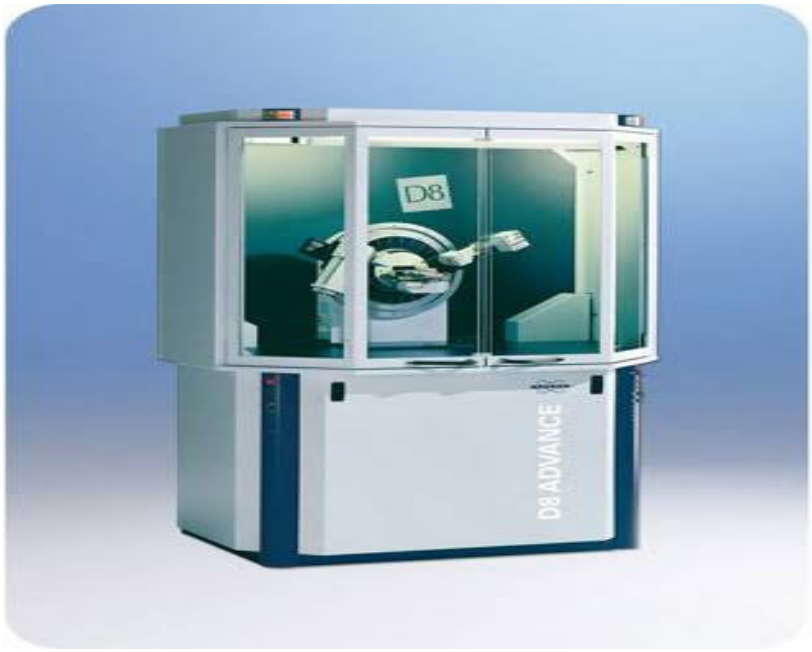


Fig.No. 5 Bruker's X-ray Diffraction D8-Discover instrument.

X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons towards a target by applying a voltage and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. These spectra consist of several components, the most common being K_α and K_β. K_α consists in part of $K_{\alpha1}$ and $K_{\alpha2}$. $K_{\alpha1}$ has a slightly shorter wavelength and twice the intensity of $K_{\alpha2}$. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Filtering, by foils or crystal monochrometers, is required to produce monochromatic X-rays needed for diffraction. $K_{\alpha1}$ and $K_{\alpha2}$ are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction, with CuK_α radiation = $1.5148A^{0.}$ These X-rays are collimated and directed onto the sample. As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and

processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor.

The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle θ while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 2θ. The instrument used to maintain the angle and rotate the sample is termed a goniometer. For typical powder patterns, data is collected at 2θ from -5^0 to 70^0, angles that are present in the X-ray scan.

Applications:

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is critical to studies in geology, environmental science, material science, engineering and biology.

Other applications include:

1. Characterization of crystalline materials
2. Identification of the fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically.
3. Determination of unit cell dimensions.
4. Measurement of sample purity.

With specialized techniques, XRD can be used to:

1. Determine crystal structures using Rietveld refinement
2. Determine of modal amounts of minerals (quantitative analysis)
3. Make textural measurements such as the orientation of grains in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction:

Strengths:

1. Powerful and rapid (<20 min) technique for identification of an unknown minerals.
2. In most cases, it provides an unambiguous mineral determination.
3. Minimal sample preparation is required.
4. XRD units are widely available.
5. Data interpretation is relatively straight forward.

Limitations:

1. Homogenous and single phase material is best for identification of an unknown
2. Must have access to a standard reference file of inorganic compounds (d-spacings, *hkls)*
3. Requires tenths of a gram of material which must be ground into a powder.
4. Foe mixed materials, detection limit is -2% of sample.
5. For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.
6. Peak overlay may occur and worsens for high angle 'reflections'.

User's Guide-Sample Collection and Preparation:

Determination of an unknown requires: the material, an instrument for grinding and a sample holder.

1. Obtain a few tenths of a gram (or more) of the material, as pure as possible.
2. Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation.
    a. Powder less than -10 μm (or 200-mesh) in size is preferred.
3. Place into a sample holder or onto the sample surface.
    a. Packing of the fine powder into a sample holder. Smear uniformly onto a glass slide, assuring a flat upper surface.
4. Pack into a sample container
5. Sprinkle on double sticky tape
    a. Typically the substance is amorphous to avoid interference
6. Care must be taken to create a flat upper surface and to achieve a random distribution of lattice orientations unless creating an oriented smear.
7. For unit cell determinations, a small amount of a standard with known peak positions (that do not interfere with the sample) can be added and used to correct peak positions.

Data Collection, Results and Presentation:

Data collection:

The intensity of diffracted X-rays is continuously recorded as the sample and detector rotate through their respective angles. A peak in intensity occurs when the mineral contains lattice planes with d-spacings appropriate to diffract X-rays at that value of θ. Although each peak consists of two separate reflections ($K_{\alpha 1}$ and $K_{\alpha 2}$), at small values of 2θ the peak locations overlap with $K_{\alpha 2}$ appearing as a hump on the side of $K_{\alpha 1}$. Greater separation occurs at higher values of θ. Typically these combined peaks are treated as one. The 2λ position of the diffraction peak is typically measured as the center of the peak at 80% peak height.

Data reduction:

Results are commonly presented as peak positions at 2θ and X-ray counts (intensity) in the form of a table or an *x-y* plot (shown above). Intensity (*I*) is either reported as peak height intensity, that intensity above background, or as integrated intensity, the area under the peak. The relative intensity is recorded as the ratio of the peak intensity to that of the most intense peak (*relative intensity =I/I_1 x 100).*

The d-spacing of each peak is then obtained by solution of the Bragg equation for the appropriate value of λ. Once all d-spacings have been determined, automated search/match routines compare the *ds* of the unknown to those of known materials. Because each mineral has a unique set of d-spacings, matching these d-spacings provides an identification of the unknown sample. A systematic procedure is used by ordering the d-spacings in terms of their intensity beginning with the most intense peak. Files of d-spacings for hundreds of thousands of inorganic compounds are available from the International Centre for Diffraction Data as the Powder Diffraction File (PDF). Many other sites contain d-spacings of minerals such as the American Mineralogist Crystal Structure Database. Commonly this information is an integral portion of the software that comes with the instrumentation.

The author used it for elemental identification and quantitative compositional information of the *LINGA CHENDURAM.*

4.3. TOXICOLOGICAL STUDIES

4.3.1. ACUTE TOXICITY STUDY IN FEMALE WISTER RATS TO EVALUATE TOXICITY PROFILE OF *LINGA CHENDURAM*

OBJECTIVES

The aim of this Study is to evaluate the toxicity of the test substance ***LINGA CHENDURAM*** , when administered orally to Female Wister Rats with different doses, so as to provide a rational base for the evaluation of the toxicological risk to man and indicate potential target organs.

Guidelines followed:

(a) OECD Guidelines No. 423 (Organisation for economic co-operation and development).

Study Design and Controls:

1) Female Wister Rats in controlled age and body weight were selected.
2) ***LINGA CHENDURAM*** was administered at **5 mg/kg, 50 mg/kg, 300 mg/kg,1000 mg/kg, 2000 mg/kg,** body weight as (Water) as suspension along with blank.
3) The results were recorded on day 0, with single oral dosing period of 14 days.

EXPERIMENTAL PROCEDURE

1. ANIMALS

Supply

A total of 15 Female Wister Rats with an approximate age of 6 weeks and purchased from Central Animal House, Arulmigu Kalasalingam college of pharmacy, krishnankoil. On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the age requested. The mean weights of Female Wister Rats were 100-150 g respectively. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 3 animals of the same sex.

All animals underwent a period of 20 days of observation and acclimatization between the date of arrival and the start of treatment. During the course of this period, the animals were inspected by a veterinary surgeon to ensure that they fulfilled the health requirements necessary for initiation of the Study.

Housing

The Female Wister Rats were housed in metabolic cages (55 x 32.7 x 19 cm), placed on racks. From the week before initiation of the treatment, each cage contained a maximum of 3 rats of the same sex and treatment group.

Each cage was identified by a card, color coded according to the dose level. This card stated the cage number, number and sex of the animals it contained, Study number, test substance code, administration route, dose level and Study Director's name, date of the arrival of the animals and initiation of treatment.

The temperature and relative humidity were continuously monitored. Lighting was controlled to supply 12 hours of light (7:00 to 19:00 hours) and 12 hours of dark for each 24-hour period.

The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

2. DIET

All the rats had free access to a pelleted rat diet. The diet was analyzed by the manufacturer to check its composition and to detect possible contaminants.

Water

The water was offered ad libitum in bottles.

3. ADMINISTRATION ROUTE AND PROCEDURE

The test substance was administered orally. The Female Wister Rats belonging to the control group were treated with the vehicle (Water) at the same administration volume as the rest of the treatment groups.

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table – 1 Numbering and Identification

Group No	Animal Marking
1	Head
2	Body
3	Tail

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals

Cage No	Group No	Animal Marking	Sex
1	I	H,B,T	Female
2	II	H,B,T	Female
3	III	H,B,T	Female
4	IV	H,B,T	Female
5	V	H,B,T	Female

Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

GROUP	DOSE
Group-I	5 mg/kg
Group-II	50 mg/kg
Group-III	300 mg/kg
Group-IV	1000 mg/kg
Group-V	2000 mg/kg

The test item was administered as single dose. After single dose administration period, all animals were observed for day 14.

Dose Preparation

LINGA CHENDURAM was added in distilled water and completely dissolved to form oral for administration. The dose was prepared of a required concentration before dosing by dissolving, in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

Administration

The test item was administered orally to each Female Wister rats as single dose using a needle fitted onto a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg bodyweight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

Observation period

All animals were observed for any abnormal clinical signs and behavioral changes. The appearance, change and disappearance of these clinical signs, if any, were recorded for approximately 1.0, 3.0 and 4.0 hours post-dose on day of dosing and once daily thereafter for14 days. Animals in pain or showing severe signs of distress were humanely killed. The cageside observation was included changes in skin, fur, eyes and mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self-mutilation, walking backwards etc were observed. At the 14th day, sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli) was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups.

Mortality and Morbidity

All animals were observed daily once for mortality and morbidity at approximately 1.0, 3.0 and 4.0 hours post dose on day of dosing and twice daily (morning and afternoon) thereafter for 14 days.

4.3.2. SUB-ACUTE TOXICITY STUDY IN WISTER RATS TO EVALUATE TOXICITY PROFILE OF LINGA CHENDURAM

Objective

The objective of this '**Sub-Acute Toxicity Study of *LINGA CHENDURAM* on Wister Rats**' was to assess the toxicological profile of the test item when treated as a single dose. Animals should be observed for 28 days after the drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

Test Guideline Followed

OECD 407 Method - Sub-Acute Toxic Class Method (Repeated Dose 28-Day Oral Toxicity Study in Rodents)

Test Item Detail

LINGA CHENDURAM

Test System Detail

The study was conducted on 5 male 5 female Wister rats. These animals were selected because of the recommended rodent species for oral studies as per followed guideline and availability of Animals 8-12 weeks old male and female rats were selected after physical and behavioral examination. The body weight range was fallen within ± 20% of the mean body weight at the time of Randomization and grouping. The rats were housed in standard laboratory condition in Polypropylene cages, provided with food and water *adlibitum* in the Animal at Central Animal House, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, government of India.

Acclimatization

The animals were selected after veterinary examination by the veterinarian. All the selected animals were kept under acclimatization for a week.

Randomization & grouping

One day before the initiation of treatment (days 0- last day of acclimatization), the selected animals were randomly grouped into three different groups containing minimum 5 males and 5 females animals per group.

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table – 2 Numbering and Identification

Case No	**Group No**	**Animal Marking**
1	CONTROL	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female
2	Low dose of *LINGA CHENDURAM* 300mg/kg	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female)
3	Middle dose of *LINGA CHENDURAM* 600mg/kg	H,B,T,HB,NM (Male) H,B,T,HB, NM (Female)
4	High dose of *LINGA CHENDURAM* 900 mg/kg	H,B,T,HB,NM (Male) H,B,T,HB ,NM (Female)

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals:

Case No	**Group No**	**Animal Marking**	**Sex**
1	CONTROL	H,B,T,HB,NM H,B,T,HB, NM	Male Female
2	Low dose of *LINGA CHENDURAM* 300mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
3	Middle dose of *LINGA CHENDURAM* 600mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
4	High dose of *LINGA CHENDURAM* 900 mg/kg	H,B,T,HB,NM H,B,T,HB ,NM	Male Female

Husbandry

Housing

The Wister rats were housed in standard polypropylene cages with stainless steel top grill. Paddy husk was used as bedding. The paddy husk was changed at least twice in a week. From the week before initiation of the treatment, each cage contained a maximum of 5 rats of the same sex and treatment group.

Environmental conditions

The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at 22±3^{0}C and the relative humidity was maintained between 30-70% with 100% exhaust facility. The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

Feed & feeding schedule

Feed was provided *adlibitum throughout* the study period, except over night fasting (18-20 hours) prior to dose administration. After the substance has been administered, food was with held for a further 3-4 hours.

Water

The water was offered *adlibitum* in bottles. There was periodically analyzed to detect the presence of possible contaminants

Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then extract was administered orally as single dose using a needle fitted on to a disposable syringe of approximate size at the following different doses.

Test Group	**Dose to Animals (mg/kg body –weight / day**	**Number of Animals**
Group – I	CONTROL	10 (5 Male and 5 Female)
Group – II	Low dose of *LC* 300mg/kg	10 (5 Male and 5 Female)
Group – III	Middle dose of *LC* 600mg/kg	10 (5 Male and 5 Female)
Group - IV	High dose of *LC* 900 mg/kg	10 (5 Male and 5 Female)

The test item was administered as single dose. After single dose administration period, all animals were observed for 28 days.

Dose Preparation

LINGA CHENDURAM was added in distilled water and completely dissolved to for oral for administration. The dose was prepared of a required concentration before dosing by dissolving ***LINGA CHENDURAM*** in distilled water.

It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

Administration

The test item was administered orally to each rat as single dose using a needle fitted on to a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg body weight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

OBSERVATIONS

These observations were also performed on week-ends. The observations included but were not limited to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central nervous and autonomous systems, somatomotor activity and behavior.

Clinical signs of toxicity

All the rats were observed at least twice daily with the purpose of recording any symptoms of ill- health or behavioral changes. Clinical signs of toxicity daily for 28 days.

Food intake

Prior to the beginning of treatment, and daily, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats was calculated.

Water intake

Water intake was checked by visual observation during the Study. In addition, the water consumption in each cage was measured daily for a period of 28 days.

Bodyweight:

The body weight of each rat was recorded one week before the start of treatment, and during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

Blood Collection

Blood was collected through retro-orbital sinus from all the animals of different groups on 28th day. The blood was collected in tubes containing Heparin/EDTA as an anticoagulant. Animals were fasted over night prior to the blood collection.

LABORATORY STUDIES

During the 4th week of treatment, samples of blood were withdrawn from the orbital sinus of 10 rats from each group, under light ether anesthesia after fasting for 16 hours. The blood samples are used to evaluate Hematological parameters like RBC, WBC, and PLATELETS etc….. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN ect……...

Hematology

The following hematological parameters were analysed using Autoanalyser

Hb	:	Haemoglobin (g %)
PCV	:	Packed Cell Volume
WBC	:	White Blood Corpuscles (x103/cmm)
RBC	:	Red Blood Corpuscles (x106/cmm)
		Blood Platelet count (x103/cmm)

Differential WBC count:

N	:	Neutrophils (%)
L	:	Lymphocytes (%)
M	:	Monocytes (%)
E	:	Eosinophils (%)
RDW	:	Red Cell Distribution Width.
MPV	:	Mean Platelet Volume

Clinical Biochemistry:

The following clinical Bio parameters were analysed using Auto analyser

Total serum protein (g/dl)

ALT/SGPT	:	Alanine amino transferase (U/L)
AST/SGOT	:	Aspartate amino transferase (U/L)
ALP	:	Alkaline serum phosphatase (U/L)
CHL	:	Cholesterol (mg/dL)
HDL	:	High density lipoprotein
TG	:	Triglyceride

TERMINAL STUDIES

Sacrifice and macroscopic examination

On completion of the 4 weeks of treatment, 18 Wister rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents both *in situ* and after evisceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

Organ weights:

After the macroscopic examination the following organs were weighed after separating the superficial fat: Brain, Heart, Spleen Kidneys, Testes, Liver, Lungs, pancreas and stomach.

4.4. PHARMACOLOGICAL STUDIES

EVALUATION OF APHRODISIAC ACTIVITY OF *LINGA CHENDURAM* IN ETHANOL TREATED MALE RATS

Aim

To evaluate the aphrodisiac potential of *LC* in ethanol treated Wistar albino male rats.

Animal procurement and maintenance

Wistar Albino rats of either sex, weighing 150 g to 200 g were purchased from King Institute of Preventive medicine Animal House, Chennai, India and they were acclimatized in Animal house of C.L Baid metha college of pharmacy, Gerugambakkam, Chennai, India at 21-23ºC. Animal ethical guidelines of CPCSEA, Ministry of Animal Husbandry and Welfare, Govt. of India were strictly followed for the care and maintenance of procured animals. The animals were fed on standard rodent pellet and RO water was provided *ad libitum.* Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The protocol for experimentation was approved by Institutional Animal Ethics Committee (IAEC).

Experimental Details

The sexually active male rats were chosen separately and divided into 4 groups; each group consisting of 6 animals. The animals in the divided groups received the treatment orally.

The sexual behavior of the experimental rats was observed in a dark light in specially designed cages that have glasses on all the sides and measuring 50×30×30cm. The male experimental rat was first placed in the cage and then two female rats in estrous phase were introduced. An initial period of 15 minutes was considered as acclimatization period. After 15 minutes, the extract or the drug was introduced and the activity of male rat in each group was recorded individually for 60 minutes, after 30 minutes of drug administration. To determine the aphrodisiac activity of the extracts, several parameters were observed. These include measuring and observing the mount frequency, mount latency, intromission frequency, intromission latency, genital grooming and anogenital sniffing

Definitions of individual parameters observed Mount frequency

Mount Frequency is corresponded to the number of mounts without intromission from the time of introduction of the female until ejaculation.

Intromission frequency

Intromission is the introduction of one organ or parts into another. e.g. the penis into the vagina. Intromission Frequency is therefore defined as the number of intromissions from the time of introduction of the female until ejaculation.

Mount latency

Mount Latency is defined as the time interval between the introduction of the female and the first mount by the male.

Intromission latency

Intromission Latency is the time between the introduction of the female and the first intromission. This is usually characterized by pelvic thrusting, and springing dismounts.

Ejaculatory latency

Ejaculatory Latency is defined as the time from the first intromission to the first ejaculation.

Post-ejaculatory interval

The post-ejaculatory interval is the time between ejaculation and the forth coming non ejaculatory intromission. The test is negative if the latency of intromission and ejaculation is greater than 20 minutes.

Sperm count (no of sperm x 10^6)

After treatment, the sperm count was carried out by using Haemocytometer (Mukherjee and Kanai, 1988). Haemocytometer is generally used for RBC as well as WBC count. It is provided with the pipettes for the dilution of the blood samples and Neaubaur□s slide with special type of ruling. The counting was done in the ruled squares on the slide. The epididymis was removed and placed in a pre-chilled petri-plate. 2 ml. of 0.9% saline was added to it and the cauda epididymis was gently minced with the help of sharp razor. This sample was used for the sperm count. The sample was pipetted out with the help of pipette provided in the Haemocytometer. A

clean and dry cover slip was kept on the Neaubaur's ruling. The ruling was loaded with the sample by touching the tip of the pipette to the slide.

Histopathological Analysis

At the end of 28th day testis were isolated for histopathological examination and fixed in 10 % formal saline (10 parts of formaldehyde and 30 parts of normal saline). Tissues were processed and embedded in paraffin wax. Sections were cut at 5 micron thickness and stained with Haematoxylin and Eosin. Light microscopic examination of the sections was then carried out and micrographs produced using Vanox-T Olympus photographing microscope. The histopathological examinations were reviewed by the pathologist.

EVALUATION OF SPERMATOGENIC ACTIVITY OF *LINGA CHENDURAM* IN ETHANOL INDUCED WISTAR MALE RATS

Aim

To evaluate the spermatogenic activity of (LC) *in Wistar* albino rats by Ethanol induced method.

Procurement and rearing of experimental animal

Adult male Wistar rats weighing 180-210 gms were used for this study. The inbred animals were procured from the animal house of Kings institute, Chennai and the study was conducted at C.L, Baid Metha College Of Nursing, Chennai, India.They were housed six per cage under standard laboratory conditions at a room temperature at 22 ± 2^0 C. The animals were subjected under standard photoperiodic condition of 12:12 hrs light: dark cycle. The animals were fed with standard rodent pellet and water *ad libitum*. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. The protocol for experimentation was approved by Institutional Animal Ethics Committee.

Experimental design
Sample Size:

24 albino rats , Experiment Duration: 60 days

Animal grouping and interventions

The animals were randomly selected and divided into four groups (I, II, III and IV) of six rats (n=6) each. Individual identification of the animal was made by marking. Group I animals served as control and received only 1ml milk, p.o. for 60 days. *Group II* served as negative control (Alcohol induced), received 0.5 ml of 25% ethanol /kg /BW /day for 60 days. Experimental groups splits into group III and IV served as the treated groups and received LC which was grounded in mortar-pestle with milk. *Group III* was treated with 25% of 0.5 ml ethanol /kg/BW/day along with 100mg/kg of LC orally once for 60 days. . *Group* IV was treated with 25%of 0.5 ml ethanol /kg/BW/day along with 200mg/kg of LC orally once for 60 days Administration was done once a day by oral gavage in the morning.

Grouping of animals for the evaluation of Spermatogenesis activity of LC

Groups	Intervention	No of Rats
Group I Normal Control	1 ml of Milk	6
Group II Negative Control	25% of ethanol (0.5 ml /kg/day)	6
Group III Treatment group	LC (100mg / kg / day) + 25% of ethanol (0.5 ml /kg/b.w/day)	6
Group IV Treatment group	LC (200mg / kg b.w / day) + 25% of ethanol (0.5 ml /kg/b.w/day)	6

Sampling, Sacrifice and Surgical procedure

Twenty-four (24) hours after the 60th day of treatment, following over-night fasting (12 hrs), the animals were sacrificed with i.p. (intraperitonial) injection of thiopentone. The abdominal cavity was opened up through a midline abdominal incision to expose the genital organs. Testes, Prostate, seminal vesicle and epididymis were excised, trimmed of all fat and other tissues, mopped with tissue paper and then weighed. Left testicles were collected to monitor the spermatozoal characteristics and Right testicles to conduct testicular and epididymal histopathology .The section was studied microscopically for changes in histo architecture or morphology. The left caudal epididymis were transferred into sterile bottles containing 2 ml of normal saline for semen analysis. Semen samples from caudal epididymis (left) were subjected to parameters such as count, motility, viability and abnormality. Counting was performed using a haemocytometer and light microscope with 100X.

Enumeration of sperm parameters Semen analysis

Examinaion of sperm count, sperm motility, viability and spermatozoal abnormalities was carried out by making small cuts in the area of the cauda epididymis close to the vas deferens and apply gentle pressure to exude epididymal contents

Sperm Count

The sample was drawn into WBC pipette and diluted to the ratio of 1:100 with the modified Krebs Ringer-bicarbonate buffer containing 0.05% collagenase (pH 7.4) followed by this 1:1000 dilution was performed with 1.8% NaCl and 2% formalin). The sperm suspension was placed in the haemocytometer with improved double Neubauer ruling was used for the counting of spermatozoa. Counts for 2-4 haemocytometer chambers were averaged. The sperm suspension was evaluated for sperm count.

Total number of sperm cells in all the four chamber = X X multiplied by 10,000 to obtain the number of cells (Y) per ml of diluted sample Y multiplied by 100 (the dilution factor) to obtain (Z) sperm cells per ml of original semen sample.

Sperm Motility

The sample was mixed with 20mm HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid contains L-Glutamine with 5% BSA (*Bovine serum albumin*) . Final sample suspension mixed with formalin and used to assess motility. Bright field microscope magnification 100x.

$$\text{Motility (\%)} = \frac{\text{Number of motile spermatozoa}}{\text{Total number of spermatozoa (Motile + Immotile)}} \times 100$$

Percentage viability

Viability was assessed by eosin Y staining (5% in saline). Forty micro litre samples of the freshly sperm suspension were placed on a glass slide, mixed with 10 µL eosin and observed under a light microscope (x400 magnification). Live sperms remained unstained following staining; whereas, those that showed any pink or red colouration were classified as dead. At least 200 sperm were counted from each sample in ten fields of vision randomly, and the percentage of live sperms was recorded

$$\text{Percentage of sperm viability} = \frac{\text{Total Viable cells (Unstained)}}{\text{Total cells (Viable + Dead)}} \times 100$$

Sper morphology

Staining

A suggested method for staining uses 1ml of sperm suspension which was transferred to a test tube. Two drops of 1% eosin Y were added to the test tube and mixed by gentle agitation. The above mixture was incubated at room temperature for approximately 45-60 minutes to allow for staining.

Slide preparation

Slides should be cleaned with detergent, washed in water followed by alcohol and dried before use. One to two drops of the stained sperm suspension were placed approximately 1cm from the frosted end of a pre-cleaned microscope slide lying on a flat surface. A second slide was held in the right hand with the Z slides□s long edge gently touching across the width of the sperm slide and pulled across to produce a sperm smear. After drying the smears were fixed with formalin.

Characterization of normal and abnormal sperm

Abnormality in sperms were calculated based on the following parameter like curved tail, Tail less head, Headless tail, looped tail and coiled tail etc. Normal sperm were calculated based on the appearance and absence of above mentioned parameters.

$$\text{Percentage of normal sperm} = \frac{\text{No of Normal sperm}}{\text{Total number of sperms in the filed}} \times 100$$

$$\text{Percentage of normal sperm} = \frac{\text{No of abnormal sperm}}{\text{Total number of sperms in the filed}} \times 100$$

Procedure for histopathology

The rats from each group were anethetized by drug with out any injury after lower pelvic region. The collected samples were washed with normal saline and fixed in 10% neutral formalin for 48 hrs for further histological observation. Paraffin section were taken at 5 μm thickness processed in alcohol-xylene series and was stained with Haematoxylin-eosin dye. The sections were examined microscopically for histopathological changes. The magnification for low power was carried out at 10 X and for high power at 45 X.

EVALUATION OF ANTIOXIDANT ACTIVITY OF *LINGA CHENDURAM* THROUGH DPPH (2, 2-DIPHENYL 1-2 PICRYLHYDRAZYL) ASSAY

The antioxidant activity of *LC* was determined using the 2, 2-diphenyl-1 picrylhydrazyl (DPPH) free radical scavenging assay.100µl of *LC* extract was mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control subsequently, at every 5 min interval, the absorption maximum of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicates

Free radical scavenging activity was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{\text{Abs of Control} - \text{Abs of Test}}{\text{Abs of Control}} \times 100$$

5. MICROBIOLOGICAL ANALYSIS

ANTI - MICROBIAL STUDIES

Aim

To study the Anti-microbial action of "*LINGA CHENDURAM* " done by **"Agar well diffusion method"** – Kirby – bauyermethod.

Components of Muller Hinton agar medium

Beef extract	-	300gms/lit
Agar	-	17 gms/lit
Starch	-	1.5 gms/lit
Casein Hydrolysate	-	17.5 gms/lit
Distilled water	-	1000 ml
PH	-	7.6

Procedure:

The method of antibacterial activity study is UPS Diffusion Method. Antibiotic discs are prepared with known concentration of antibiotic are placed on agar plates that has been inoculated with the known pathogenic micro organism. The antibiotic diffuses through the agar producing an antibiotic concentration, gradient antimicrobial susceptibility is proportional to the diameter of the inhibitory zone around the disc. If the microorganism which grows up to the edge of the disc are resistant to the antimicrobial agent. The recommended medium in this method is Muller Hinton Agar, its PH should be between 7.2-7.6 and should be poured to uniform thickness of 4mm in the petri plate (25ml).

Methodology:

Muller Hinton Agar plates are prepared and Pseudomonas, Staphylococcus aureus, Escherichia coli, is inoculated separately.

The prepared disc of *LINGA CHENDURAM* are placed over the incubated plate using sterile forceps and incubated for 24 hours at 37 degree celcius. The plates after 24 hours incubation are observed for the zone of inhibition.

6. RESULTS AND DISCUSSION

6.1. STANDARDISATION OF *LINGA CHENDURAM*

The test drug ***LINGA CHENDURAM*** had been subjected to various studies to establish the works of Siddhar`s to be true. Literary collections, physico-chemical and elemental analysis, pharmacological study, toxicological study and antimicrobial study are done to prove the activity of ***LINGA CHENDURAM*** as aphrodisiac, spermatogenesis and anti-oxidant activities.

Table – 3 Physico Chemical Standardisation.

SL. NO.	PARA METER	RESULTS
1.	Organo leptic characters	
	a. Color	Red
	b. Odour	Odourless
	c. Sense of touch	Soft
	d. Appearance	Powder
1.	Physico chemical standard	
	a. Loss on drying at 70°C	7.20 %
	b. Ash	
	i. Total ash	8.20%
	ii. Acid insoluble ash	0.85 %
	iii. Water soluble	8.65 %
	c. Extractive value	
	i. Ethanol soluble extractive	8.10 %
	ii. Water soluble extractive	9.30 %
	d. pH value (1% solution)	7.840

Interpretation:

The physical parameters like colour, odour, touch, appearance revealed that *LINGA CHENDURAM* is a Red, Odourless, having the PH 7.840 slightly alkaline Ph.

Determination of loss of drying normal:

The loss on drying test is designed to measure the amount of volatile matters in a sample when the sample is dried under specified conditions moisture is one of the major factors. Responsible for the deterioration of the drugs and formulations low moisture content is always desirable for higher stability of days.

The percentage of loss on drying was within acceptable range to thus implying that the formulation can be stored for a long period and would not easily be attacked by microbes.

Microbial Limit Tests

The total bacterial count and the total fungal count of the drug were found to be within the WHO prescribed limits which indicate that the drug is free from microbial contamination. The other pathogens like Escherichia coli, Salmonella sps, Pseudomonas aeruginosa and Staphylococcus aureus were found to be completely absent in the drugs.

Total Ash:

Ash values are helpful in determining the quality and purity of crude drugs. in this trial drug *LINGA CHENDURAM* (The minerals that present in the trial drug are calcium, chloride, sulphate). The salts, Ca+, Cl-, Sulphate are not harmful one. In this trial drug *LINGA CHENDURAM* is used as a condensation from water extraction . So only water soluble trace elements present here in a very few trace levels. The total ash was 8.20%.

Acid insoluble Ash:

Acid insoluble ash values represents detecting the presence of silica and oxalate in a drugs. In my drug the silica and oxalate that is the acid insoluble ash is very low on 0.85 %. So the drug has high quality.

Water soluble ash:

Water soluble ash also indicate the purity of the drug water soluble ash higher than acid insoluble ash represents good quality of the drug which is *LINGA CHENDURAM* is 8.65 %. So water soluble ash is higher than acid insoluble ash.

b)Water soluble extractive

Proceed as directed for the determination of Alcohol-soluble extractive , using chloroform water instead of ethanol water soluble extractive LINGA CHENDURAM is 9.30 %.

c)Alcohol soluble extractive

Macerate 5g of the air dried drug, coarsely powdered, with 100ml of alcohol of the specified strength in a closed flask for 24 hrs, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105^{o},to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug. Alcohol soluble extractive LINGA CHENDURAM is 8.10 %.

d) Determination of pH

5 gms of LINGA CHENDURAM was weighted accurately and placed in clear 100ml beaker. Then 50ml of distilled water was added to it and dissolved well. after 30 minutes it was then applied into pH meter at standard buffer solution of 4.0, 7.0 and 9.0. Repeat the test 4 times and average was recorded. The pH of LINGA CHENDURAM is 7.840.

Microbial Limit Tests

S.No.	Test Particulars	Colony Counts (CFU/ g)	Limits Value (CFU/g)
1.	Total Viable Aerobic Bacterial Count	4×10^2	1×10^5
2.	Total Viable Fungal Count	3.5×10^2	1×10^3

Results of Specific Pathogens Test

S.No.	Test for Specified Pathogens	Colony Counts (CFU/ g)	Limits Value (CFU/g)
1.	*Salmonella* sp.	No growth	-
2.	*Staphylococcus aureus*	No growth	-
3.	*Escherichia coli*	No growth	-
4.	*Pseudomonas aeruginosa*	No growth	-

Disintegration:

The disintegration of test sample under the specifications not more than 15 minutes. In the present analysis the *LINGA CHENDURAM* disintegration on only 10m 50 sec.

BIO CHEMICAL ANALYSIS

Table – 4 Results of Preliminary test for basic and acidic radicals

S.NO	EXPERIMENT	INFERENCE
1.	Test for Calcium	**Present**
2.	Test for Sulphate	**Present**
3.	Test for Chloride	**Present**
4.	Test for Carbonate	Absent
5.	Test for Starch	Absent
6.	Test for Ferric Iron	Absent
7.	Test for Ferrous Iron	**Present**
8.	Test for Phosphate	Absent
9.	Test for Albumin	Absent
10.	Test for Tannic Acid	Absent
11.	Test for Unsaturated Compounds	Absent
12.	Test for Reducing Sugar	Absent
13.	Test for Amino Acid	Absent
14.	Test for Zinc	Absent
15.	Test for Mercury	**Present**

INTERPRETATION:

The biochemical analysis of *LINGA CHENDURAM* contains the following chemical constituents, Calcium, Sulphate, Chloride, Ferrous iron, Mercury.

CALCIUM:

- Calcium is involved in muscle contraction. That it reduces fatigue. calcium ions have an apparently paradoxical effect on sperm motility. In the epididymis, calcium ions stimulate immature sperm, whereas in ejaculated semen, calcium ions. Calcium binding substances and Calcium transport inhibitors are secreted by male accessory sexual organs and mixed with sperm during ejaculation. In the female genital tract sperm acquire full capacity of fertilize the ovum. Finally, Calcium ions trigger the acrosome reaction and facilitate sperm penetration into the ovum.

SULPHATE:

- It significantly improves the joint function. It helps in reduce the pain and inflammation.
- Sulphate's primary biological role in halting or reversing joint degeneration.
- Nutritionally essential element
- Functional in the form of sulphur containing amino acids.

FERROUS IRON :

- Iron is easily soluble and readily absorbed from intestine and involved.

CHLORIDE:

- Calcium- activated chloride channels (Ca-Cl) are thought to regulate neuronal excitability and recently chloride regulation in DRG (Dorsal root ganglion) neurons has attracted much attention in pain research.
- Chloride forms the chief anion of the extracellular fluid and exists along with sodium mostly.
- Regulates acid base balance.
- Formation of HCl in gastric juice
- Help to preserve normal neuromuscular irritability by maintaining a state of equilibrium, on account of their relative proportion in ECF and ICF.

PHYTOCHEMICAL STUDY OF *LINGA CHENDURAM*

The *LINGA CHENDURAM* was subjected to qualitative chemical investigation Details of the various tests performed for the presence of phytoconstituents is shown in Table 5.

Table – 5 Phytochemical tests for *LINGA CHENDURAM*

Tests	*LINGA CHENDURAM*
Alkaloids	
Mayer's test	-ve
Dragendroff's test	-ve
Hager's test	+ve
Carbohydrates and glycosides	
Molisch test	-ve
Legal's test	-ve
Borntrager's test for anthraquinones	-ve
Phytosterols	
Liebermann-Burchard test	-ve
Salkowski test	-ve
Flavanoids	
Shinoda test Magnesium turnings and hydrochloric acid (Presence of red color)	-ve
Fluorescence test	-ve
Tannins	
Ferric chloride test	-ve
Potassium dichromate test	-ve
Lead acetate test	-ve
Proteins	
Millon's test	-ve
Biuret test	+ve
Ninhydrin test	-ve
Fixed oils and fats	
Spot test	-ve
Saponification test	-ve
Lignin	
Phloroglucinol test	-ve
Saponins	
Frothing test	-ve

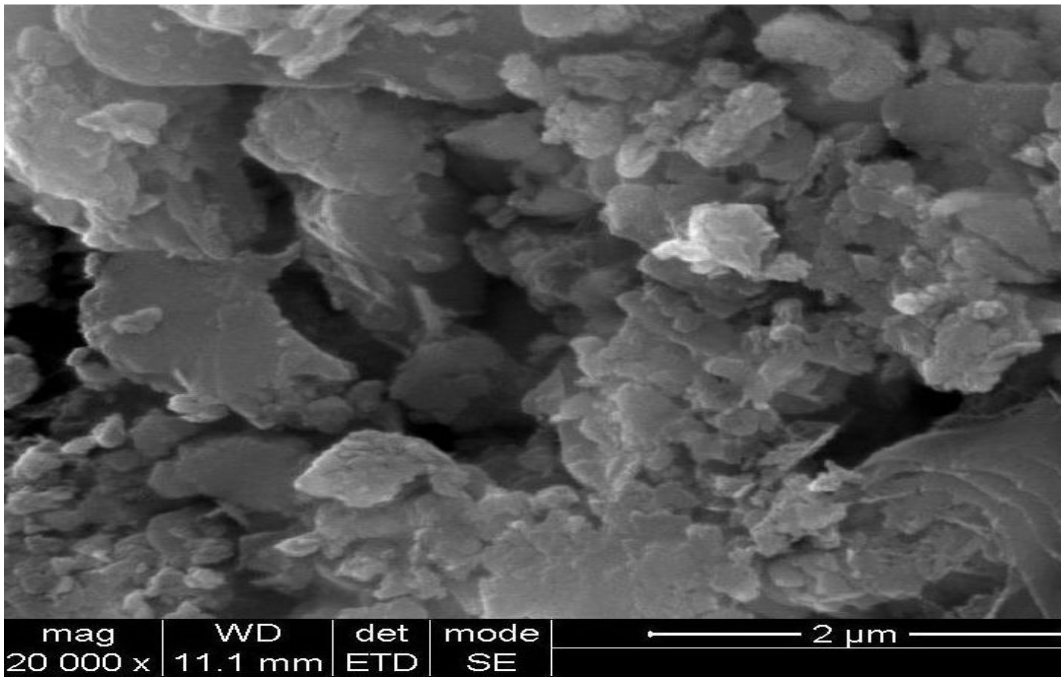
(+ve) indicates the presence of phytochemical, (-ve) indicates the absence of phytochemical.

INTERPRETATION:

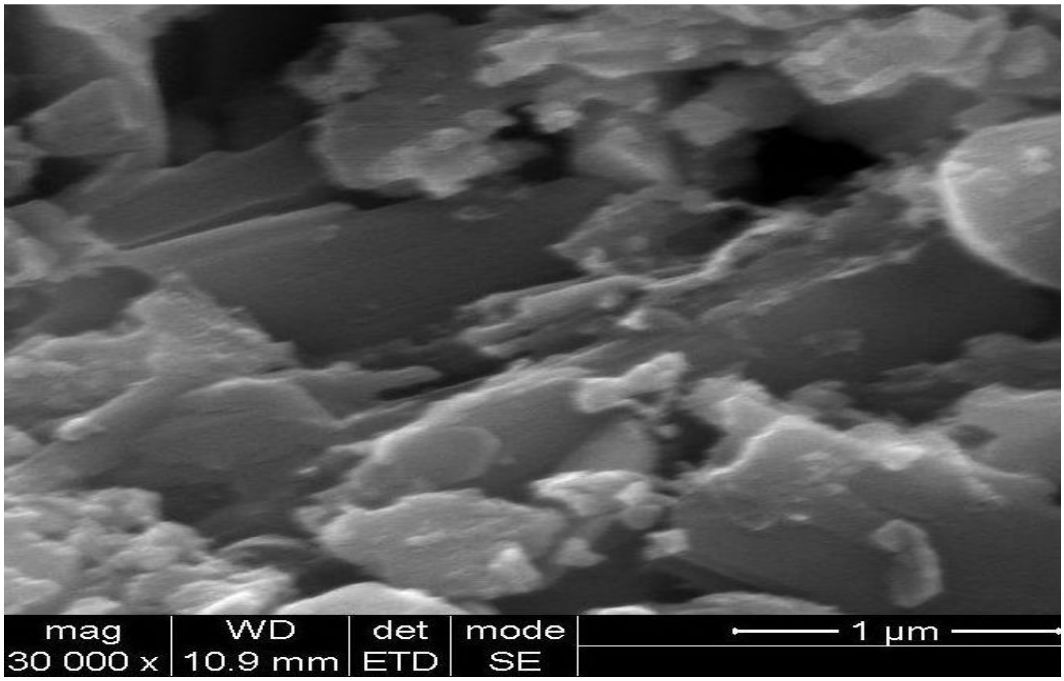
- Alkaloids-decreased gastric acid secretion and inhibit the gastric motility.
- Tannins-Tannins react with tissue promote tissue proteins.
- This study revealed the presence of active phytochemicals in LINGA CHENDURAM such as alkaloids, tannins.

INSTRUMENTAL ANALYSIS

SCANNING ELECTRON MICROSCOPE (SEM)



SEM -20000 Magnification



SEM -30000 Magnification

Figure - 6 Showing SEM Results of Trial Drug

(LINGA CHENDURAM)

INTERPRETATION :

The morphology of the *LINGA CHENDURAM* samples can be determined by Environmental SEM (FEI Quanta). A representative portion of each sample must be sprinkled onto a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination. The SEM photographs revealed that particles were spherical in shapes and sizes were in the range from 1µm to 5µm.Although the particle sizes of different batches showed similarity, it seems that these particles were aggregates of much smaller particles.

When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gave these particles a tendency to aggregate together to form larger particles. ***LINGA CHENDURAM*** exhibited larger sizes and agglomeration of the particles. Therefore, the comparatively larger size may be due to the agglomeration of the particles by repeated cycles of calcinations involved in preparation. SEM analysis of the ***LINGA CHENDURAM*** shows most of the particles present in the sample are nano size, average particle size is **2 - 1µm**.

FOURIER TRANSFORM-INFRARED SPECTROSCOPY(FTIR)

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis results in absorption spectra that provide information about the functional group and molecular structure of a material IR relates with the sample and the bonds among atoms in the molecule stretch and bend, absorbing infrared energy and creating the infrared spectrum. It is of two kinds of bending and stretching.

FT-IR is a very useful tool in the recognition of the functional groups of bio molecules, thus aiding in their structural elucidation, so confirming the presence of active molecules responsible for the therapeutic activity of Siddha drugs. The results of Table no: 6 and Fig no:7 shows the presence of functional group and inorganic compounds of ***LINGA CHENDURAM***

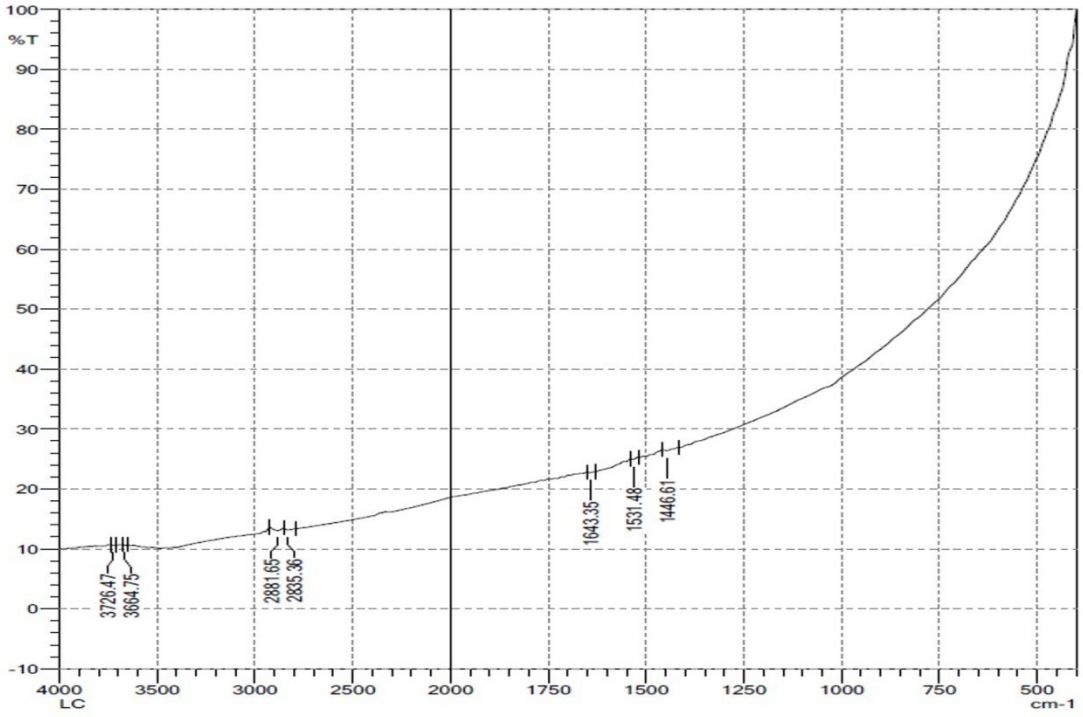


Figure -7 Showing FTIR Image of *LINGA CHENDURAM*

Table – 6 Interpretation of FTIR Spectrum

S.No	Frequency	Bond	Functional Group
1.	3726.47	-	Unknown compound
2.	3664.75	O-H Stretch	Alcohol
3.	2881.65	O-H Stretch	Alcohol
4.	2835.36	N-H Stretch	Amine salt
5.	1643.35	C=N Stretch	Imine/oxime
6.	1531.48	N-O Stretch	Nitro compound
7.	1446.61	O-H bend	Carboxylicacid

INTERPRETATION:

1. FTIR instrumental analysis was done. The test drug was identified to have 7 peaks. They are the functional groups present in the trial drug *LINGA CHENDURAM* .
2. It confirms that *LINGA CHENDURAM* constitutes Alcohol, Amine salt, Imine/Oxime, Nitro compound, Carboxylic acid as functional groups.

Carboxylic acid :

It is a classical organic compounds that are characterised by the presence of carboxyl groups (-COOH) in them. They make up series fatty acids which are extremely good for human health.The omega3 and the omega 6 are the essential fatty acids which are not produced by the body. They help in maintaining the cell membrane and control nutrient use along with metabolism.

- AMINES:Acts as a neuro transmitter.Involved in protein synthesis.Amines play an important role in reducing abdominal pain,bloating.
- ALKANES:They protect against bacteria and fungal infections.
- ALCOHOLS:Has anti microbial action.Acts as a antiseptic agent.

ICP-OES of *LINGA CHENDURAM*

LINGA CHENDURAM (wt:0.41210g)

Elements	Wavelength (nm)	Concentration
Al	396.152	BDL
As	188.979	BDL
Ca	315.807	10.180 mg/l
Cd	228.802	BDL
Cu	327.393	BDL
Fe	238.204	11.376 mg/l
Hg	253.652	BDL
K	766.491	03.821 mg/l
Mg	285.213	01.104 mg/l
Na	589.592	14.320 mg/l
Ni	231.604	BDL
Pb	220.353	BDL
P	213.617	86.341 mg/l
S	180.731	201.204 mg/l
Zn	206.200	3.256 mg/l

BDL: Below Detectable Limit(Normal-1ppm)

1% = 10000ppm,

1ppm = 1/1000000 or 0.0001%

Toxic metals and the permissible limits

Heavy metals	WHO & FDA limits
Arsenic(As)	10ppm
Mercury(Hg)	1ppm
Lead (pb)	10ppm
Cadmium (Cd)	0.3ppm

INTERPRETATION:

1. Optical Emission spectrometry is based on the principle that atoms or ions in an excited state tend, to revert back to the ground state and in so doing emit characteristic wavelength and intensity of that light is proportional to the concentration of that particular element in the sample solution.
2. This technique is used for quantitative and qualitative determination of the metals and mettaloids, in the biological preparation.
3. This results shows Below detection limit(BDL) of As(arsenic),Hg(Mercury), Cd (Cadmium), Pb(Lead), Ni(Nickel), Al(Aluminium), Cu (Copper).It is evident that the effectiveness of *siddha* medicine has been proved by the modern scientific way.
4. This result indicates the presence of Calcium, Ferrous, Magnesium, Potassium, Sodium, Phosphorus.

1.Calcium :

Calcium is a major component of bones and teeth. It is essential for vital functions like neuronal activity,muscle contraction ,cardiac activity,secretions in glands ,blood coagulation etc.The normal blood calcium level ranges between 9 – 11mg/dl.It forms about 1.5% of total body weight.

2. Ferrous iron

Ferrous sulphate provides the iron needed by the body to produce red blood cells.It is used to treat or prevent Iron deficiency Anaemia.A condition that occurs when the body too few red blood cells because of pregnanacy, poor diet, excess bleeding or other medical problems.

3.Potassium :

Pottasium channel openers comprise a diverse group of chemical agents which open Plasmalemmal k-channels. The type of k-channel involved in the actions of both exogenous k- channel openers is still uncertain, although a prime candidate in smooth muscle seems similar to the (ATP)modulated k-channel in the pancreatic p-cell. This is focused in attention on the action of these agents in vascular smooth muscle and on the possible clinical exploitation of the ir poverful vasorelaxant properties. This property is very useful for the penial erection during the coitus.

4.Magnesium :

Magnesium may offer blood building support without potential to do harm. Infact, where magnesium is a necessary catalyst in over 300 enzymatic reactions in the human body. Magnesium deficiency can lead to anemia as a lack of magnesium causes the red blood cell(erythrocyte) membrane to become more fragile and easily damaged.

5.Sodium :

Sodium is an electrolyte that helps regulate the movement of water throughout the body. It also maintains the blood pressure and nerve and muscle function.

6.Phosphorus :

Phosphorus plays a major role in physiological functioning , including energy production,cellular replication, and bone mineral metabolism.phosphorus alone has been implicated in inhibiting red blood cell production.

7. Zinc

Zinc is an essential trace mineral for the normal functioning of the male reproductive system. Zinc supplementation was found to significantly increase the semen volume, sperm motility and the percentage of normal sperm morphology.

XRD (X-Ray Diffraction):

X-Ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions.

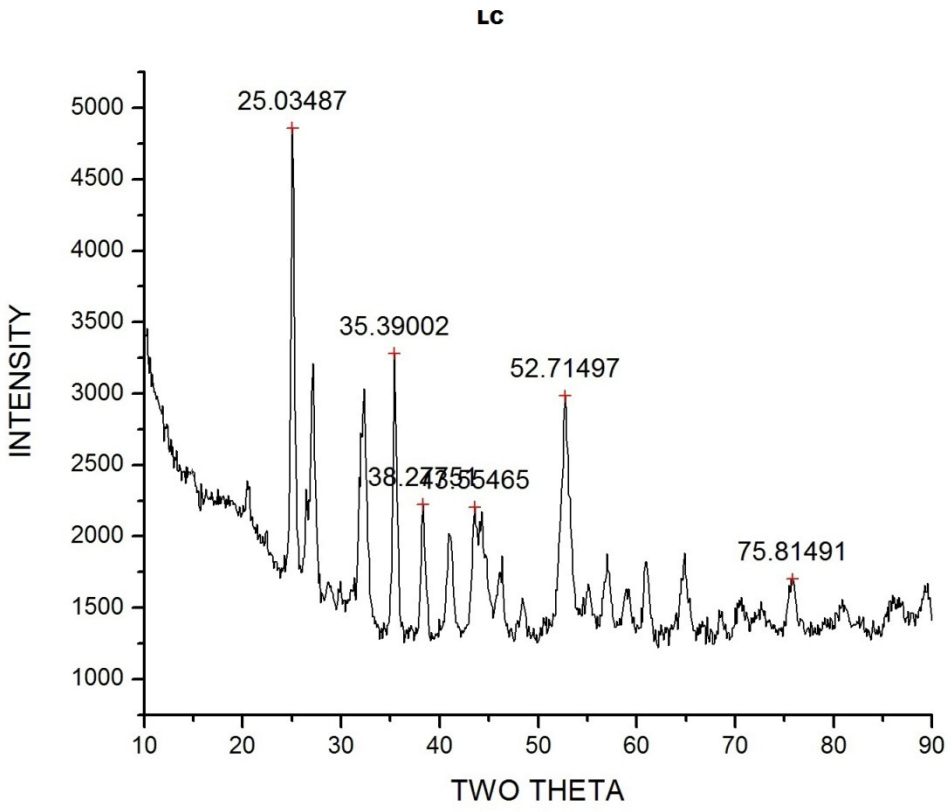


Fig. No. 8 XRD –Results of *LC*

This XRD finger print shows both the similarities and differences of the sample successfully and is a valuable primary tool for checking the quality control of Herbo-mineral formulations. The different peaks show the presence of minerals in the samples.

TOXICITY STUDIES

EVALUATION OF ACUTE TOXICITY STUDY OF *LINGA CHENDHURAM*

Table no –7 Effect of Acute Toxicity Study (14 Days) of *Linga Chendhuram*

Physical and behavioral examinations.

Group no.	Dose(mg/kg)	Observation sign	No. of animal affected.
Group-I	5mg/kg	Normal	0 of 3
Group- II	50mg/kg	Normal	0 of 3
Group-III	300mg/kg	Normal	0 of 3
Group-IV	1000mg/kg	Normal	0 of 3
Group-V	2000mg/kg	Normal	0 of 3

Table no- 8 Home cage activity

Functional and Behavioural observation	Observation	5mg/kg Group (G-I)	50mg/kg (G-II)	300mg/kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Body position	Normal	3	3	3	3	3
Respiration	Normal	3	3	3	3	3
Clonic involuntary Movement	Normal	3	3	3	3	3
Tonic involuntary Movement	Normal	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

Table no-9 Hand held observation

Functional and Behavioral observation	Observation	Control	5 mg/ kg (G-I)	50 mg/kg (G-II)	300mg/ kg (G-III)	1000mg/ kg (G-IV)	2000mg/ kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3	3
Salivation	Normal	3	3	3	3	3	3
Piloerection	Normal	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

Table no-10 Mortality

Group No	Dose no(mg/kg)	Mortality
Group-I	5(mg/kg)	0 of 3
Group-II	50(mg/kg)	0 of 3
Group-III	300(mg/kg)	0 of 3
Group-IV	1000(mg/kg)	0 of 3
Group-V	2000(mg/kg)	0 of 3

RESULT:

From acute toxicity study it was observed that the administration of *LINGA CHENDHURAM*at a dose of 2000 mg/kg to the rats do not produce drug-related toxicity and mortality. So No-Observed-Adverse-Effect- Level (NOAEL) of *LINGA CHENDHURAM*is 2000 mg/kg.

DISCUSSION

LINGA CHENDURAM was administered single time at the dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal

clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioural signs of any toxicity due to administration of ***LINGA CHENDURAM*** at the doses of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominaltone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups.Food consumption of all treated animals was found normal as compared to normalgroup.

Body weight at weekly interval was measured to find out the effect of ***LINGA CHENDURAM*** on the growth rate. Body weight change in drug treated animals was found normal.

INTERPRETATION:

LINGA CHENDURAM was administered single time at the dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behaviou ral signs of any toxicity due to administration of ***LINGA CHENDURAM*** at the doses of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats.

At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch

response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominaltone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups.Food consumption of all treated animals was found normal as compared to normalgroup.

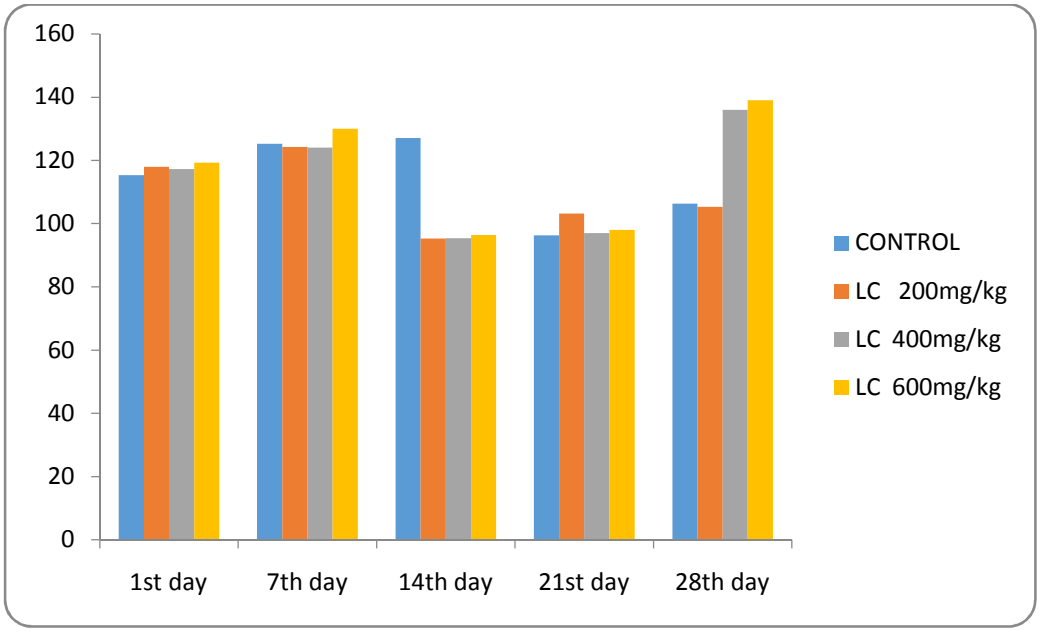
Body weight at weekly interval was measured to find out the effect of ***LINGA CHENDURAM*** on the growth rate. Body weight change in drug treated animals was found normal.

SUB-ACUTE TOXICITY STUDY IN WISTAR RATS TO EVALUATE TOXICITY PROFILE OF *LINGA CHENDHURAM*

Table :11 EFFECT OF SUB- ACUTE DOSE (28 DAYS)OF *LINGA CHENDHURAM ON* BODY WEIGHT IN GRAM

GROUP	CONTROL	LOW	MID	HIGH
1st day	115.3±1.03	118±1.543	117.3±2.231	119.3±2.23
7th day	125.3±1.03	124.3±1.343	124±2.113	130±2.11
14th day	127.1±1.004	95.3±1.12	95.4±2.012	96.4±2.012
21st day	96.3±2.120	103.2±1.501	97±1.131	98±1.13
28th day	106.3±1.041	105.3±1.202	136±2.0405	139±2.040

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

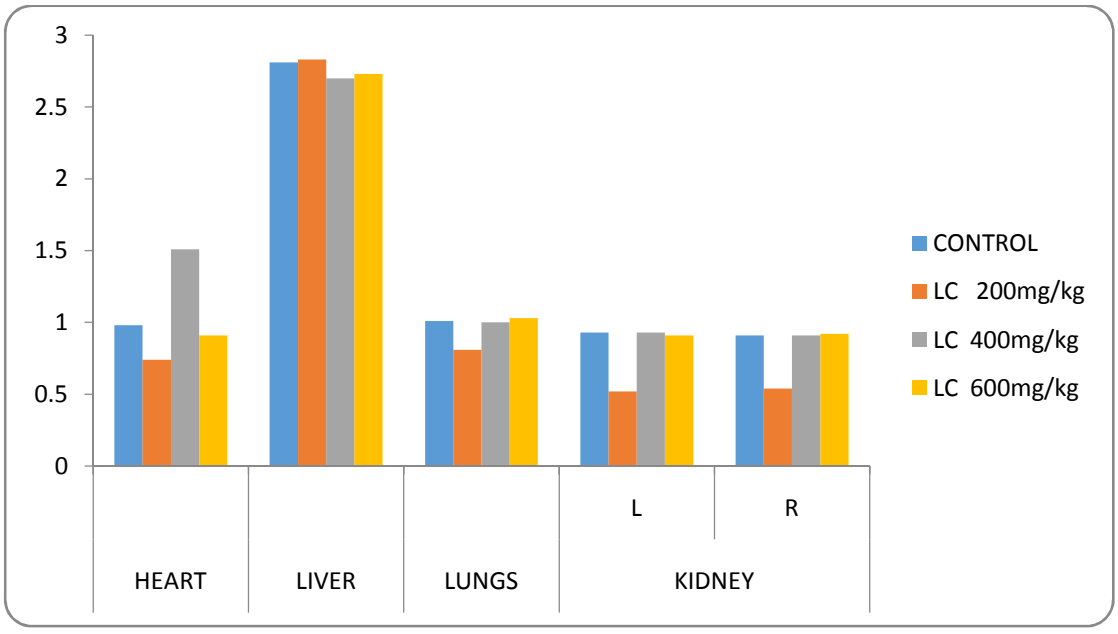


EFFECT OF SUBACUTE DOSE (28 DAYS)OF *LINGA CHENDURAM*

Table : 12 *LINGA CHENDURAM* ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM

GROUP		CONTROL	LOW	MID	HIGH
HEART		0.98±0.52	0.74±0.54	1.51±0.61	0.91±0.52
LIVER		2.81± 0.73	2.83±0.73	2.70±0.51	2.73± 0.73
LUNGS		1.01±0.60	0.81±0.64	1.00±0.74	1.03±0.60
KIDNEY	L	0.93±0.52	0.52±0.53	0.93±0.52	0.91±0.52
	R	0.91±0.524	0.54±0.52	0.91±0.524	0.92±0.024

Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by Dunnett's(n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

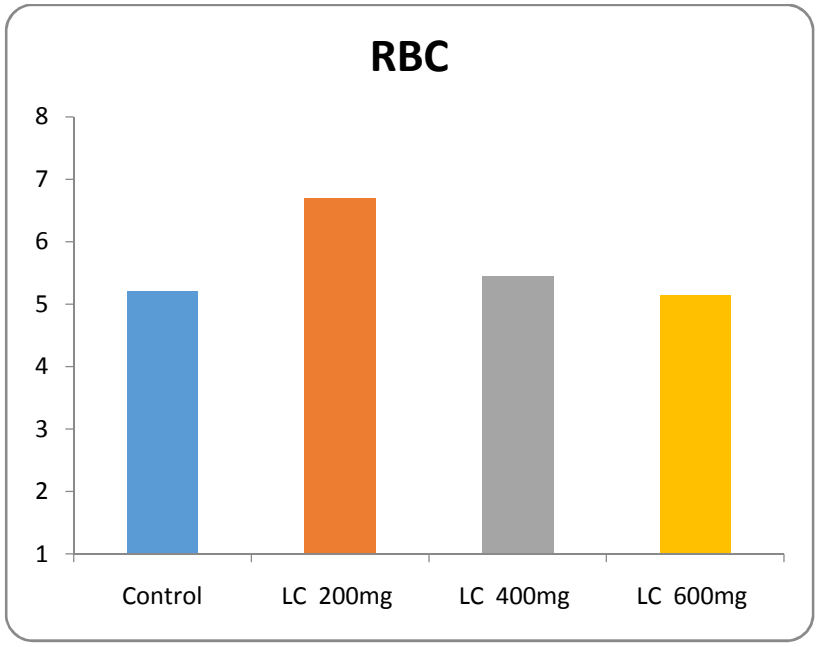


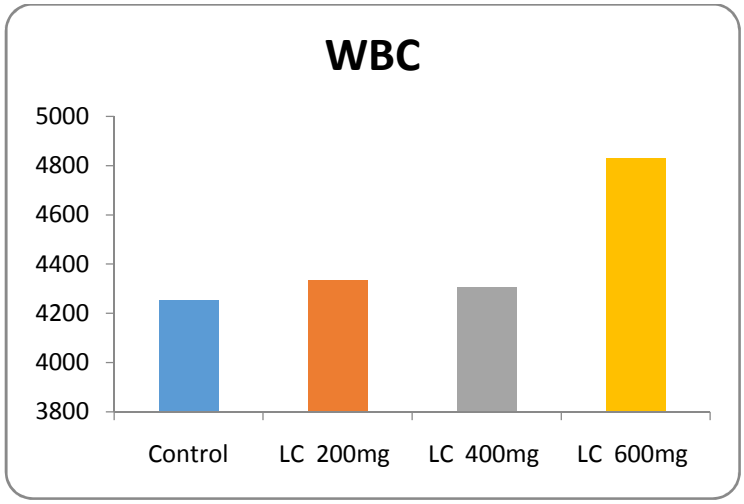
EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *LINGA CHENDURAM ON* HAEMATOLOGICAL PARAMETERS

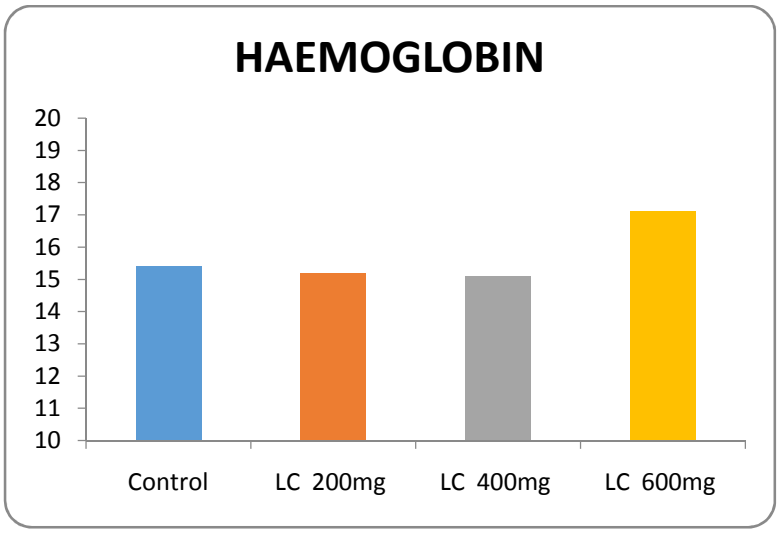
Table no 13

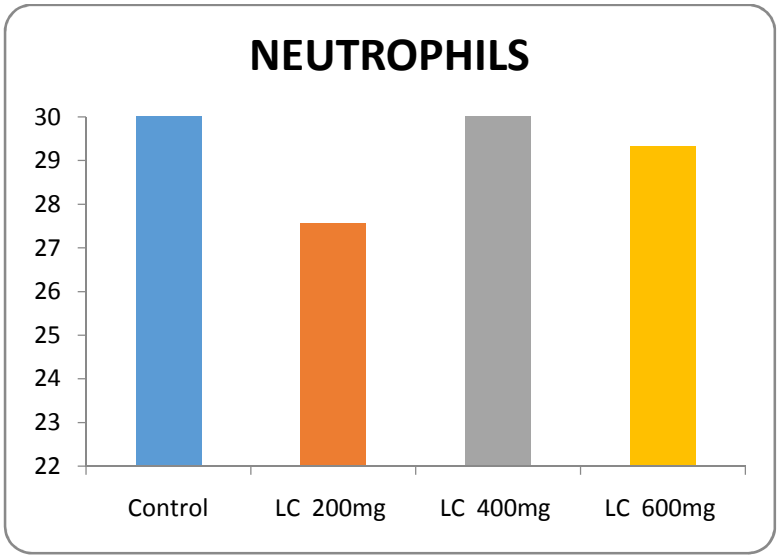
Drug treatment	RBC million cells/cmm	WBC cells/cmm	Haemoglobin gm %	Differential count %			
				Neutrophils	Eosinophils	Monocyte	Lympocyte
Control	5.21±0.50	4253.42±24.32	15.40±0.46	32.28±1.20	2.54±0.11	0.5±0.15	24.14±3.32
LOW	6.69±0.40	4335.05±24.22	15.20±0.44	27.56±1.41	3.11±0.14	0.62±0.30	24.23±3.51
MID	5.44±0.32	4305.26±33.35	15.11±1.04	31.33±2.22	2.45±0.12	0.72±0.40	24.14±3.32
HIGH	5.15±0.32	4829.26±33.35	17.11±1.04	29.33±2.22	2.51±0.12	0.54±0.40	25.14±3.32

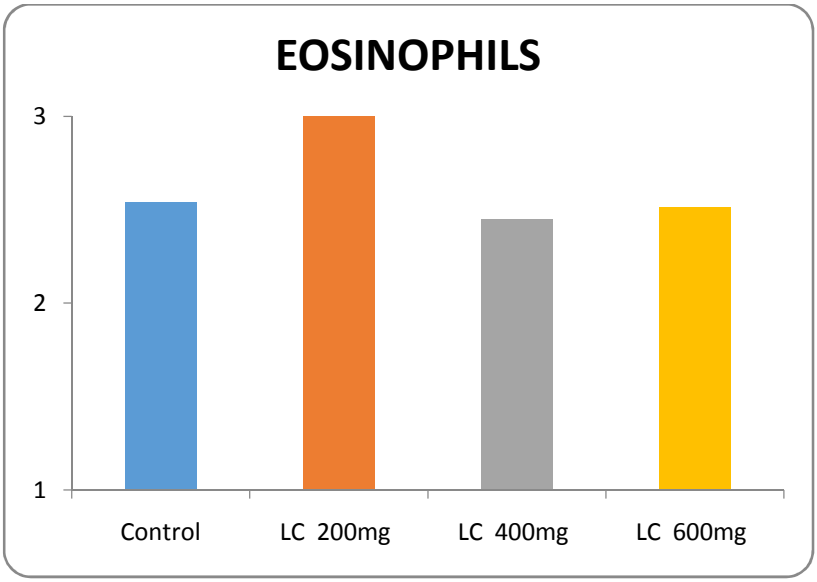
Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

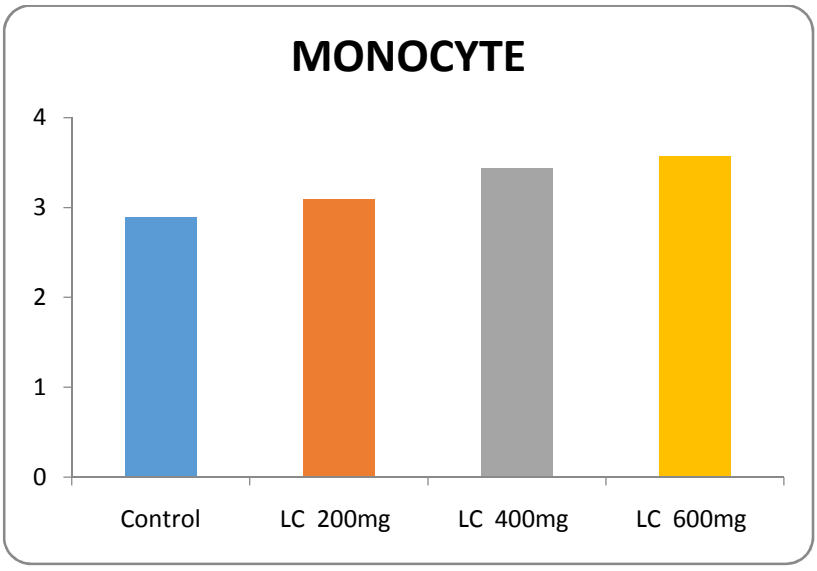



WBC
5000
4800
4600
4400
4200
4000
3800
Control
LC 200mg
LC 400mg
LC 600mg


HAEMOGLOBIN
20
19
18
17
16
15
14
13
12
11
10
Control
LC 200mg
LC 400mg
LC 600mg


NEUTROPHILS
30
29
28
27
26
25
24
23
22
Control
LC 200mg
LC 400mg
LC 600mg


EOSINOPHILS
3
2
1
Control
LC 200mg
LC 400mg
LC 600mg


MONOCYTE
4
3
2
1
0
Control
LC 200mg
LC 400mg
LC 600mg

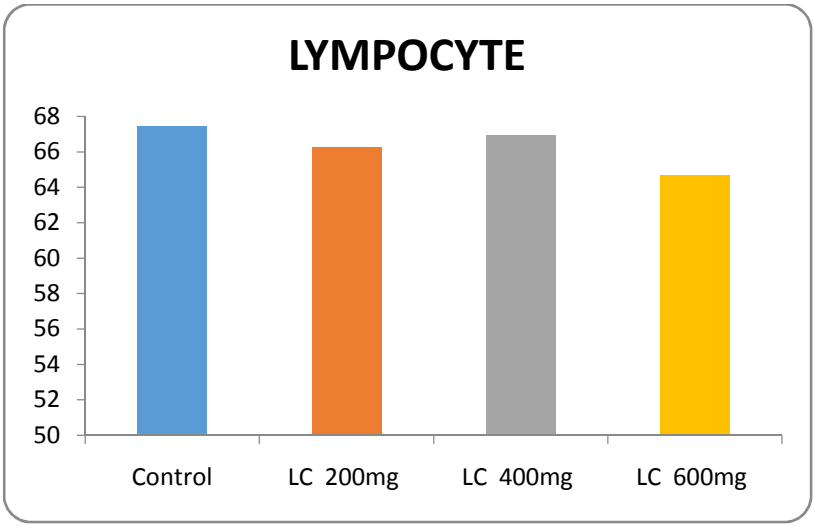
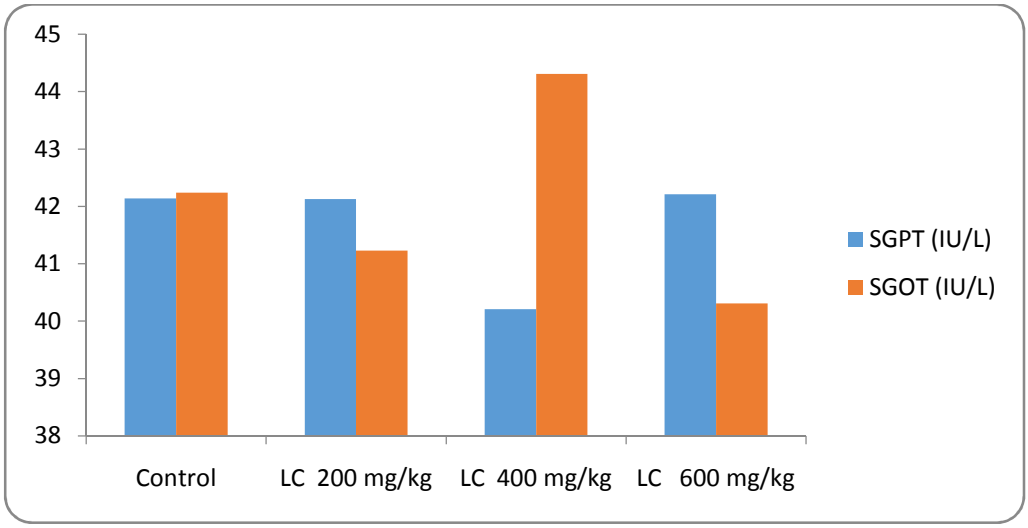
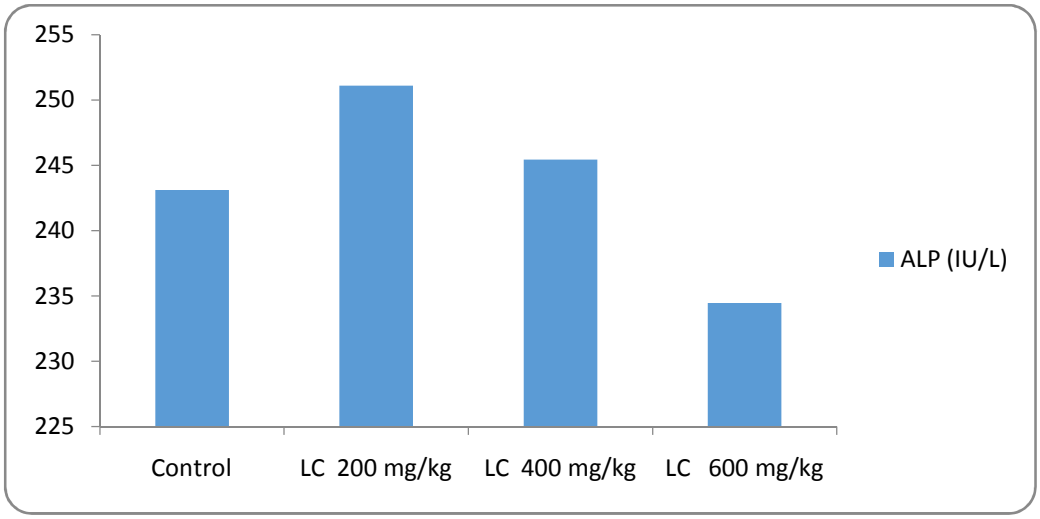
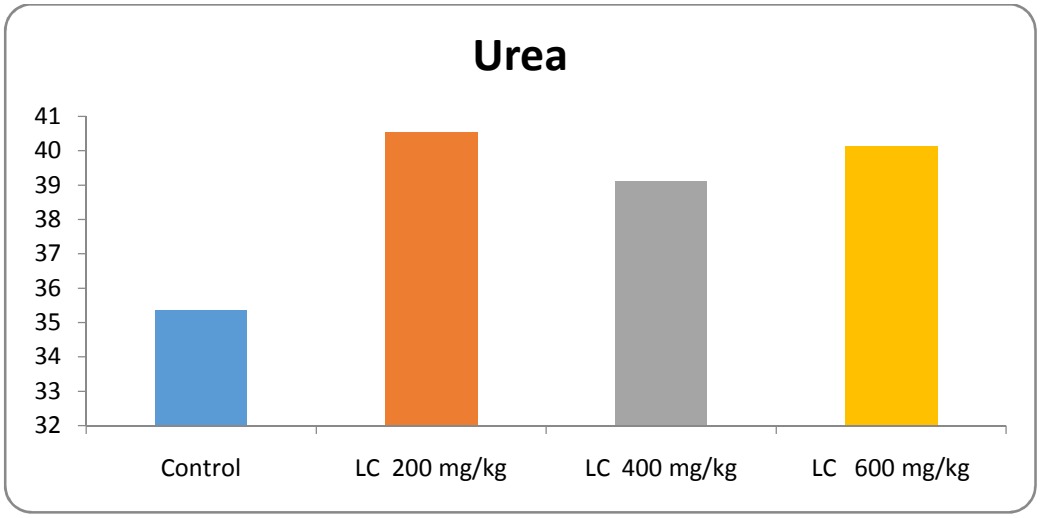

LYMPOCYTE
68
66
64
62
60
58
56
54
52
50
Control
LC 200mg
LC 400mg
LC 600mg

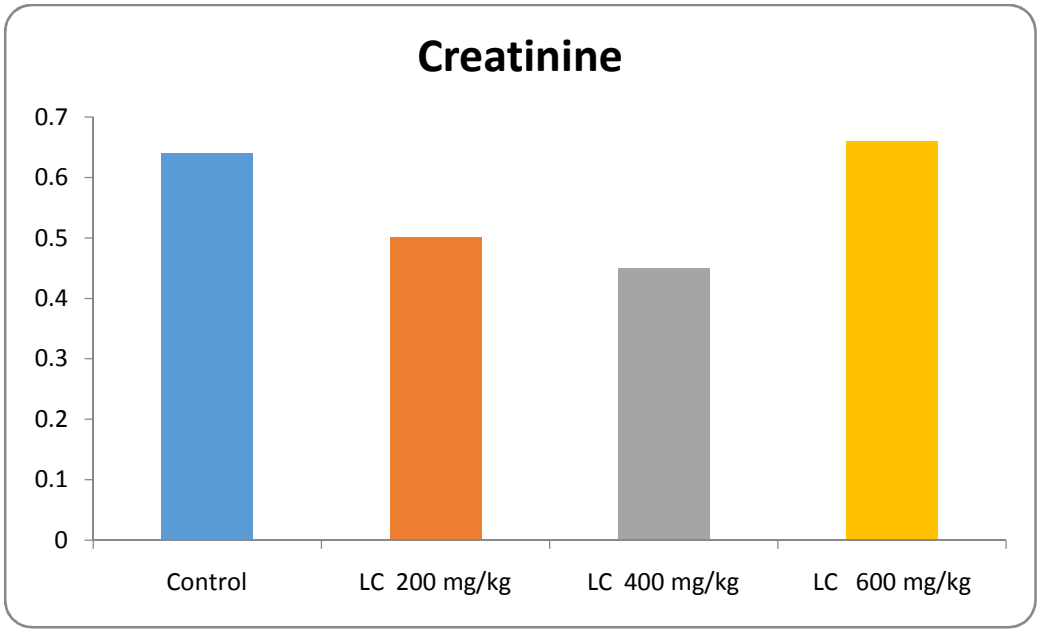
Table :14 EFFECT OF SUB- ACUTE DOSE(28 DAYS)OF *LINGA CHENDHURAM*ON BIOCHEMICAL PARAMETER

Drug Treatment	SGPT (IU/L)	SGOT(IU/L)	ALP(IU/L)	Urea (mg/dl)	Creatinine(mg/dl)
Control	42.14±3.02	42.24±4.31	243.12±11.32	35.35±3.00	0.64±0.03
LOW	42.13±3.22	41.23±4.01	251.11±12.42	40.53±2.42	0.50±0.04
MID	40.21±4.44	44.31±2.21	245.45±4.14	39.12±2.22	0.45±0.04
HIGH	42.21±4.44	40.31±2.21	234.45±4.14	40.12±2.22	0.66±0.04






Urea
41
40
39
38
37
36
35
34
33
32
Control
LC 200 mg/kg
LC 400 mg/kg
LC 600 mg/kg


Creatinine
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
LC 200 mg/kg
LC 400 mg/kg
LC 600 mg/kg

EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *LINGA CHENDHURAM*BIOCHEMICAL PARAMETERS

GROUP	CONTROL	*LINGA CHENDURAM* (200mg/kg)	*LINGA CHENDURAM* (400mg/kg)	*LINGA CHENDURAM* (600mg/kg)
TOTAL BILIRUBIN (mg/dl)	0.508±0.2457	0.758±0.27	0.88±0.6	0.904±0.19

Values are

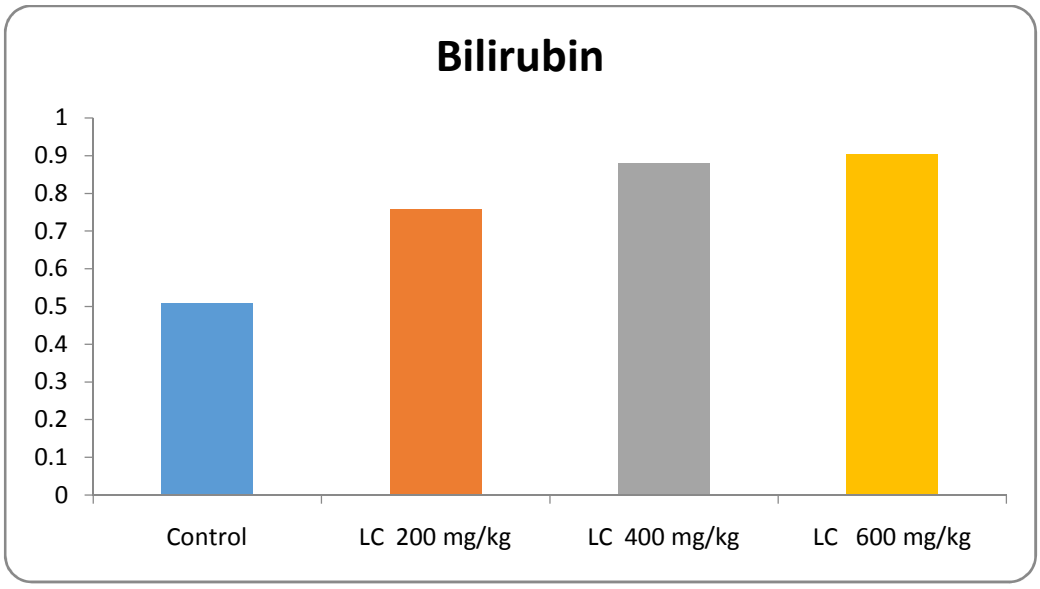


Table:15 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF ON FOOD INTAKE IN GRAM

GROUP	CONTROL	Low	mid	high
1st DAY	18.33±13.5110	19.1672±14.3	12.10±21.71	17.5±7.62
7th DAY	15.5±11.	10.863±12.67	16.73±9.853	11.17±14.41
14th DAY	18.83±8.72	10.83±14.28	10±13.96	19.72±8.981
21st DAY	11.87±12.4	15±8.466	15.88±9.43	19.17±8.02
28th DAY	12.10±11.38	18.38±11.50	10±8.90	10±7.57

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group

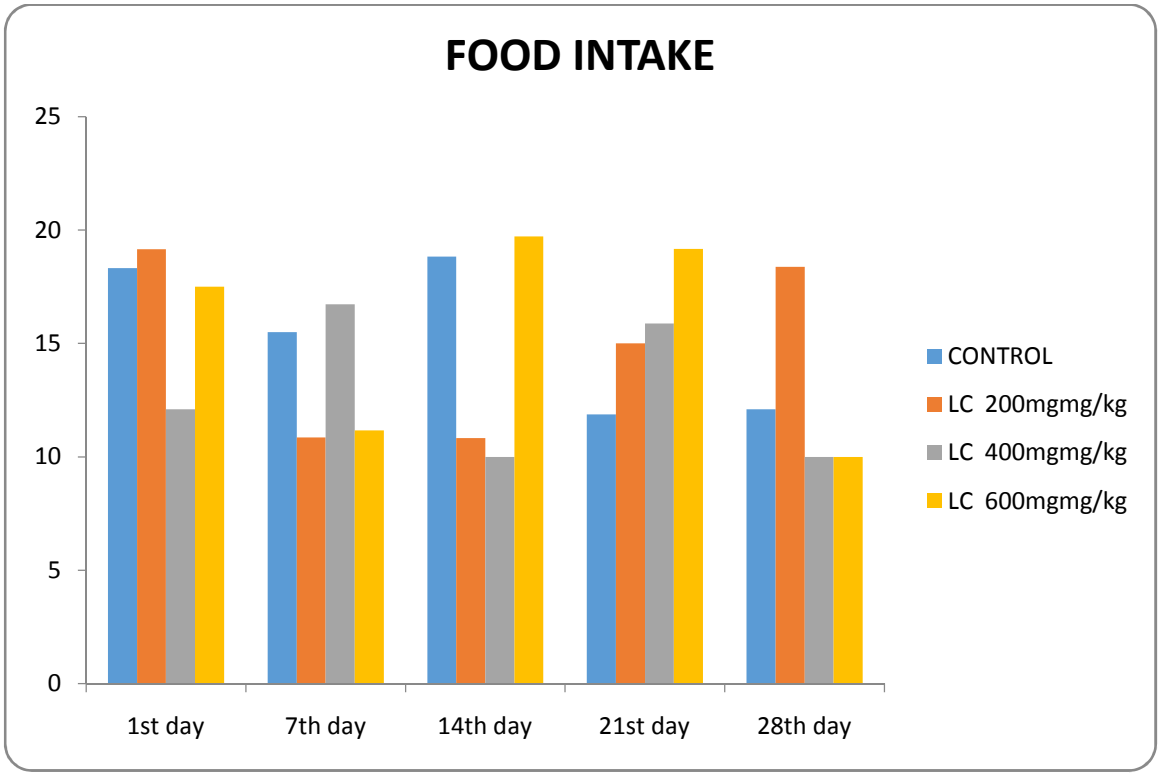


Table:16. EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF *LINGA CHENDURAM* ON WATER INTAKE IN ML

GROUP	CONTROL	*LC* (200mg/kg)	*LC* (400mg/kg)	*LC* (600mg/kg)
1st DAY	98.8±13.50	89.12±14.26	102.10±21.99	67.5±7.63
7th DAY	85.5±11.79	100.863±12.60	76.63±9.363	81.6717±14.450
14th DAY	58.3383±8.72817	90.8363±14.2812	80±13.9692	89.1672±8.0
21st DAY	91.6687±12.4	85±8.66	65.8±9.550	89.7±8.62
28th DAY	82.10±11.384	88.3348±11.4	80±8.961	70±7.573

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); ^{ns}p>0.05, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups

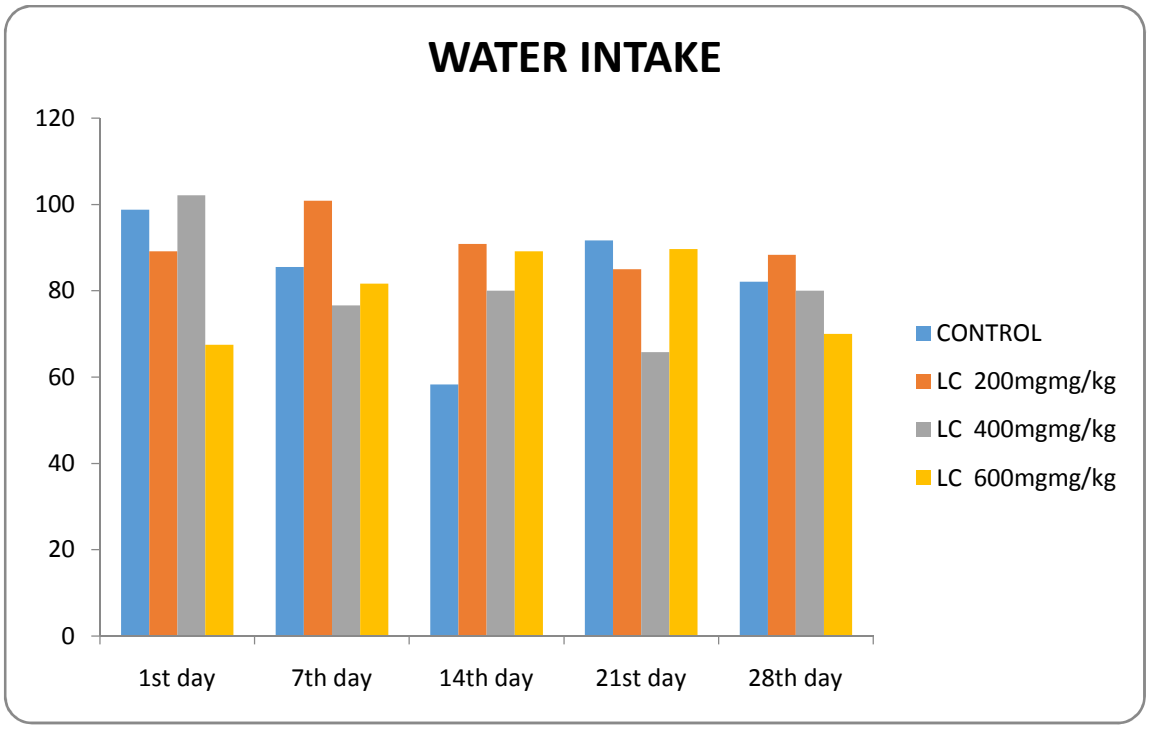
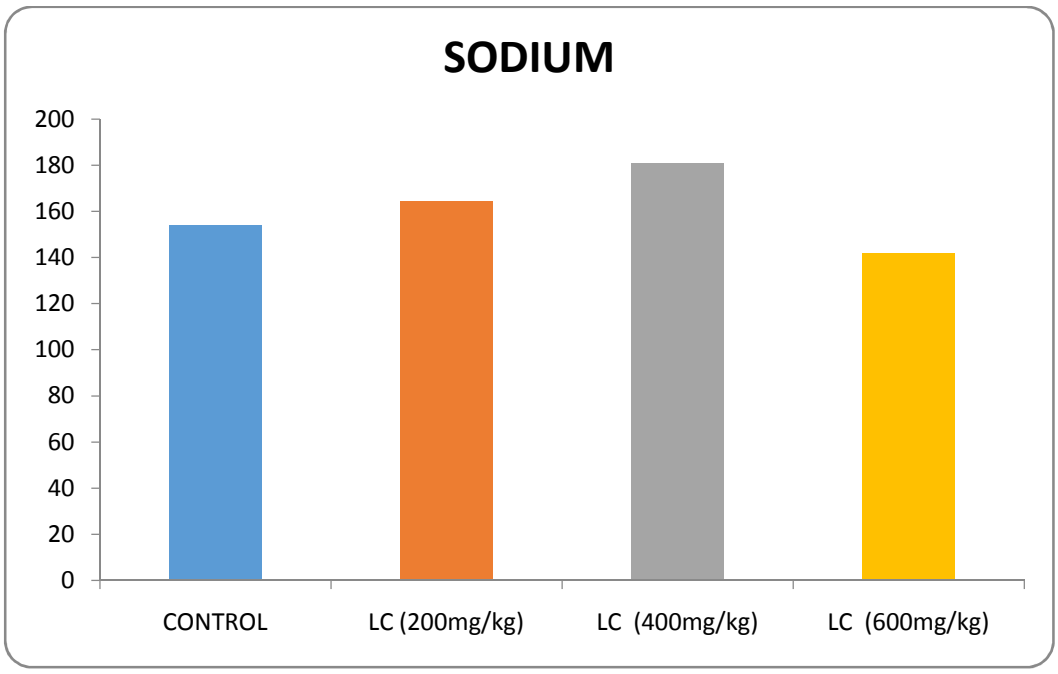
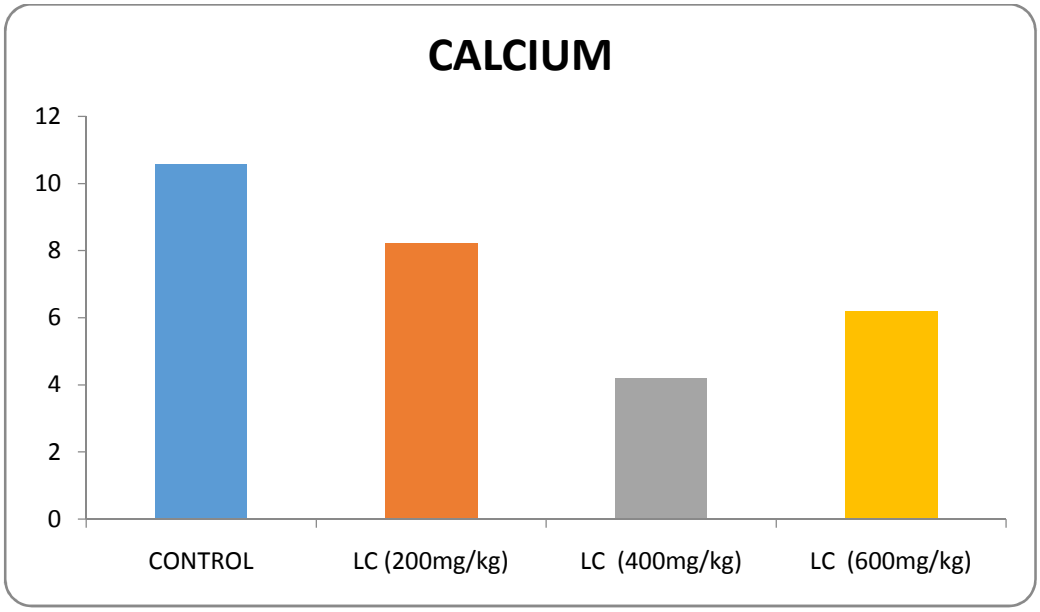


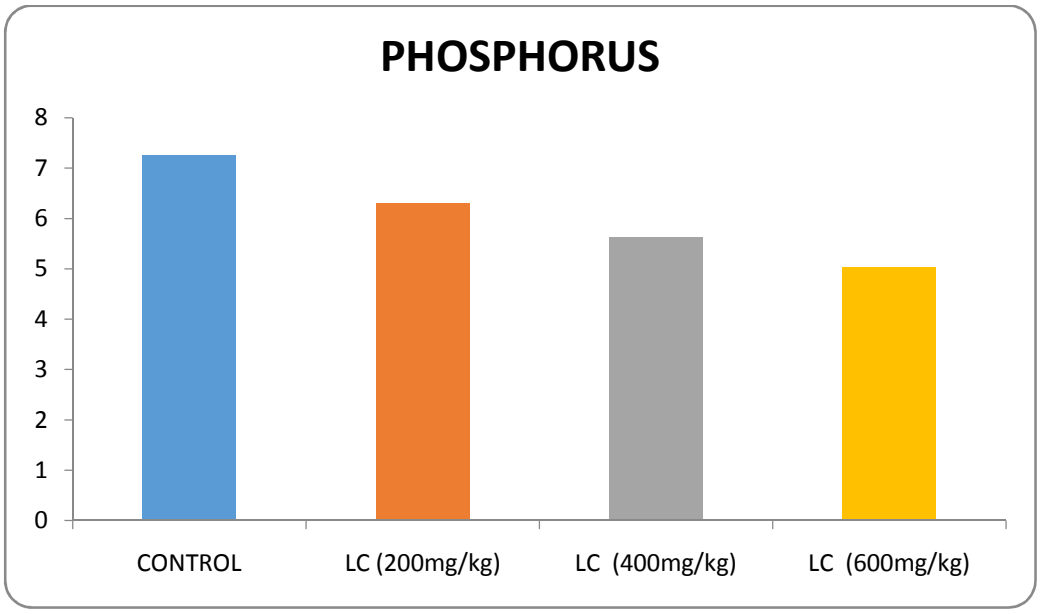
Table: 17 EFFECT OF SUB ACUTE DOSES (28 DAY) OF *LINGA CHENDHURAM*ON ELECTROLYTES: -

GROUP	CONTROL	*LC* (200mg/kg)	*LC* (400mg/kg)	*LC* (600mg/kg)
Sodium (mg/dl)	154.10±0.5	164.30±0.62	181±0.71	141.80±0.70
Calcium(mg/dl)	10.580±0.19	8.20±3***	4.2***	6.180±0.191***
Phosphorus (U/L)	7.278±0.027	6.3010 ± 0.0115^{ns}	5.630 ± 0.091^{ns}	5.037±0.32*

Values are expressed as mean ± SEM Statisticalsignificance (p) calculated by one-way ANOVA followed by Dunnett's(n=6); NS- non-significant, *p<0.05, **p<0.01, ***p<0.001




CALCIUM
12
10
8
6
4
2
0
CONTROL
LC (200mg/kg)
LC (400mg/kg)
LC (600mg/kg)


PHOSPHORUS
8
7
6
5
4
3
2
1
0
CONTROL
LC (200mg/kg)
LC (400mg/kg)
LC (600mg/kg)

6.0 RESULTS:

CLINICAL SIGNS:

All animals in this study were free of toxic clinical signs throughout the dosing period of 28 days.

Mortality:

All animals in control and in all the treated dose groups survived throughout the dosing period of 28 days.

Body weight:

Results of body weight determination of animals from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

Food consumption:

During dosing and the post-dosing recovery period, the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

Organ Weight:

Group Mean Relative Organ Weights (% of body weight) are recorded in Table No.22 Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable similarly.

Hematological investigations:

The results of hematological investigations conducted on day 29 revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

Biochemical Investigations:

Results of Biochemical investigations conducted on the day 29th and recorded in Table no 24, 25 revealed the following significant changes in the values of hepatic serum enzymes studied. When compared with those of respective control. However, the increase or decrease in the values obtained was within normal biological and laboratory limits.

INTERPRETATION:

All the animals from control and all the treated dose groups up to 15ml/kg survived throughout the dosing period of 28 days.

1. No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.
2. Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.
3. Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days
4. Haematological analysis conducted at the end of the dosing period on day 29th, revealed no abnormalities attributable to the treatment.
5. Biochemical analysis conducted at the end of the dosing period on day 29^{th}, no abnormalities attributable to the treatment.
6. Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.

PHARMACOLOGICAL RESULTS

EVALUATION OF APHRODISIAC ACTIVITY OF LINGA CHENDURAM IN ETHANOL TREATED MALE RATS

Table No.18. Mount frequency observed in the evaluation of Aphrodisiac activity of LC

Groups	Mount frequency (Sec)
Control	6.16±0.32
Ethanol treated rats	0.56±0.12
Ethanol+ *LC*100mg/kg	3.54±0.26
Ethanol+*LC*200mg/kg	4.13±0.14

*Values are expressed as mean S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

Fig. 9. Mount frequency observed in the evaluation of Aphrodisiac activity of LC

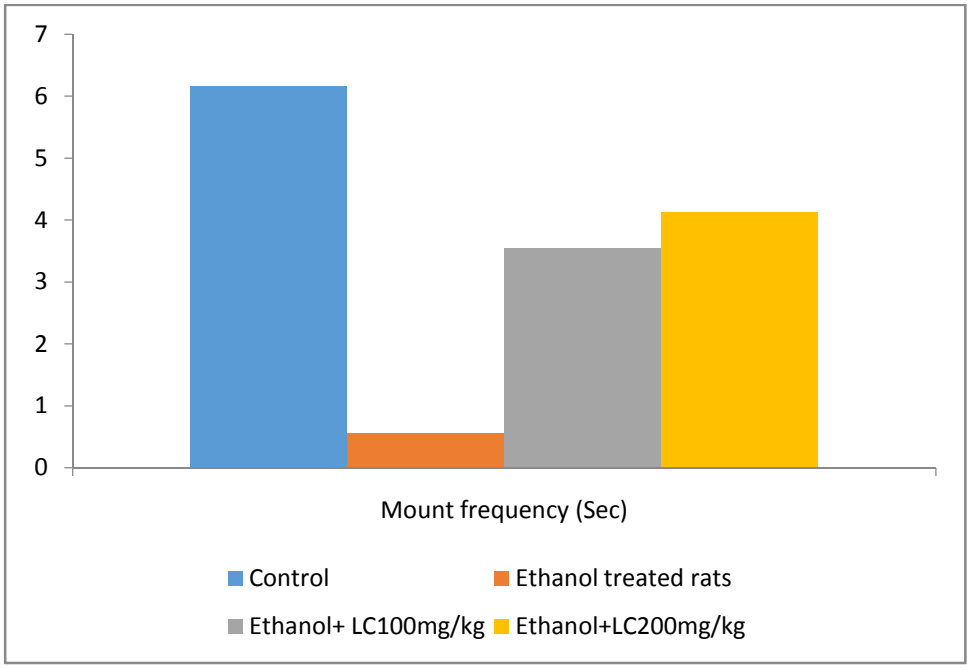


Table No.19. Intromission frequency observed in the evaluation of Aphrodisiac activity of LC

Groups	Intromission frequency (Sec)
Control	3.28± 0.26
Ethanol treated rats	0.56±0.9
Ethanol+ *LC*100mg/kg	1.64±0.22*
Ethanol+*LC*200mg/kg	2.29±0.22**

*Values are expressed as mean S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

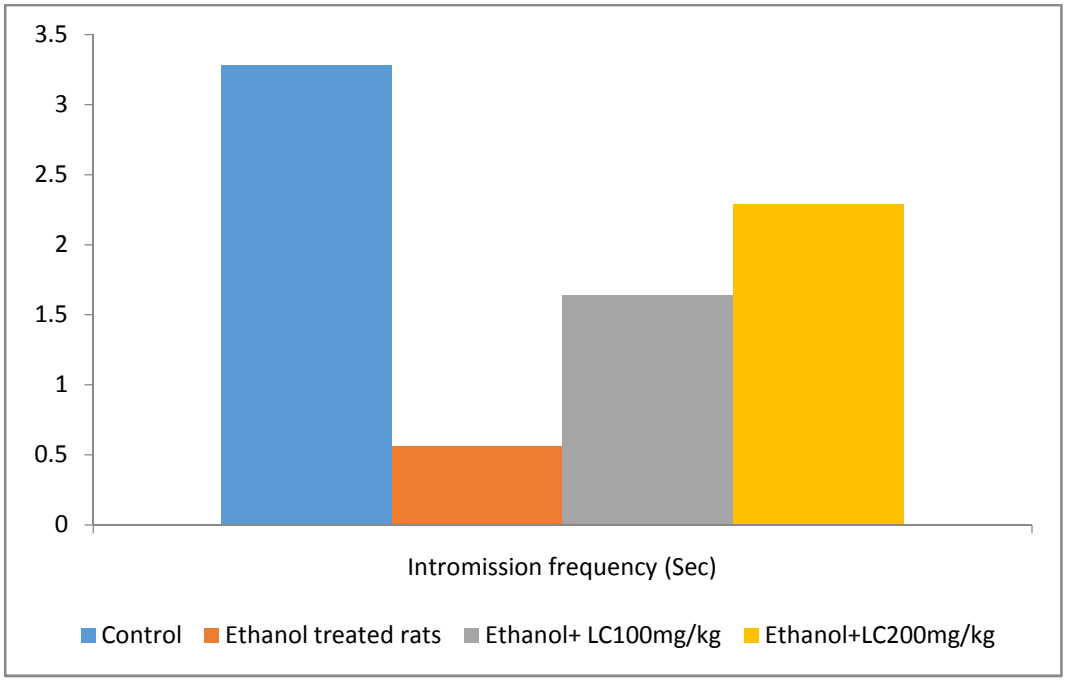


Table No.20. Mount latency observed in the evaluation of Aphrodisiac activity of LC

Groups	Mount latency (sec)
Control	244.21±7.16
Ethanol treated rats	379.14±8.15
Ethanol+ *LC*100mg/kg	276.19±5.22*
Ethanol+*LC*200mg/kg	242.00±2.12***

*Values are expressed as mean S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

Table No.21. Intromission latency observed in the evaluation of Aphrodisiac activity of LC

Groups	Intromission latency (sec)
Control	227.18±26.06
Ethanol treated rats	936.26±318.64
Ethanol+ *LC*100mg/kg	114.16±120.44**
Ethanol+*LC*200mg/kg	202.32±58.15***

*Values are expressed as mean S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

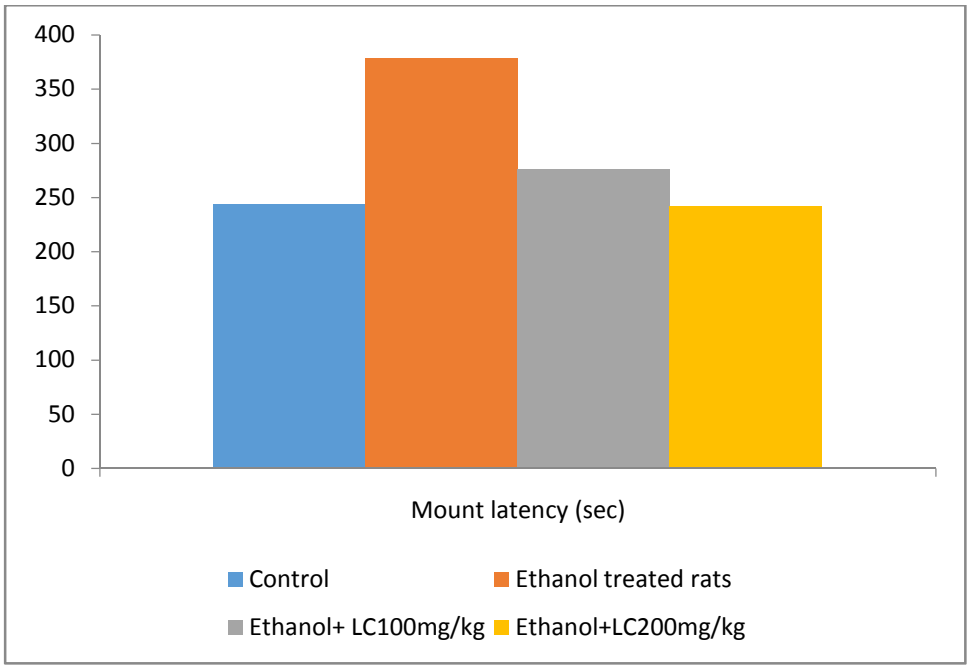


Table No.22 Ejaculation latency observed in the evaluation of Aphrodisiac activity of LC

Groups	Ejaculation latency (min)
Control	4.09±0.58
Ethanol treated rats	1.12±0.69
Ethanol+ *LC*100mg/kg	3.43±0.56**
Ethanol+*LC*200mg/kg	3.98±0.85***

*Values are expressed as mean S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

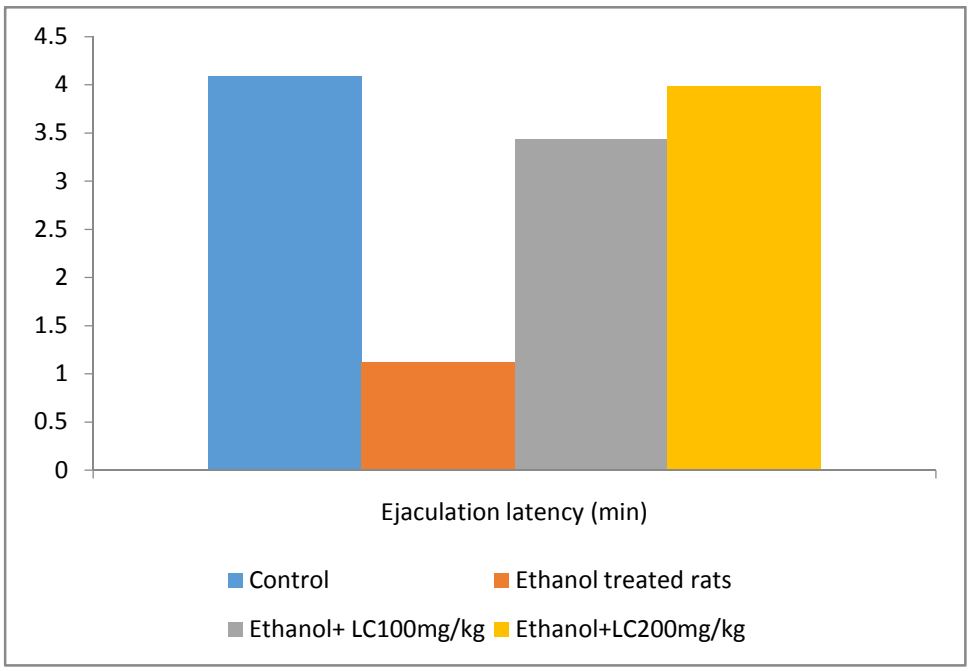
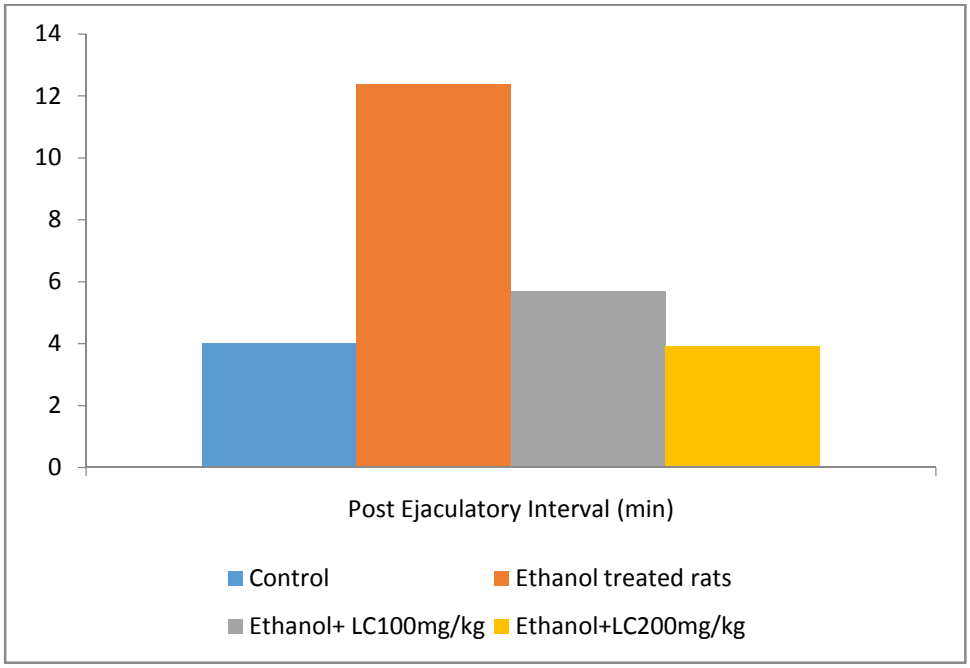


Table No: 23 Post Ejaculatory Interval observed in the evaluation of Aphrodisiac activity of LC

Groups	Post Ejaculatory Interval (min)
Control	4.02±0.77
Ethanol treated rats	12.4±1.07
Ethanol+ *LC*100mg/kg	5.7±0.15**
Ethanol+*LC*200mg/kg	3.92±0.42***

*Values are expressed as mean S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*



Interpretation

From the observed results of mount frequency, intromission frequency, mount latency, intromission of latency, ejaculation latency and post ejaculation latency can confirmed that the trial drug *LC* has the property of Aphrodisiac. The study concluded that the cumulative dose of *LC* could enhance overall sexual function and performance in male rats by increasing the spermatozoa concentration and hormonal levels such as FSH, Testosterone, LH. The drug *LC* showed more potent aphrodisiac activity at the dose level of 100mg/Kg BW and 200mg/Kg BW. The results suggest that the prepared *LC* may be a new promising aphrodisiac combination, which can be used to improve the sex life of many troubled men. This aphrodisiac property may be due to possible synergistic action of selected plants used in this *LC*

EVALUATION OF SPERMATOGENIC ACTIVITY OF *LINGA CHENDURAM* IN ETHANOL INDUCED WISTAR MALE RATS

Table No 24. Effect of on sperm count and motility

Groups	Intervention	Sperm Count	Sperm Motility(%)
Group I[C]	1 ml of Milk	216.22±1.187	96.12±1.83
Group II [IG]	25% of ethanol (0.5 ml/kg/day)	80.79±0.765	36.21±1.67
Group III [TG-1]	LC (100mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	140.96±2.51*	72.28±1.783*
Group IV [TG-2]	LC (200mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	161.86±1.86**	77.17±1.96**

*Values are expressed as mean S.E.M (Dunnett's test). *P<0.05, **P<0.01, ***P<0.001 vs control; N=6*

Fig . No 10. Effect of on sperm count and motility

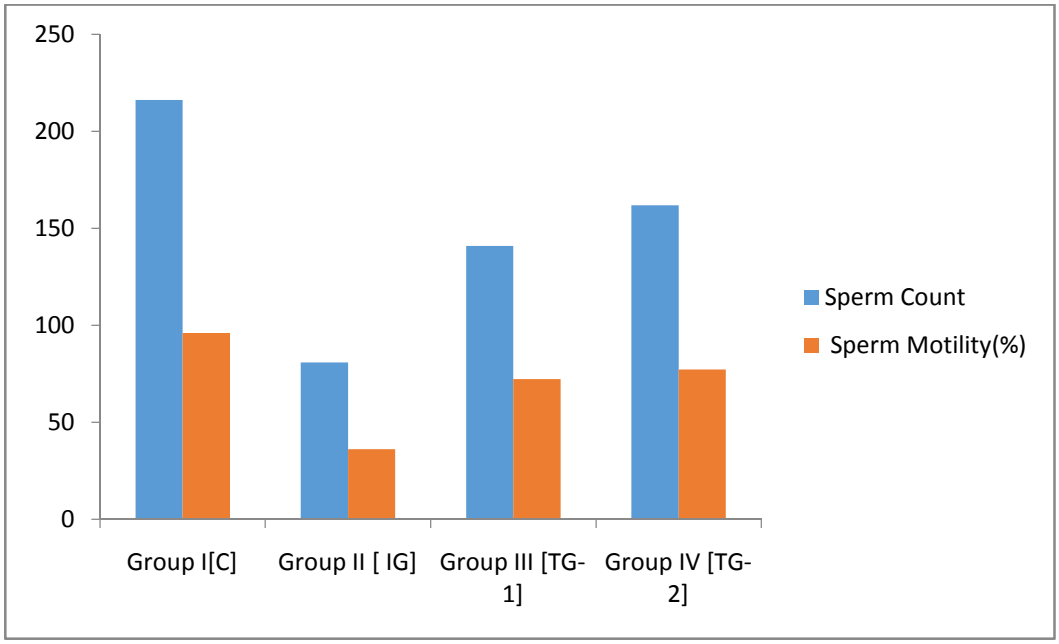


Table No.25. Effect of LC on Sperm morphology and viability

Intervention	Morphology		Viability
	Normal	Abnormal	
1 ml of Milk	86.66±4.52	30.82±1.07	78.31±2.66
25% of ethanol (0.5 ml/kg/day)	38.13±0.65	79.69±0.99*	56.71±1.90
LC (100mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	80.31±2.78*	33.52±1.03	74.43±2.08*
LC (200mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	86.82±2.92**	29.42±0.45	76.73±1.26*

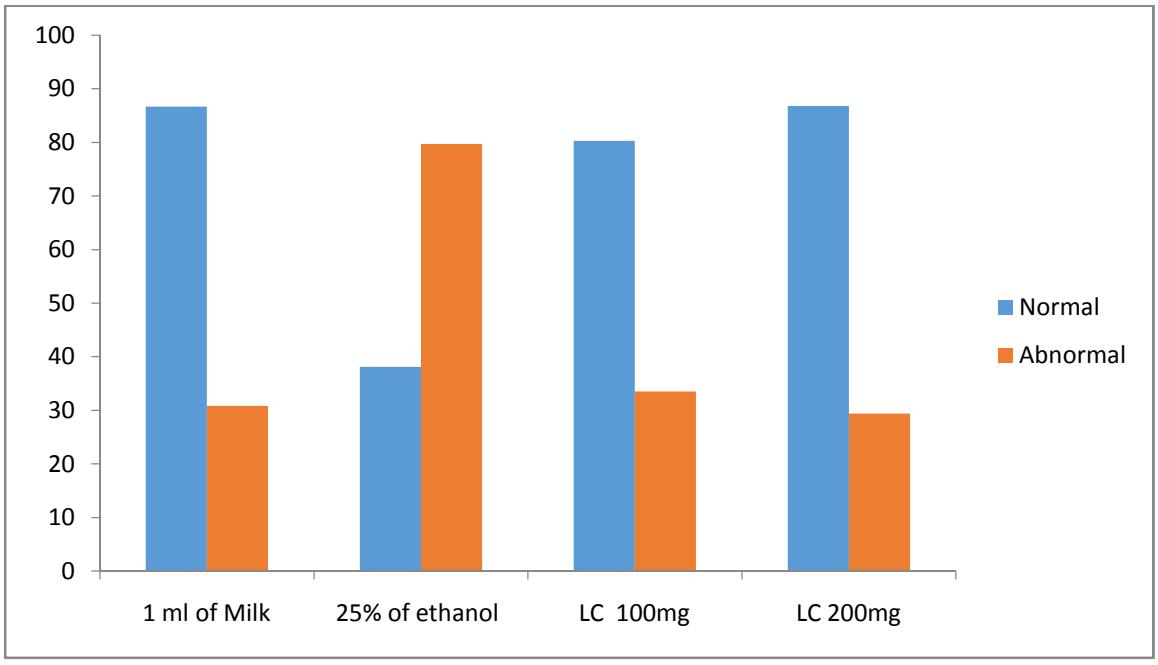
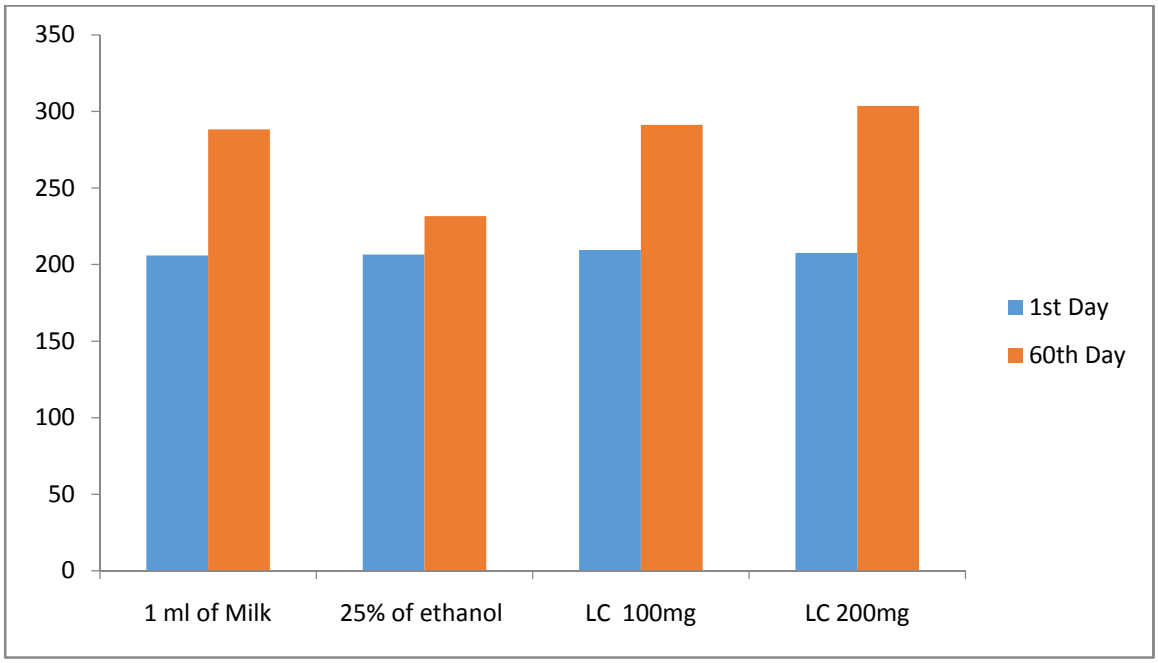


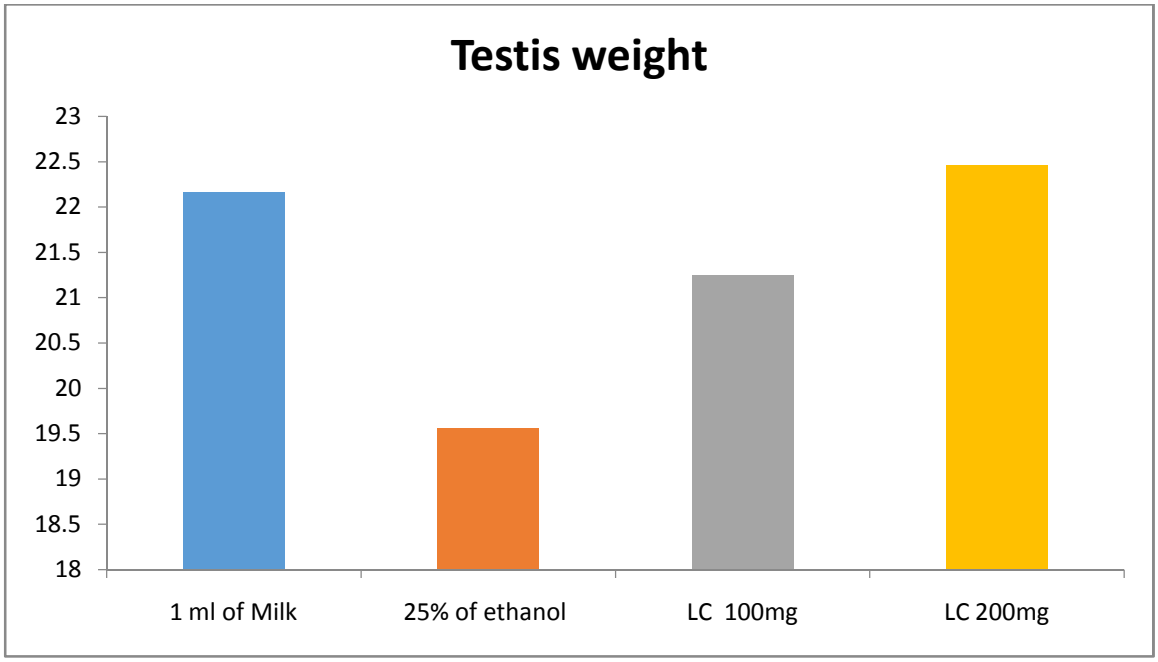
Table No. 26. Effect of LC on Body and Testis weights

Intervention	Body weight(g)		Testis weight(g)
	1st day	60th day	
1 ml of Milk	205.88±5.02	288.39±3.13	22.16±0.419
25% of ethanol (0.5 ml/kg/day)	206.60±3.25	231.71±1.98	19.563±0.308
LC (100mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	209.52±3.90	291.23±3.54	21.25±0.801**
LC (200mg / kg / day) + 25% of ethanol (0.5 ml/kg/day)	207.64±2.08*	303.62±4.56*	22.46±0.96

Values are expressed as mean S.E.M (Dunnett's test).

P<0.05,P<0.01,***P<0.001 vs control; N=6*





INTERPRETATION

From the above obtained results, can conclude that the drug *LINGA CHENDURAM* exhibits the spermatogenic activity by increasing the number of spermatozoa in somniferous tubules. The trial drug possess significant Spermatogenic activity at the dose levels of 100 and 200 mg/kg of body weight. usally plant products play a major role in the functioning of spermatogenesis like ashwagandha. Likewise the drug *LINGA CHENDURAM* also possess potent spermatogenic activity. Already the ingredients of this LC formulation cinnabar and nelumbo nucifera were proved as a potent drug for spermatogenesis. So, this evaluation of spermatogenic activity study will lead the way for the clinical use of the drug.

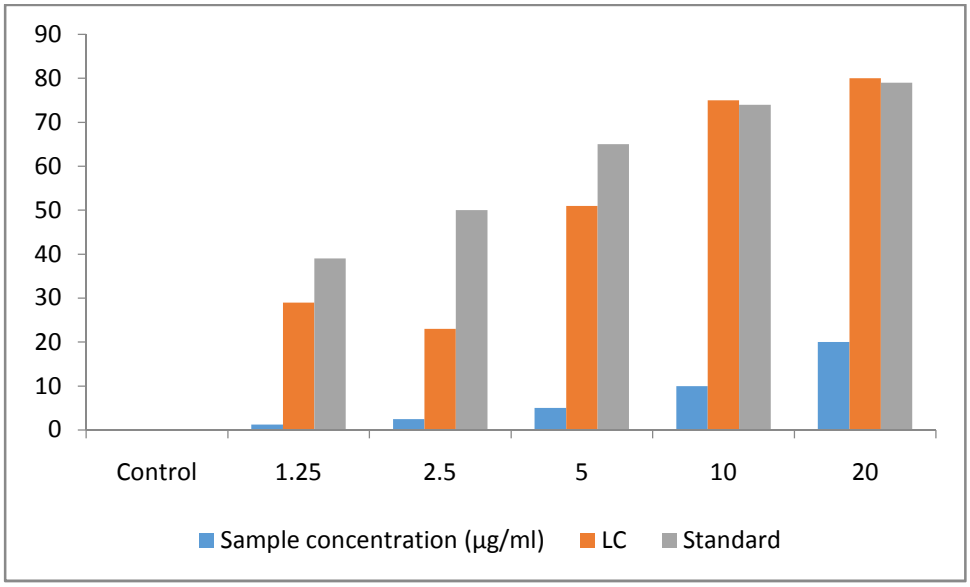
EVALUATION OF ANTIOXIDANT ACTIVITY OF *LC* THROUGH DPPH (2, 2-DIPHENYL 1-2 PICRYLHYDRAZYL) ASSAY

Table : 27 Evaluation of Antioxidant Activity

Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	LC	Standard	LC	Standard
Control	0.5201	0.364	-	-
1.25	0.4881	0.220	29%	39%
2.50	0.3955	0.181	23%	50%
5	0.2522	0.124	51%	65%
10	0.1250	0.092	75%	74%
20	0.1005	0.076	80%	79%

µg/ml: microgram per millilitre. Drug: LC (1.25-20µg/ml). Standard: BHT (10mg/ml)

Fig : 11 Evaluation of Antioxidant Activity



INTERPRETATION

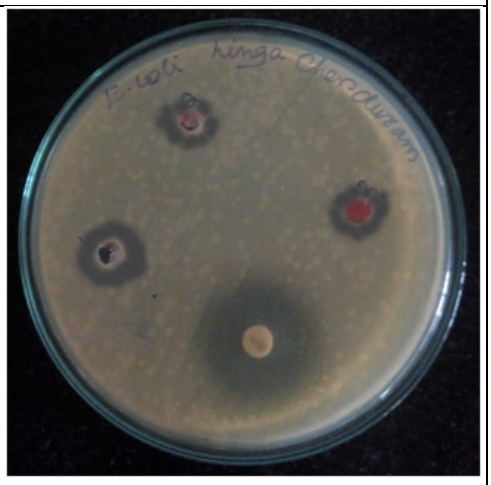
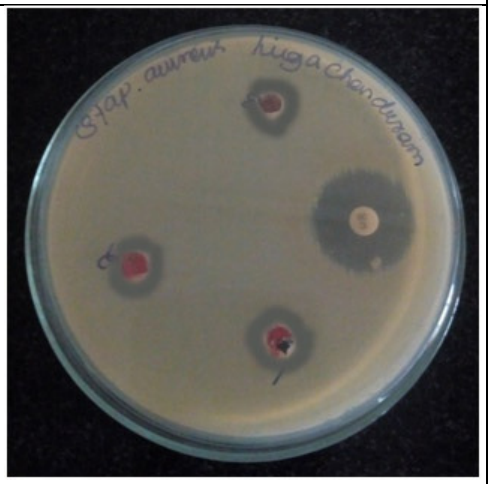
From the investigation of DPPH radical scavenging assay of LC it was concluded that the test drug has shown promising antioxidant activity and exhibits significant percentage inhibition against DPPH radicals when compared to that of standard BHT. Because of this high antioxidant therapeutic nature the drug *LINGA CHENDURAM* will helps to treat the male infertility. Antioxidants play a major role in the treatment of male infertility. So the presence of antioxidant property of LC will be highly useful for the treatment of male infertility.

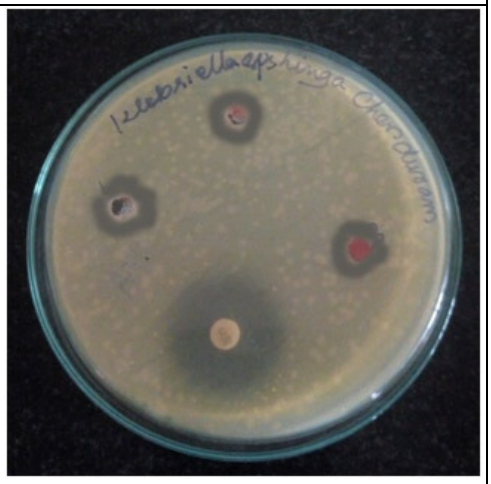
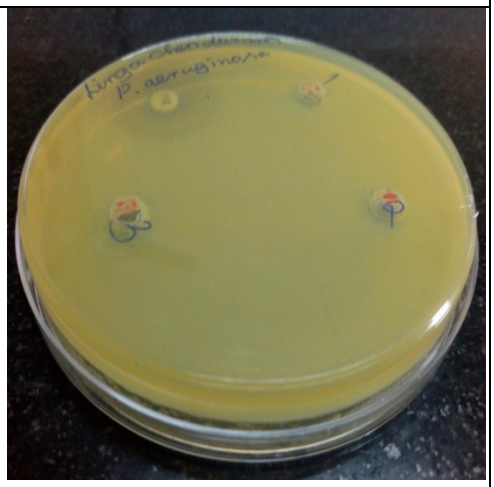
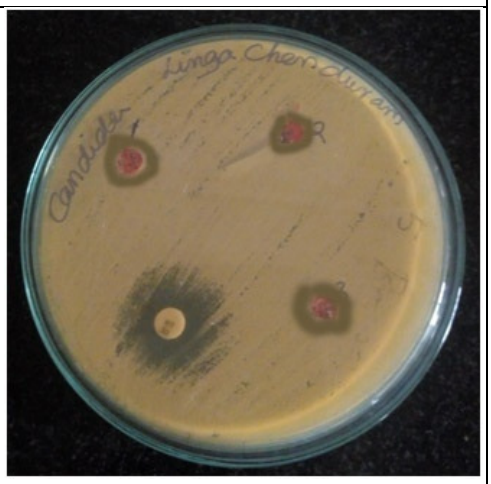
ANTIMICROBIAL ACTIVITIES OF LINGA CHENDURAM BY AGAR WELL DIFFUSION METHOD

Table : 28

S.No.	Test Pathogens	Result	Zone of Inhibition (mm) at 30µl	
			Positive Control (Chloromphenical)	**Size of Inhibition**
1.	*Escherichia coli*	Sensitive	25 mm	21 mm
2	*Klebsiella pneumoniae*	Sensitive	20 mm	12 mm
3	*Staphylococcus aureus*	Sensitive	20 mm	10 mm
4	*Pseudomonas aeruginosa*	Resistant	23 mm	5 mm
5.	*Candida sp (Ketaconozole)*	Sensitive	24 mm	10 mm

Figure No: 12

S.No.	Bacterial Pathogens	Plates
1.	*Escherichia coli*	
2.	*Staphylococcu aureus*	

S.No.	Bacterial Pathogens	Plates
3.	*Klebsiella sps.*	
4.	*Pseudomonas aeruginosa*	
5.	*Candida sp*	

INTERPRETATION :

Both gram positive, gram negative bacteria E.Coli, Klebsiella, Pneumonia and Staphylococcus aureus and fungus Candida species were found to be sensitive when compared to the standard drug Chloromphenical and Ketaconozole. The bacteria Pseudomona aeruginosa were found to be resistance. The mineral drug **Linga Chenduram** exhibited broad spectrum activity against bacterial and fungal pathogenes at 100 mg/ml concentration of the drug.

7. SUMMARY

- The trial drug "Linga chenduram" was selected from the text Anuboga Vaithiya Navaneetham" for the validation of safety, efficacy and its potency in Aphrodisiac, Spermatogenisis, Anti oxidant activities.
- The ingredient *lingam* were collected from Gobala aasan shop Nagerkovil, Kanyakumari District. The *Thamarai Kizhangu* were collected from Sri lanka. The raw drugs were identified and authenticated by experts of PG Gunapadam department, Government Siddha Medical College, Palayamkottai.
- The trial drug was prepared as per the standared operting procedure.
- Various literacy collections of siddha and modern sciences about the ingredients of the trial drug supports the fact of Linga chenduram has good effect in thathu viruthi.
- The results of physio-chemical analysis confirm that the Linga chenduram is a red colour and low moisture content and did not contain free metals or microbs. Thus ensuring its safety efficacy.
- In physico-chemical analysis shows, all the parameters are within acceptable range. So it can be stored for a long period and would not easily be attacked by microbes.
- Linga chenduram when subjected to preliminary tests for basic and acidic radicals showed the presence of calcium, chloride, sulphate, ferrous iron, mercury of these calcium and chloride play major role in maintaining neuromuscular excitability the principle mechanism of aphrodisiac activity.
- As per siddha literature sukkilavadham is due to decreased vadha humour and increased kabha humour leads to severe emaciation of the body affects the venneer (spermmotility). Rhizome of nelumbo nucifera have sweet and astringent taste both taste normalise the vadha humour, lingam (cinnabar) normalise the deranged kapha humour, treats sperm abnormalities.
- Calcium is involved in muscle contraction. Thus it reduces fatigue, calcium ions have an apparently paradoxical effect on sperm motility. In the epididymis, calcium ions stimulate immature sperm, whereas in ejaculated semen, calcium ions inhibit sperm motility, maturation processes change the response of sperm to calcium ions. Calcium binding substances and calcium transport inhibitors are secreted by male accessory sexual organs and mixed

with sperm during ejaculation. In the female genital tract sperm acquire full capacity of fertilize the ovum. Calcium binding substances and calcium transport inhibitors are removed during the process known as capacitating. Finally, calcium ions trigger the acrosome reaction and facilitate sperm penetration into the ovum.

- The main ingredient of the drug is red sulphate, but ICP-OES results of final product shows below detection limit of calcium, zinc, potassium, magnesium, ferrous iron. It is evident that the effectiveness and safety of siddha medicine has been proved by modern scientic way.
- Zinc directly involved in sperm production, maturation, activation or capacitation process the impact of speculative activity on sperm zinc is mainly due to the direct effect of zinc on sperm.
- Calcium is important for normal muscle contraction and blood vessel structure. Calcium is a cell signaling mineral, which means it plays a vital role in cell-to cell communication.
- Pottassium channel openers comprise a diverse group of chemical agents which open plasmalemmal K-channels. The type of K-channel involved in the actions of both exogenous K-channel openers is still uncertain, although a prime candidate in smooth muscle seems similar to the (ATP) modulated K-channel in the pancreatic P-cell. This is focused in attention on the action of these agents in vascular smooth muscle and on the possible clinical exploitation of their powerful vasorelaxant properties. This property is very useful for the penial erection during the coitus.
- Instrumental analysis FTIR showed the peak value presence of alcohol, Amine salt, Nitro compount, Carboxylic acid, Imine/oxime.
- The acute toxicity study shows that Linga chenduram did not produce any toxic effect at dose of 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 2000 mg/kg to rats. So No-observed- Adverse – Effect – Level (NOAEL) of linga chenduram at dose of 2000 mg/kg.
- In sub acute toxicity study test drug Linga chenduram can be considered as safe, as it did not cause either any lethality or adverse changes with general behaviour of rats and also there were no observable detrimental effects (200 to 600mg/kg body weight) over a period of 28 days. Our results have

demonstrated that the Linga chenduram is relatively safe when administered orally in rats.

- Pharmacological analysis shows that the test drug “Linga chenduram” has got good significant Aphrodisiac, Spermatogenesis and antioxidant activities when compared to the standard drug.
- Both gram positive and gram negative bacteria E.coli, staphylococcus aureus, klebsiella and candida sp were found to be sensitive when compared to the standard drug chloramphenicol (broad spectrum).
- It has been summarized that Linga chenduram is very effective in treating pre mature ejaculation without causing any adverse effects.
- Finally all the parameters and histopathological studies results revealed the drug was safe in rats. Acute oral toxicity of LC observed no toxicity. Acute and subacute toxicity study of LC represents non toxic and safe drug in wistar albino rats. Biochemical and histopathological results it concluded that the dose level of 130-195mg mentioned in the siddha literature “Anupoga Vaithiya Navaneetham” is the safety dose for human consumption the test drug of LC hope fully use for human trails.

8. CONCLUSION

The trial drug *Linga Chenduram* is a herbo mineral, selected from the text book of *Anuboga Vaithiya Navaneetham, Part – IV*, authored by Abdhula Sayubu PM, Thamarai Noolagam, Chennai (2006) 106 for Aphrodisiac, Spermatogenesis, Anti-oxidant Activities and the results supported the study.

From the literature review, physico-chemical analysis, Bio-Chemical analysis, Pharamacological studies, Microbiological analysis, Instrumental analysis and toxicological studies it is concluded that the test drug of LC is safe and effective for Premature Ejaculation and in safer to continue even for a long duration.

9. FUTURE SCOPE

Preclinical evaluation of the test drug Linga Chenduram has been done by bio-chemical, physio-chemical, instrumenta, pharmacologica, toxicological and microbial standard presecribed procedures. In future the drug has to validated by extensive clinical trials as per WHO guidelines. This Linga Chenduram is to be used very much to treating Premature Ejaculation.

Having made up of nano particles, Linga Chenduram holds extra ordinary promise for the presentation and treatment Premature Ejaculation. Thus the ancient wisdom siddhars will remains as one important source of future medicine and therapeutics

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