PRECLINICAL EVALUATION OF SIDDHA DRUG

PADIGA LINGA CHENDURAM

FOR ITS

ANTI-DAIRRHOEAL, ANTI-PYRETIC, AND

ANTI-SPASMODIC, ACTIVITIES

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GOVT. SIDDHA MEDICAL COLLEGE,

PALAYAMKOTTAI-02

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Pre-clinical study of Siddha Drug Padiga linga chenduram for its Anti-diarrhoeal, Anti-pyretic, and Anti-spasmodic activities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.A.Kingsly, M.D.(S).,** Reader, Post Graduate Department of *Gunapadam*, Govt.Siddha Medical College, Palayamkottai,Tirunelveli-02 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"Pre-clinical study of Siddha Drug Padiga linga chenduram for its Anti-diarrhoeal, Anti-pyretic, and Anti-spasmodic activities"** is a bonafide work done by **Dr.F.Sophia Anthuvanth** a candidate of GSMC, palayamkottai, in partial fulfilment of the University rules and regulations for award of MD (S) Gunapadam under my guidance and supervision during the academic year of 2016

Seal & Signature of the HOD **Seal &Signature of the Principal**

Date:

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ABBREVATIONS

%	percentage
CHL	Cholesterol
CPCSEA	Committee for the Purpose of Control and Supervision of
E	Eosinophils
EDTA	Ethylene Diamine Tetra Acetic Acid
	Experiments on Animals
FTIR	Fourier transform infrared spectroscopy
g	Gram
g%	Gram percentage
Hb	Haemoglobin
HDL	High density lipoprotein
IAEC	Institutional Animal Ethical Committee.
ICP-OES	Inductively coupled plasma optical emission spectrometry
Kg	Kilogram
L	Lymphocytes
LD_{50}	Lethal Dose $_{50}$
M	Monocytes
Mg	Milligram
ML	Milliliter
MLD	Minimum Lethal Dose
MPV	Mean Platelet Volume
MTD	Maximum Tolerated Dose
N	Neutrophils
No.	Number
NOAEL	No-Observed-Adverse-Effect-Level
OECD	Organisation of Economic Co-operation and Development
p.o	peros
PC	Padigalinga Chenduram
PCV	Packed Cell Volume
R&D	Research and Development
RBC	Red Blood Corpuscles

RDW	Red Cell Distribution Width.
SEM	Scanning Electron microscope
TG	Triglyceride
TLC	Thin Layer Chromatography
WBC	White Blood Corpuscles

1.INTRODUCTION

Siddha system of medicine is the most primitive medical system in India. The term *"Siddha"* means achievements and "Siddhars" were saintly persons who achieved results in medicine. Siddha system of medicine is originated by first *siddhar* called Lord Siva. It is practiced and taught to next generation by *guru* disciple method.The father of Siddha medicine is the primordial *Guru, Agasthiar*.

The physiological functioning of based on principle of 96 thathuvam which includes the functions of sensory and motor systems psyco somatic and psyco immune system 6 vital centers, 5 kosam(system) which include Circulatory, Respiratory , Excretory, Reproductory and Nervous system.

The fundamental principles of *Siddha* system include theories of Five Elements *(Aimpootham)*, and three Vital humor*(Mukkuttram). "Health sustainment or decline is defined by the Normal or abnormal state of the Humors"* .The doshas within any person keep changing constantly due to lifestyle, foods and environment.The loss of balance among the humors causes energy disharmony and physical and mental disequilibrium which may appear at any time and become the cause of diseases. According to the *Siddhar 'Yugi muni'*, the diseases are widely classified into 4448 types. *Siddha* system insists that, the physician should enquire into the nature of the disease, its cause and its method of cure and treat it faithfully. *Envakai Thervukal* (The Eight Methods of Examination) are used to determine diagnosis, etiology, treatment and prognosis of disease.

This art of healing incorporates a variety of holistic practices and remedies. The drugs are categorized into three groups, namely herbal products, metal, mineral and animal products.32 types of internal medications and 32 external medications are adopted.

“வேர்பாரு தலைபாரு மிஞ்சினக்கால் மெல்ல மெல்ல

பற்பம் செந்தூரம் பாரே”

In Siddha system of medicine, *kazhichal* is also known as *Athisaaram. Bedhi, kirani.* As *chenduram* made from minerals have a long shelf life period when compared to herbal products, I have taken *Padigalinga chenduram* (for my dissertation), as a medication for *Kazhichal.*

2. AIM AND OBJECTIVES

AIM

The aim of this study is to do a scientific review to validate the Anti diarroeal, Anti spasmodic, and Anti pyretic activity of *'PADIGA LINGA CHENDURAM'* for Diarrhoeal by pre-clinical studies.

OBJECTIVES:

The main objective of the present study is to high light the safety and efficacy of *PADIGA LINGA CHENDURAM* in the treatment of *KALICHAL***,** the following methodology was adopted to evaluate the drug and its standardization studies.

- Collection of literature evidence regarding the trail medicine.
- Identification of the drugs in the *padiga linga chenduram*
- Preparation of the trail medicine as per the text.
- Physico-chemical analysis of Test drug.
- Evaluation of the toxicity of Test drug.
- Evaluation of Anti- diarrhoeal activity of the Test drug by in vivo and vitro methods
- Evaluation of Anti- spasmodic activity of Test drug by in-vitro method.
- Evaluation of Anti-pyretic activity of Test drug by in-vivo methods

3. REVIEW OF LITERATURE

3.1.LINGAM (CINNABAR)

3.1.1. GUNAPADAM ASPECT

SYNONYMS

இலிங்க பாசாணம் பேரியற்ற கேளு
பேற்று ஈஞ் சலாட்ட கனமான வன்னி கெற்பந்
தலிங்கம் பஞ்சரவான் சிந்தையான் (சிந்தான்)
தான்றன் நன்னியதோர் மாசமாரிலே – சாகுங்
கலிங்கமாங் காரணமா மிலங்கமாகும்
காராஞ் சானியம் மலபவுஞசமாகு
மலிங்க மாமணி நாகர மென்று சொல்லு
மருவிய தோர் பேரெல்ரா மலிங்கம் பாரே

- Bhogar nigandu 1200

Vanni

Karpam

Pansaravaan

Sindaan

Kalingam

Maamani

Naathakaram aankuri

Maniraagam

Mileatcham

Inkulingam

Raasam

Kadai vanni

Kaanjanam

Kaaranam

Sandagam

Samarasam

Saaniyam

Chendooram

Maniraagam

Malainasam

Maninagam

TYPES

Generally lingam is divided in two

1. Natural lingam
2. Artificial lingam

NATURAL LINGAM

This type of lingam very rare.

ARTIFICIAL LINGAM

- Ulantha lingam
- Rubi lingam
- Mathulai lingam
- Bombai lingam (or) Mesiri lingam
- Nattu (or) china ligam

- Now- a- days we are getting pure lingam. In sharps we can get (found)
- In some areas like panjab, Kashmir Rubi lingam.
- In areas like Chennai , we can't able to found that type of lingam and its very rare to get that.
- And there may be difficult in transport to get lingam from long distance. So prepare ourselves means that would be fine. So here some preparation method (*vaipu murai*).

1. BOMBAI LINGA VAIPU:

Purified *rasam* (mercury) – 7 part

Purified kandhagam – 2 part

Purified *kanthagam* have to grind both well until *rasam* mix with it and change of colour onto black.

After that do 7 *seelaiman* then dry it well and put it into glass jar. A medicine should be filled half portion of the glass jar. The mouth of the glass jar must be closed with *maakal,* and it have to sealed by 7 *seelaiman.* Fill a 4 inch of sand into

Earthernpot and pan the jar on it and fill the sand upto the neck of the jar, then closed with mud plate gain do 7 s*eelaiman* and dry it up. Heat it for 16 *samam* (48 hours) upto till the sand become red hot, and allow it cool. If we break the glass jar means, we can find deporited lingam at mouth of the jar. Then it can be purified and used the lingam obtained by this Process is said to be *(bombai) lingam or Misiri lingam.*

2. NAATTU LINGA VAIPU:

Purified *rasam* (mercury) – 1 part

Purified kandhagam – 1 part

Do it as said before.

3. ROUMI LINGAM:

Purified rasam – 12 parts

Purified kanthagam – 8 parts

Manoselai – 5 parts

Do it as said before.

4. MEALAINATTU LINGAM:

Purified rasam – 16 ounce

Purified kanthagam – 5 ounce

Both are melt, after the process continue before.

5. MAATHULAI LINGA VAIPU

Purified rasam - 1 part

Purified thuttipadanam - 1 part

Grind both well and as proceed as before.

Anubaha vaidya navaneetham part:4 pg no 4-5.

6. METHOD OF PREPARATION

Purified mercury – 280gm

Sulphur _ 70gm

Potassium nitrate _ 70mg

Mercury is thoroughly mixed and triturated with sulphur. Potassium nitrate is then added, placed in a conical flask and burnt for 18 hours. After cooling, the red sulphide of mercury is collected out.

Siddha meteria medica (mineral and animal section)

CHARACTER

It is hard when it is put into fire it becomes smoke, not soluble in water, it has no smell, taste and has hot potency.

ACTION:

Tonic

THERAPEUTIC EFFECTS : பொது குணம்

"பேதிசுரஞ் சந்தி பெருவிரண நீரொடுத
காதகடு காவங் கரப்பான்புண் - ணோத
வுருவிலிங்க சங்கதமா யூறுகட்டு யும்போங்
குருவிலிங்க சங்கமத்தைக் கொள்

ஆதி யிரதவுருக் காதலாற் சாதிலிங்க
மோதி லிரதக முற்றுடலிற் சாதிலிங்க
குட்டங் கிரந்தி கொடுஞ்டசூலை றீதுபுரி
லுட்டங்கு நோய்களையோட் டும்"

It is indicated to diarrhea, pyrexia, delirium, urticarial, diuresis, tuberculosis, scabies, unknown insect bites, syphilis, leprosy, eczema, skin diseases, throbbing pain (*soolai*) and *vatha* disease

"நிலத்தி லெழுந்தபிணி நீங்காக் கிரந்தி
சலத்துடனே சூலைவெடி தானகற்றும் - பலத்ததாம்
சாதிலிங்கத் தின்குணத்டதைச் சாற்றினேன் சன்னிமுதல்
ஓதுசுரைம் போமே ஒளிந்து"

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

PURIFICATION

1. Alangium bark (Alangium savlifolium) – 1400gm is powdered and added with vinegar 5.2 litres and placed in dews in the night. The next day it is rubbed and kindled well. 35 gm of cinnabar is tied ell 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 for 24 hours. Then the cinnabar is taken soaked individually with the whole plant of vitis lanata (puli karunai) and indian sarasaparilla root as stressed in the following tamil verses.

சொல்லக்கேள் புலத்தியனே மகனே யிந்தத்
துறையான சாதிலிங்க சுத்தி தானே
வெல்லக்கே ளழிஞ்சில்புளிங் கருனை யோடு
மேலான நன்னாரிக் காடித் தண்ணீர்
புல்லக் கேளதிலோர் மூன்று வைகல்
புகையாமல் விளக்கிலெரி யழுக்கு நீங்கும்
வெல்லக்கே ளளவுதொடிக் கொன்றே வீசை
வெறுந் தண்ணீர் நாலுபடி வீத மாமே
மேகப் பிணிமுதல் போக வென்றால்
மேலாம் லிங்கம் சுத்தி செய்ய
மாகத் தமிஞ்சி கருனை நாரி
வாய்ந்த புளிநீ ரொன்பா நாள்
பாகத தழலெரி தீவி கைபோற்
பாம்புப் பிடகர் கைப்பண் பிதென
வேகமதை நீக்கென றாடாய் பாம்பே
மேலாஞ் சாக்கிதென் றாடாய் பாம்பே.”

2. 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.

3. 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

Siddha materia medica (mineral and animal section) pg no 222 to 224

4. *Jathi lingam* – 35 gm (should be a single piece)

Have to place it an nice plate and heat in low flame of hare *sire surukittu*. Breast milk for 2 *samam* (6 hours). We can do more than 2 *samam*, while doing like this, lingam will be purified well.

5. Take sufficient amount of lingam and break into small pieces, and tied it up in cloth and have it up in small vessels. Fill up ¾ of vessels with bile juice of goat. The lingam should be dipped into bile juice in the depth of 4 inches. Boil it in low flame

upto water gets drain up. When it gets cool do the same process but have to pour 25 palam of distilled water that obtained from *uvarman.* Again boil it in low flam upto that water gets evaporates. Do the same process for 7 types. If it done for 14 times mean that would be fine and good for medicine.

6. *Lingam* – 1part

Lemon juice – 10 parts

Have to powder the lingam in kalvam and pour a lemon juice little by little and grind it well. Then make it into small dough; dry it well have to do process for 10 times, if this is way to purify the lingam. Instead of lemon juice can use goats milk or with breast milk.

7. Auother method:

Lingam	:	1 palam (single Bar)
Breast milk	:	1 Aazhakku
Honey	:	1 Aazhakku
Onin juice	:	1 Aazhakku
Lemon juice	:	1 Aazhakku

Place lingam in iron plate and give *surukku* by above juice in correct method. This is also method of purification of lingam.

8.*Auother method:*

Take sufficient amount of lingam, have to grind well with lemon juice in *kalvam.* In this lemon juice should be add little by little and have to grind for 2 *samam.* After that make that into one piece of ball and tied up in cloth and make it to hang in pot. In that pot lemon juice should be filled ¼ th for the lingams weight. The lingam should be hang and dipped into lemon juice in the depth of 4 inches, and it should not touch the bottom of the pot. After this process boil it in low flame up to the juice drains completely. Take lingam and place it on glass bowl and make it into dry and powered it and preserved it well in separate jar (or) bowl.

TOXIC SYMPTOMS OF LINGAM

Loss of taste, difficulty in eating and drinking water.

Ulcers in the buccal floor,

Uvula (base of the mouth),

Inner portion of the tongue,

Larynx and large intestine,

Foul odour from the mouth,

Discharge of viscous,

Whitish saliva,

Difficult to speak

Burning sensation are the toxic features of red silphide of mercury

ANTIDOTE:

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

- Siddha materia medica (mineral and animal section) pg no 233

OTHER PREPARAIONS WHICH CONTAIN *LINGAM* AS CHIEF INGREDIENTS:

1. Padigalinga chenduram

Dose : 390mg to 650mg

Adjuvant : Ghee or Butter

Indication : Dysentery, Diarrhea with bleeding, Menorrhagia, Cholera and Diarrhea associated with fever.

- Siddha materia medica (mineral and animal section) : pg no 231

2. Linga kattu

Adjuvant : *Thirikadugu Chooranam*

Indication : Heart Attack, Diarrhea, Abdominal Distension, Delirium And Syncope.

- Siddha materia medica (mineral and animal section) pg no 230

3. Sandarasa parpam

Dose : 488mg

Indication : Diarrhoea, Cholera, Flatulence, Dysentery, Dropsy, Anasarca, Jaundice,etc.

4. Linga karuppu

Dose : 130mg to 260

Adjuvant : Honey and Ghee

Indication : Fever, Diarrhea, Urinary disease.

- Anubhava vaidya navaneetham pg no 9

5. Linga mathirai

Dose : One tablet

Adjuvant : *Sukku kasayam* (dry ginger decoction)

Indication : All type of fever

- Koshaye anubhoga vaidya pirama ragasiyam pg no.17

6. Linga kattu

Adjuvant : Honey, Mothers milk

Indication : Fever , Headache and *Sanni*

- Koshaye anubhoga vaidya pirama ragasiyam pg no.25

7. Linga parpam

Dose : 488mg

Adjuvant : Honey, Ghee, Butter

Indication : Megam, Thadippu, Vayu

- Koshaye anubhoga vaidya pirama ragasiyam pg no.28

8. Valai sangeevi kgulikai

Dose : One tablet

Adjuvant : Piper battle

Indication : Fever.

- Koshaye anubhoga vaidya pirama ragasiyam pg no.84

9. Jathi linga mathirai

Dose : One tablet

Adjuvant : Mother's milks

Indication : *Sanni, Seethala suram*

- Koshaye anubhoga vaidya pirama ragasiyam pg no.88

10. Agasthiyar linga guzhampu

Dose : 488mg

Adjuvant : *Pirandai saaru*

Indication : Abdominal pain

- Koshaye anubhoga vaidya pirama ragasiyam pg no.93

11. Panja padana chenduram

Dose : One rice wight

Adjuvant : Honey and Ginger

Indication : Fever, *Sanni, kuttam*, Oral cancer. Cervical cancer.

- Koshaye anubhoga vaidya pirama ragasiyam pg no.105

12. Linga chenduram

Dose : One rice

Adjuvant : Honey and Ginger

Indication : *Sagala vayu*

- Koshaye anubhoga vaidya pirama ragasiyam pg no.106.

13. Linga parpam

Adjuvant : Honey and Ginger

Indication : *Sunni, Kulir suram*

- Koshayea anubhoga vaidya pirama ragasiyam pg no.112.

14. Panja sootha mezhugu

Dose : 1 to 2 rice

Adjuvant : Pamjaggery and Butter

Indication : Fever and Diarrhoeal.

- Anubava vaidya navaneedham part 7 pgno: 55

15. Linga chenduram

Dose : ½ to 1 rice

Adjuvant : Pamjaggery

Indication : All type of diarrhoeal

- Anubava vaidya navaneedham part 4 pgno: 74

LINGAM (CINNABAR)

3.1.2.GEO CHEMICAL ASPECT

Metonymy

Eng	:	Cinnabar, Vermilion.
Tam	:	Lingam
San	:	Hingula
Tel	:	Ingileekan
Mal	:	Chayilyam
Can	:	Inglika
Hin	:	Sinjraph

INTRODUCTION

Cinnabar is one of important mineral derative of mercury. Cinnabar has been used for 3000 years ago in Indian medicine. Vermilion is the name given to red pigment derived from either natural or artificial cinnabar. This is combination of mercury and sulphur. It is pinkish red in colour (Scarlet red) in colour. It is naturally available in the mines. But recent days artificially prepared lingam is available for the medicine preparations. This sulphide occurs in nature as a fine grained dark red, very heating mercury with sulphur in a retort. However, the natural cinnabar is different from artificial cinnabar with impurities and colour changes. The other preparations from mercury are:

1. Cinnabar
2. Kijjali, the red preparation called rasa sinduram.
3. Rasakarpura.

OCCURRENCE:

It occurs in many parts of the world particularly in China, United states and Spain.

GENERAL PROPERTIES

Chemical formula	:	HgS
Chemical Name	:	Mercury II sulphide
Specific gravity	:	8
Shape	:	Hexagonal
Colour	:	Scarlet red
Solubility	:	Insoluble in water
Transparency	:	Crystals are translucent to transparent

CINNABAR HAS TWO VARIETIES

1. Red variety
2. Black variety

Cinnabar decomposed by Hot concentrated sulphuric acid, soluble in aquaegia ith separation of sulphur and in warm HCL with evaluation of hydrogen sulphide.

ACTION:

Tonic
Alterative
Purgative
Indirect cholagogue
Anti septic.

USES

1. Mercury preparations have been used for many pears as tonic and alterative in western medicine.
2. Cinnabar is also used to recover gold sediments.
3. Mercury is used as small proportion in thermometers and Sphygmanometers.

Medicinal uses:

1. Cinnabar preparations are used in treatment for cirrhosis of liver, dyspepsia, chronic diarrhea & dysentery.
2. Cinnabar is used in the treatment where the stools are deficient in bile.
3. Cinnabar is used in nervous disorders.
4. Cinnabar used in the treatment for fever, ascites, asthma and other forms of difficulty in breathing.
5. Used for skin disease like ringworm, eczema, psoriasis etc
6. Cinnabar used in the treatment of syphilis.
7. Powders of cinnabar dusted into the eyes are useful collyrium to cure ophthalmia.

TOXICITY OF CINNABAR

1. Over dose or long continued use of mercurial preparations produce a set of symptoms called as mercurialism.
2. In such cases, profuse salivation, swollen and spongy gums, swelling of tongue, loosening of teeth etc.
3. The long term uses of cinnabar medicines are result in renal dysfunction due to deposition of mercury in kidney.
4. Most of the soluble salts of mercury are absorbed slowly from intact mucous membrane of alimentary tract and finally excreted into caecum and faeces and produce their toxic effects.

3.1.3.LATERAL RESEARCH

LINGAM

Exposure to Low Dose of Cinnabar (a Naturally Occurring Mercuric Sulfide (HgS)) Caused Neurotoxicological Effects in Offspring Mice

In conclusion, our results provide a toxicological basis for cinnabar-induced neurotoxic and ototoxic effects in offspring mice, which may be extrapolated to adults and children exposed to therapeutic dosage in TCM. Changes in NO_x levels and Na^+/K^+-ATPase activities appear to be the underlying mechanism of the toxicological effects of cinnabar, which may supply an important and useful biomarker in offspring exposure to low dose and long-term mercuric compounds-induced neurotoxicity.

http://www.hindawi.com/journals/bmri/2012/254582/

Neurotoxic Mechanism of Cinnabar and Mercuric Sulfide on the Vestibulo-Ocular Reflex System of Guinea Pigs

http://toxsci.oxfordjournals.org/content/67/2/256.full

3.2.*PADIKARAM* (ALUM)

3.2.1. GUNAPADAM ASPECT

SYNONYMS

“சீனத்தின் பேர்தனையே சொல்லக் கேளு
சிறந்த வெண் காரிதான் பழகிசீனி
மானத்தின் மின்போல வெள்ளச்சியாகும்
மகத்தான பாணிச்சி குருச்சியாம்
பானுத்தி னுப்புக்கு சத்துரு வேயாகும்
பாங்கான வெண்நீலி கடுஞ் சுண்ணத்தி
கானத்தின் கடுஞ் சீன பேருமாகுங்
கடுஞ்சுண்ண முறைகளுக்கு யதனை நாட்டே”

Boghar nigandu 1200

Vennkari

Cheeni

Panitchi

Uppusathru

Kadumsunnathy

Padugi

Vellatchi

Kurutchi

Venneeli

Cheena kaaram

Chenam

OCCURRENCE

Nepal

Kathiyawar

Panjab

Bihar

PANJABOOTHAM

“பலித்திட்ட சவுக்காரட் பஞ்ச பூதப்
பயனாகப் பண்ணியே பார்த்த நேர்மை
பேலித்திட்டபிருதிவிமண் கல்லுப் பாச்சு
பேரான வப்புசலஞ் சத்திச் சாரம்
தெலித்திட்ட தேயுவது வெடுயுப் பாமே
செயநீர்தான் தீயென்றே செப்ப லாகும்
வலித்திட்ட வாயுவது காற்றுச் சீன
மகத்தான வாகாசம் பூநீ றாச்சே”

Agathiya Vazhalai Pannirandu

The pancha bhootha salts are:

- Earth (nilam) - Kalluppu (Crystalline salt)
- Water (neer) - Sathicharam (An acid salt)
- Fire (thee) - Vediyuppu (Potassium nitrate)
- **Air (kaatru) - Seenam (Alum)**
- Sky Pooneeru (fuller’s earth)

CHARACTER

White in colour

Transparent crystals

TASTE

Sweet

Sour

Astringent

ACTIONS

Astringent

Antiseptic

Antispasmodic

Siddha material medica (mineral and animal section)

Caustic

Haemostatic

Irritant and purgative in large doses

Emetic in repeated doses

Indian meteria medica vol – 2 pg no 2

THERAPEUTICAL EFFECT:

“சீனமெனுங் காரமது சீறிவரு பல்லரணை

ஆனைப்பால் கண்ணோய் அனிலமொடு – மாநிலத்தில்

துன்மாங் கிசம்வாயு தோலாத உள்ளழலை

குன்மமிவை போக்கு மெனக் கூறு”

Gunapadam thathu geevam

It cure, gingivitis, eye diseases, ophthalmia, elephantiasis, vayu, tumour, seen of heat, gastric ulcer, hypertension, haemorrhage, dysentery, diarrhea, children’s vomiting, diarrhea, whooping cough, spittle cough with expectorant, pharyngitis, menorrhagia and gonorrhea.

PURIFICATION AND DETOXIFICATION

The alum is dissolved in water filtered, boiled when it attains consistency of jelly. It is cooled to get the purified form.

USES

1. Alum (130 mg) is dissolved in 1 ounce of water and on washing it controls eye diseases.

2. Alum (35gm) dissolved in 10.4 liters of water is used as a mouth wash and for washing the ulcers.

3. When a cloth is soaked in alum dissolved water is applied over the cut injuries, it may arrests bleeding.

4. Alum, acacia catechu and bark of cinnamomum zeylanica (equal Quantities) are powdered and mixed with 975 mg of honey and give to control diarrhea due to tuberculosis.

5. It also controls vomiting when given at a dose of 65 mg.

6. When the alum 195 gm is dissolved in rose water 14ml and administered twice dily, the cough associated with asthma is controlled.

7. Alum (65 to 130 mg) is dissolved in distillated of (Trachyspermum ammi) bishop's weed *theeneer* and administered in whooping cough to control effectively.

8. For whooping cough and chronic hiccup that produce vomiting it is given with honey in the dose of 4 gm.

9. Alum (4.7 gm) dissolved in butter milk is given for snake bite.

10. For severe head injury, alum (130) is administered along with sugar.

11. Alum (2.6 gm) is given with sugar syrup for guinea worm infestation.

12. In leucorrhoea with bleeding, alum is given with the juice of *Adathoda vasica* thrice daily.

13. Alum is boiled along with and filtered; the filtrate effective in the management of excessive menstrual blood flow; it is also effective in the treatment of toxic fever and heamorrhoid.

14. Alum is dissolved in water and applied tropically to control the bleeding in nose, teeth and penis.

15. Alum is used in hair dye preparations.

16. Alum is powdered and sprinkled on the pulp of aloes to get its juice.

USES:

It is useful in leucorrhoea, haematuria, haeoptysis, menorrhagia, gastric and intestinal catarch & other haemorrhages, in chronic diarrhoea and dysentery and in atonic discharges generally.

In chronic diarrhoeas, a mixture containing 10 grains of alum, 5 drops of laudanum and 1 ½ ounces of infusion of acorus root, given thrice daily is useful.

In the diarrhoea preceding eholera and in the diarrhoea of phthisis, a compound powder of alum, catechu and cinnamon each 10 grain mixed with honey is given in reprated doses. It is useful also in strangery and vomiting in small doses i.e 2 to 10 grains of it arrests the spasms of asthuma.

In narcotic poisoning in children it is a good and efficient antidote. In whooping cough, after the first or acute stage has passed, alum in doses of 2 – 4 grains according to age of the child, given twice or thrice a day in the form of powder or in solution in *omum* water (1 in 60) in doses of a teaspoonful to a dessertspoonful for a child from 1 to 4 years old, given thrice a day is most beneficial. For asthuma & cough alum 5 grains in half an ounce of rose water is given twice a day.

In obstinate cases of malaria desiccated alum in 5 grain doses with some aromatic compound powder to disguise the taste given 2 hours before the expected rigour with only a teaspoonful of water has given very satisfactory results.

In obtained hiccup one drachm doses given two or three times a day induce vomiting and stop hiccup.

Alum 45 grains mired with treack is given internally for guinea – worm.

Alum in 5 grain doses thrice a day with the juice of adhathoda vasica works wonderfully in certain form of leucorrhoea, especially when the flow is tinged with blood.

Alum whey or "line whey" prepared by boiling for 10 minutes 2 drachms of powdered alum in a pint of milk and strained in beneficial in doses of ½ to 2 ounces thrice daily in menorrhagia and bleeding piles. "As a haemostatic, its use is recommended in bleeding from the nose and other mucous surfaces." Dr.H.C. Sen has "derived satisfactory results" from alum – whey in cases of enteric fever.

EXTERNALLY

Alum forms one of the in gredients of some hair dyes and hair lotions.

It is applied in a saturated solution i.e 5 percent in bleeding from nose, gums, vagina or the rectum, as a styptic in leech bites, cuts etc. in protopses ani and prolopsus uteri.

Locally applied it checks weats in the armpits, groins and soles of the feet.

Weak solution is used as a lotion to ulcers and chilblains; as "an astringent gargle in a strength of 2 draehms to a pint of decotion of gall or Babul dark or of plain water". It is used in relaxed or ulcerated sore – throut, aphonaia, atony of the larynx spongy or bleeding gums, loose teeth, ulcers of the mouth and tongue, fissures of the tongue in consumption, in excessive salivation etc, it is locally applied in diphtheria, croup and pharyngitis; as a collyrium it is used in chronic and purulent ophthalmia, chronic conjunctivitis.

"Alum lotion, internally is administed to check haemorrhage from lungs, stomach, kidney and other organs or to arrest excessive menstrual flow"

In inflammation round the ear a paste made of alum and gypsum equal parts and gile Armani, is applied, in otorrhoea it may be dropped into the ear.

In apththae and thrush, sporgy gums and other affections of the mouth powdered alum with honey is used with benefit. It is often sprinkled over indolent ulcers, especially chronic umbilical ulcers of infants and used as a snuff in eoistaxis or a gauze wet with alum lotion is plugged in the nose.

In cases of past partum hemorrhages or menorrhagia, sterilized cotton plugs saturated with alum powder or sterilized alum lotion immediately stop the bleeding.

In discharges from the urethra, caused by a sore or excoriated surface between the prepuce and the head of the penis often confounded with gonorrhea, a 4 P.C Solution applied twicw or thrice daily is very beneficial.

For bed sores or where these are likely to occur, a meture of 30 gains of burnt alum & the white of an egg, is painted over the past.

Indian meteria medica vol 2 pg no: 2-6.

ARTIFICIAL ALUM

"சீன வைப்பு செல்லுவேன் சீனம் தொகுகுறிபோல்
நல்லு வருப்புங் கல்லுப்பு ரெண்டு
மல்லும் பழச்சாறும் தயிர் விட்டு
பல்லுறுவாக பாண்டத்திர் மூடே
மூடியே சாணி முழுவதுமே பூசி
நாடியே ஆவின் பதிதானிற்றாக்குக்
கூடியே மண்டலங் குறிப்பாக வைத்துத்
தேடியெடுக்க சினமு மாச்சே"

"பாரப்பா சரக்கினுட வைப்புக்கேளு
பாருப்பு தன்னுனே கல்லுப்புங் கூட்டி
ஊரைப்பா பழச்சாறு தயிருங்கூட்டி
உடன் சேர்த்து பாண்டத்தில் விட்டு மூடி
காரப்பா குழிவெட்டி யெருவைப் போட்டு
கருவான பாண்டமதை யதன்மேல் வைத்து
சேரப்பா எருப்போட்டு சமாதி வைத்தால்
திறமாக மண்டலத்தில் சீனமாச்சே"

Agathiyar amudha kalaienanam 1200, pg no 647

1. *Padigam vaippu*

Annapaethy	-	*4 parts*
Yellow yolk	-	*1 part*
Pioneer	-	*1 part*
Water	-	*72 parts*

Mix all those things and dry it up by flaming it well undisturbed this upto 5 months. After 5 months have pour water for 10 to 15 days dry it again and use it medicine.

2. Yellow alum

- The method to prepare yellow *padigaram* as said before in *padigaram vaippu* by using yellow *annapaethy*.
- *F*or a sufficient amount of *padigaram* take 18 portion of water and mix with it then filter it well. Take 1 portion of that distilled water and for this required amount of yellow yolk is 9 portions. In 1:9 ratio mix well and flame it well.

3. Green alum

- Take sufficient amount of white *padigaram* and mix it in 18 portions of water, and filter it without silt. Mix this distilled water with *Jangal green* in the ration of 1:4, and dry it up by flaming or by sunlight.
- Sufficient amount yellow *padigaram* and mix it with 18 portions of water. If we put copper powder in this water it will turns into green colour. After the change of colour make it filter without silt and preserved in glass jar.
- Take sufficient amount of white *padigaram* in glass bowl, and mix 18 portions of water and filter it well pour this filtered water in copper pot. Put ½ *navatcharam* into it, and leave it for few days. After that dry it by flamming.
- As said before, By mixing yellow *padigaram* and water then take distilled water. Add half portion of *jangal green* powder to the weight of yellow *padigaram*. Mix well and filter it without silt and flame it well and then use it for medicine.

4. Red alum

- Sufficient amount of white *padigaram* and to add 18 portions of water and filter it. In this distilled water (10 portions) and add 2 portion of *jangal* green and mix well. It will turns into red colour, after that flame it and used it for medicine.

- Sufficient amount of *venkaram* and to this pour 10 portions of water, mix well and distilled it without silt. Take 4 portion distilled water and to this add 1 portion of white *padigaram*; then mix well and flame it well and take it and dry it up by sunlight.

OTHER PREPARAIONS WHICH CONTAIN ALUM AS CHIEF INGREDIENTS:

1. Padigara parpam

   Dose : Pepper (*milagu)*

   Adjuvant : Butter

   Indication : Leucorrhoea

   - Koshaye anubhava vaidya piramaragaciyam pg no 30

2. Unani chenduram

   Adjuvant : Ghee and Butter

   Indication : Menorrhagia and Leucorrhoea

   - Koshaye anubhava vaidya piramaragaciyam pg no 36

3. Padigara parpam

   Dose : 130mg to 260 mg

   Adjuvant : Butter

   Indication : Leucorrhoea and diarrhea.

   - Uyir kakkum siddha Maruthuvam pg no : 526

4. Sandarodhaya kulikai

   Dose : 2

   Adjuvant : Honey

   Indication : Fever

   - Saraboji vaidya thirattu pg no 68

5. Padiga thalaga parpam

   Dose : *Dhuvarai*

   Adjuvant : Honey

   Indication : Fever, cough and Asthma

   - Saraboji vaidya thirattu pg no 87

6. Padigalinga chenduram

Dose	:	488mg
Adjuvant	:	Ghee and Honey
Indication	:	diarrhea and chest disease

Saraboji vaidya thirattu pg no 99

7. Ukkira veera chenduram

Dose	:	488mg
Adjuvant	:	Ghee and Butter
Indication	:	leucorrhea, menorrhagia, *Madhu megam* (DM)

Saraboji vaidya thirattu pg no 103

8. Suvarna boopathy kuligai

Dose	:	1
Indication	:	*Sanni, yelai, kasam*

Kannusamy sigitcha rathina deepam pg no 44

9. Padigara parpam

Dose	:	130mg to 195mg
Adjuvant	:	Butter
Indication	:	Vomiting

Kannusamy sigitcha rathina deepam pg no 214

10. Padigara mathrai

Dose	:	*Milagu to pattani*
Adjuvant	:	Honey
Indication	:	Diarrhoea, cough and abdominal discomfort.

Kannusamy paramparai vaidyam pg no 148

11. Padikavengara parpam

Dose	:	1 to 4 *Kundri*
Adjuvant	:	Ghee, Butter
Indication	:	*Neer kattu, Thasai adaippu*

Siddha vaidya thirattu pg no: 127

PADIKARAM (ALUM)

3.2.2.GEO CHEMICAL ASPECT

ALUMEN (ALUM)

Potassium alum

$K_2\ SO_4\ Al_2(So_4)_3\ 24H_2\ O$

An alum is a double sulphate formed by the union of a sulphate of aluminum, chromium, manganum or Ferrum with a Sulphate of an alkaline metal of group like Potassium, Sodium or Ammonium.

The official alum is Aluminum and Potassium Sulphate or potassium Alum which becomes Alumen Exsiccatum. Dried Alum when the molecules of water are driven off.

PHYSICAL PROPERTIES

Solubility	:	water soluble
Diapheny	:	Transparent
Lustre	:	Vitreous
Colour	:	Colour less, white
Luminescence	:	Non-Fluroscent
Hardness(moh's)	:	2-Gypsum
Cleavage	:	Indistinct
Streak	:	white

CHEMICAL PROPERTIES

Chemical formula : $K\ Al\ (So_4)_2\ 12H_2\ O$

CHARACTERISTICS

Potassium alum crystallizes in regular octahedral with flattened corners, and is very soluble in water. The solution reddens litmus and is an astringent. When heated to nearly a red heat it gives a porous, friable mass which is known as "burnt alum." It fuses at 92 °C (198 °F) in its own water of crystallization. "Neutral alum" is obtained by the addition of as much sodium_carbonate to a solution of alum as will begin to cause the separation of alumina.

MINERAL FORM AND OCCURRENCE

Potassium alum or alum-(K) is a naturally occurring sulfate mineral which typically occurs as encrustations on rocks in areas of weathering and oxidation of sulfide_minerals and potassium-bearing minerals. In the past, alum was obtained from alunite, a mineral mined from sulfur-containing volcanic sediments source.[6] Alunite is an associate and likely potassium and aluminium source.[1][7] It has been reported at Vesuvius, Italy, east of Springsure, Queensland, Alum Cave, Tennessee, Alum Gulch, Santa Cruz County, Arizona and the Philippine island of Cebu. A related mineral is *kalinite*, a fibrous mineral with formula $KAl(SO_4)_2 \cdot 11H_2O$

USES

Chemical

- in tanning of leather to prepare the hide
- as a mordant for dyes
- in the clarifying of turbid liquids, including post-storm treatment of lakes to precipitate contaminants
- as a fire retardant in textile products
- (historically) as a hardener for photographic emulsions (films and papers), usually as part of the fixer. Modern alternatives are superior.

Medicinal

- As an astringent/styptic and antiseptic.
- As a natural deodorant by inhibiting the growth of the bacteria responsible for body_odor.
- After shaving to prepare the skin
- To reduce bleeding in minor cuts and abrasions, nosebleeds, and hemorrhoids.

CULINARY

- As an additive to baking_powder to provide a second leavening phase at high temperatures.
- To preserve and add crispness to fruit and vegetable especially when pickling.

TRADITIONAL

- In Ayurveda, where it is called phitkari or saurashtri
  In traditional Chinese medicine it is called *ming fan*.

TOXICOLOGY AND SAFETY

Alum**,** any of a group of hydrated double salts, usually consisting ofaluminum sulfate, water of hydration, and the sulfate of another element. A whole series of hydrated double salts results from the hydration of the sulfate of a singly charged cation (*e.g.,* K^+) and the sulfate of any one of a number of triply charged cations (*e.g.,* Al^{3+}). Aluminum sulfate can thus form alums with sulfates of the singly charged cations of potassium, sodium, ammonium, cesium, and other elements and compounds. In similar fashion, sulfates of the triply charged cations of iron, chromium, manganese, cobalt, and other metals may take the place of aluminum sulfate. The most important alums are potassium aluminum sulfate, ammonium aluminum sulfate, and sodium aluminum sulfate. Potassium aluminum sulfate, also known as potassium alum or potash alum, has a molecular formula of $K_2(SO_4)\cdot Al_2(SO_4)_3\cdot 24H_2O$ or $KAl(SO_4)_2\cdot 12H_2O$.

Alums can easily be produced by precipitation from an aqueous solution. In producing potassium alum, for example, aluminum sulfate and potassium sulfate are dissolved in water, and then upon evaporation the alum crystallizes out of the solution. A more common production method is to treat bauxite ore with sulfuric acid and then with potassium sulfate.Ammonium alum is produced by the evaporation of a water solution containing ammonium sulfate and aluminum sulfate. It can also be obtained by treating a mixture of aluminum sulfate and sulfuric acid with ammonia. Alums occur naturally in various minerals. Potassium alum, for example, is found in the minerals kalinite, alunite, and leucite, which can be treated with sulfuric acid to obtain crystals of the alum.

Most alum has an astringent and acid taste. They are colourless, odourless, and exist as a white crystalline powder. Alums are generally soluble in hot water, and they can be readily precipitated from aqueous solutions to form large octahedral crystals.

3.2.3LATERAL RESEARCH

ALUM

SPECIAL FEATURE: IMMUNOLOGICAL ADJUVANTS

The problem with pure recombinant or synthetic antigens used in modern day vaccines is that they are generally far less immunogenic than older style live or killed whole organism vaccines. This has created a major need for improved and more powerful adjuvants for use in these vaccines. With few exceptions, alum remains the sole adjuvant approved for human use in the majority of countries worldwide. Although alum is able to induce a good antibody (Th2) response, it has little capacity to stimulate cellular (Th1) immune responses which are so important for protection against many pathogens. In addition, alum has the potential to cause severe local and systemic side-effects including sterile abscesses, eosinophilia and myofascitis, although fortunately most of the more serious side-effects are relatively rare. There is also community concern regarding the possible role of aluminium in neurodegenerative diseases such as Alzheimer's disease. Consequently, there is a major unmet need for safer and more effective adjuvants suitable for human use. In particular, there is demand for safe and non-toxic adjuvants able to stimulate cellular (Th1) immunity. Other needs in light of new vaccine technologies are adjuvants suitable for use with mucosally-delivered vaccines, DNA vaccines, cancer and autoimmunity vaccines. Each of these areas are highly specialized with their own unique needs in respect of suitable adjuvant technology. This paper reviews the state of the art in the adjuvant field, explores future directions of adjuvant development and finally examines some of the impediments and barriers to development and registration of new human adjuvants.

http://www.nature.com/icb/journal/v82/n5/full/icb200475a.html

3.3.DISEASE REVIEW

3.3.1.*KAZHICHAL* - SIDDHA ASPECT

Vernacular names:

Migu Kalichal

Athisaaram

Introduction

Intaken foods are completely or incompletely digested sometimes immediately after the food taken. It can be evacuate through intestine without storage, all ingested foods are frequently evacuated is termed as perunkalichal.

Aetiology

- Excess of Carbohydrates

  Eg : *maapandam*

- Excess of fatty foods

  Eg : oil., ghee., Non vegetarian items

- Decomposed fish Vegetables
- Easily undigestable foods

  Eg: *pulak., kichadi.*

- Excess of worm infections

Types:

Perunkalichal is divided into

1. *Vatha Perunkalichal*
2. *Pitha Perunkalichal*
3. *Kabha Perunkalichal*
4. *Mukutra Perunkalichal*
5. *Sura Perunkalichal*
6. *Paya Perunkalichal*

7. *Thukka Perunkalichal*
8. *Kuruthi Perunkalichal*

1. Vatha Perunkalichal

Flatulence

Frequent evacuation

Stools with blackish or yellowish colour and unpleasant odour

Stomach pain

Belching

2. **Pitha perunkalichal**

Flatulence

Vomiting

Pain around umbilicus

Stools with blood yellowish &frothy

Oedema in legs &hands

3. Kabha Perunkalichal

Abdominal pain during evacuation

Sputum like stools appearance

Frothy, unpleasant odour in stools.

4. Mukutra Perunkalichal

The symptoms occurs in the combination of *vadha, pitha and kabha perunkalichal*

5. Sura Perunkalichal

High fever

Sometimes like typhoid & thyphus

6. Paya & Thukka Perunkalichal

Perunkalichal occurs in fear

7. Thoda Perunkalichal

Perunkalichal occurs in children

8. Kuruthi perunkalichal

Spicy foods,

Mercury, arsenic preparations are taken in high doses irritate the intestine and produce *kuruthi kalichal.*

Over doses of internal medicine like serankottai and kundrimani.

நாடி

1. **“சிறப்பான வாதத்தில் உஷ்ணந் தானே சேர்ந்த தெனில் அதிசாரம்”**
2. **“தழைப்பான பித்ததட்தில் உஷ்ணம் சேர்ந்தால் ஆதிசாரந்தான்”**
3. **உறுதியுள்ள பித்தமது தோன்றில்.. அதிசாரம்**
4. **தானமுள்ள சேத்துமந்தா னிளகில்…….. அதிசாரம்**
5. **“சிறப்பான சேத்துமத்தில் பித்தந்தான் எழுந்தணுகிடல் அதிசாரந் தானாகுமே”**

(சதகநாடி)

Some of the medicines for diarrhea:

Kudineer

Maramanjal kudineer

Athividaya kudineer

Thippiliyathi kudineer

Chooranam

Thayeerchundi chooranam

Sunndaivatral chooranam

Thaalisa podi

Anna podi

Vadagam

Thaalisaathi vadagam

Pirandai vadagam

Vaazhaipoo vadagam

Mathirai

Kabaada mathirai

Kattuvaathi mathirai

Ouzhi mathirai

Vajrakabaada mathirai,

Jathikkai mathirai

Parpam

Naaga parpam

Pavala parpam

Chenduram

Uppu chenduram

Annapaethi chenduram

Lingathuvar

Manapaagu

Vilva manapaaagu

Chemparuthi manapaagu

Kudasa manapaagu

Ilagam

Saathikaai ilagam

Inji ilagam

Vilvathy ilagam

3.3.2.MODERN ASPECT

DIARRHOEA

INDRUDUCTION

Diarrhoeal disease is the second leading cause of death in children under five years old, and is responsible for killing around 760 000 children every year. Diarrhoea can last several days, and can leave the body without the water and salts that are necessary for survival. Most people who die from diarrhoea actually die from severe dehydration and fluid loss. Children who are malnourished or have impaired immunity as well as people living with HIV are most at risk of life-threatening diarrhoea.

Diarrhoea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). Frequent passing of formed stools is not diarrhoea, nor is the passing of loose, "pasty" stools by breastfed babies.

Diarrhoea is usually a symptom of an infection in the intestinal tract, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection is spread through contaminated food or drinking-water, or from person-to-person as a result of poor hygiene.

Interventions to prevent diarrhoea, including safe drinking-water, use of improved sanitation and hand washing with soap can reduce disease risk. Diarrhoea can be treated with a solution of clean water, sugar and salt, and with zinc tablets.
There are three clinical types of diarrhoea:

- acute watery diarrhoea – lasts several hours or days, and includes cholera;
- acute bloody diarrhoea – also called dysentery; and
- persistent diarrhoea – lasts 14 days or longer.

SCOPE OF DIARRHOEAL DISEASE

Diarrhoeal disease is a leading cause of child mortality and morbidity in the world, and mostly results from contaminated food and water sources. Worldwide, 780

million individuals lack access to improved drinking-water and 2.5 billion lack improved sanitation. Diarrhoea due to infection is widespread throughout developing countries.

In developing countries, children under three years old experience on average three episodes of diarrhoea every year. Each episode deprives the child of the nutrition necessary for growth. As a result, diarrhoea is a major cause of malnutrition, and malnourished children are more likely to fall ill from diarrhoea.

❖ SECRETORY

Secretory diarrhea means that there is an increase in the active secretion, or there is an inhibition of absorption. There is little to no structural damage. The most common cause of this type of diarrhea is a cholera toxin that stimulates the secretion of anions, especially chloride ions. Therefore, to maintain a charge balance in the lumen, sodium is carried with it, along with water. In this type of diarrhea intestinal fluid secretion is isotonic with plasma even during fasting. It continues even when there is no oral food intake.

❖ OSMOTIC

Osmotic diarrhea occurs when too much water is drawn into the bowels. If a person drinks solutions with excessive sugar or excessive salt, these can draw water from the body into the bowel and cause osmotic diarrhea. Osmotic diarrhea can also be the result of maldigestion (e.g., pancreatic disease or Coeliac disease), in which the nutrients are left in the lumen to pull in water. Or it can be caused by osmotic laxatives (which work to alleviate constipation by drawing water into the bowels). In healthy individuals, too much magnesium or vitamin C or undigested lactose can produce osmotic diarrhea and distention of the bowel. A person who has lactose_intolerance can have difficulty absorbing lactose after an extraordinarily high intake of dairy products. In persons who have fructose malabsorption, excess fructose intake can also cause diarrhea. High-fructose foods that also have a high glucose content are more absorbable and less likely to cause diarrhea. Sugar alcohols such as sorbitol (often found in sugar-free foods) are difficult for the body to absorb and, in large amounts, may lead to osmotic diarrhea.[11] In most of these cases, osmotic diarrhea stops when offending agent (e.g. milk, sorbitol) is stopped.

❖ **EXUDATIVE**

Exudative diarrhea occurs with the presence of blood and pus in the stool. This occurs with inflammatory bowel diseases, such as Crohn's disease or ulcerative colitis, and other severe infections such as *E. coli* or other forms of food poisoning.

❖ **INFLAMMATORY**

Inflammatory diarrhea occurs when there is damage to the mucosal lining or brush border, which leads to a passive loss of protein-rich fluids and a decreased ability to absorb these lost fluids. Features of all three of the other types of diarrhea can be found in this type of diarrhea. It can be caused by bacterial infections, viral infections, parasitic infections, or autoimmune problems such as inflammatory bowel diseases. It can also be caused by tuberculosis, colon cancer, and enteritis.

❖ **DYSENTERY**

If there is blood visible in the stools, it is also known as dysentery. The blood is trace of an invasion of bowel tissue. Dysentery is a symptom of, among others, *Shigella*,*Entamoeba histolytica*, and *Salmonella*

DEHYDRATION

The most severe threat posed by diarrhoea is dehydration. During a diarrhoeal episode, water and electrolytes (sodium, chloride, potassium and bicarbonate) are lost through liquid stools, vomit, sweat, urine and breathing. Dehydration occurs when these losses are not replaced.

The degree of dehydration is rated on a scale of three.

1. Early dehydration – no signs or symptoms.
2. Moderate dehydration:
    - thirst
    - restless or irritable behaviour
    - decreased skin elasticity
    - sunken eyes
3. Severe dehydration:
    - symptoms become more severe
    - shock, with diminished consciousness, lack of urine output, cool, moist extremities, a rapid and feeble pulse, low or undetectable blood pressure, and pale skin.

Death can follow severe dehydration if body fluids and electrolytes are not replenished, either through the use of oral rehydration salts (ORS) solution, or through an intravenous drip.

CAUSES

- **INFECTION:**

Diarrhoea is a symptom of infections caused by a host of bacterial, viral and parasitic organisms, most of which are spread by faeces-contaminated water. Infection is more common when there is a shortage of adequate sanitation and hygiene and safe water for drinking, cooking and cleaning. Rotavirus and *Escherichia coli* are the two most common etiological agents of diarrhoea in developing countries.

- **MALNUTRITION:**

Children who die from diarrhoea often suffer from underlying malnutrition, which makes them more vulnerable to diarrhoea. Each diarrhoeal episode, in turn, makes their malnutrition even worse. Diarrhoea is a leading cause of malnutrition in children under five years old.

- **SOURCE:**

Water contaminated with human faeces, for example, from sewage, septic tanks and latrines, is of particular concern. Animal faeces also contain microorganisms that can cause diarrhoea.

- **OTHER CAUSES:**

Diarrhoeal disease can also spread from person-to-person, aggravated by poor personal hygiene. Food is another major cause of diarrhoea when it is prepared or stored in unhygienic conditions. Water can contaminate food during irrigation. Fish and seafood from polluted water may also contribute to the disease.

PATHOPHYSIOLOGY OF DIARRHEA

Diarrhea is an increase in the volume of stool or frequency of defecation. It is one of the most common clinical signs of gastrointestinal disease, but also can reflect primary disorders outside of the digestive system. Certainly, disorders affecting either the small or large bowel can lead to diarrhea.

For many people, diarrhea represents an occasional inconvenience or annoyance, yet at least 2 million people in the world, mostly children, die from the consequences of diarrhea each year.

There are numerous causes of diarrhea, but in almost all cases, this disorder is a manifestation of one of the four basic mechanisms described below. It is also common for more than one of the four mechanisms to be involved in the pathogenesis of a given case.

❖ **Osmotic Diarrhea**

Absorption of water in the intestines is dependent on adequate absorption of solutes. If excessive amounts of solutes are retained in the intestinal lumen, water will not be absorbed and diarrhea will result. Osmotic diarrhea typically results from one of two situations:

- *Ingestion of a poorly absorbed substrate:* The offending molecule is usually a carbohydrate or divalent ion. Common examples include mannitol or sorbitol, epson salt ($MgSO_4$) and some antacids ($MgOH_2$).
- *Malabsorption:* Inability to absorb certain carbohydrates is the most common deficit in this category of diarrhea, but it can result virtually any type of malabsorption. A common example of malabsorption, afflicting many adults humans and pets islactose intolerance resulting from a deficiency in the brush border enzyme lactase. In such cases, a moderate quantity of lactose is consumed (usually as milk), but the intestinal epithelium is deficient in lactase, and lactose cannot be effectively hydrolyzed into glucose and galactose for absorption. The osmotically-active lactose is retained in the intestinal lumen, where it "holds" water. To add insult to injury, the unabsorbed lactose passes into the large intestine where it is fermented by colonic bacteria, resulting in production of excessive gas.

A distinguishing feature of osmotic diarrhea is that it stops after the patient is fasted or stops consuming the poorly absorbed solute.

❖ **Secretory Diarrhea**

Large volumes of water are normally secreted into the small intestinal lumen, but a large majority of this water is efficienty absorbed before reaching the large intestine. Diarrhea occurs when secretion of water into the intestinal lumen exceeds absorption.

Many millions of people have died of the secretory diarrhea associated with cholera. The responsible organism, *Vibrio cholerae*, produces cholera toxin, which strongly activates adenylyl cyclase, causing a prolonged increase in intracellular concentration of cyclic AMP within crypt enterocytes. This change results in prolonged opening of the chloride channels that are instrumental in secretion of water from the crypts, allowing uncontrolled secretion of water. Additionally, cholera toxin affects the enteric nervous system, resulting in an independent stimulus of secretion.

Exposure to toxins from several other types of bacteria (e.g. *E. coli* heat-labile toxin) induces the same series of steps and massive secretory diarrhea that is often lethal unless the person or animal is aggressively treated to maintain hydration.

In addition to bacterial toxins, a large number of other agents can induce secretory diarrhea by turning on the intestinal secretory machinery, including:

- some laxatives
- hormones secreted by certain types of tumors (e.g. vasoactive intestinal peptide)
- a broad range of drugs (e.g. some types of asthma medications, antidepressants, cardiac drugs)
- certain metals, organic toxins, and plant products (e.g. arsenic, insecticides, mushroom toxins, caffeine)

In most cases, secretory diarrheas will not resolve during a 2-3 day fast.

❖ **Inflammatory and Infectious Diarrhea**

The epithelium of the digestive tube is protected from insult by a number of mechanisms constituting the gastrointestinal, but like many barriers, it can be breached. Disruption of the epithelium of the intestine due to microbial or viral pathogens is a very common cause of diarrhea in all species. Destruction of the epithelium results not only in exudation of serum and blood into the lumen but often

is associated with widespread destruction of absorptive epithelium. In such cases, absorption of water occurs very inefficiently and diarrhea results. Examples of pathogens frequently associated with infectious diarrhea include:

- Bacteria: *Salmonella, E. coli, Campylobacter*
- Viruses: rotaviruses, coronaviruses, parvoviruses (canine and feline), norovirus
- Protozoa: coccidia species, *Cryptosporium, Giardia*

The immune response to inflammatory conditions in the bowel contributes substantively to development of diarrhea. Activation of white blood cells leads them to secrete inflammatory mediators and cytokines which can stimulate secretion, in effect imposing a secretory component on top of an inflammatory diarrhea. Reactive oxygen species from leukocytes can damage or kill intestinal epithelial cells, which are replaced with immature cells that typically are deficient in the brush border enyzmes and transporters necessary for absorption of nutrients and water. In this way, components of an osmotic (malabsorption) diarrhea are added to the problem.

❖ **Diarrhea Associated with Deranged Motility**

In order for nutrients and water to be efficiently absorbed, the intestinal contents must be adequately exposed to the mucosal epithelium and retained long enough to allow absorption. Disorders in motility than accelerate transit time could decrease absorption, resulting in diarrhea even if the absorptive process per se was proceeding properly.

Alterations in intestinal motility (usually increased propulsion) are observed in many types of diarrhea. What is not usually clear, and very difficult to demonstrate, is whether primary alterations in motility are actually the cause of diarrhea or simply an effect

PREVENTION AND TREATMENT

Key measures to prevent diarrhoea include:

- access to safe drinking-water;
- use of improved sanitation;
- hand washing with soap;

- exclusive breastfeeding for the first six months of life;
- good personal and food hygiene;
- health education about how infections spread; and
- rotavirus vaccination.

Key measures to treat diarrhoea include the following:

- Rehydration: with oral rehydration salts (ORS) solution. ORS is a mixture of clean water, salt and sugar. It costs a few cents per treatment. ORS is absorbed in the small intestine and replaces the water and electrolytes lost in the faeces.
- Zinc supplements: zinc supplements reduce the duration of a diarrhoea episode by 25% and are associated with a 30% reduction in stool volume.
- Rehydration: with intravenous fluids in case of severe dehydration or shock.
- Nutrient-rich foods: the vicious circle of malnutrition and diarrhoea can be broken by continuing to give nutrient-rich foods – including breast milk – during an episode, and by giving a nutritious diet – including exclusive breastfeeding for the first six months of life – to children when they are well.
- Consulting a health professional, in particular for management of persistent diarrhoea or when there is blood in stool or if there are signs of dehydration.

4. MATERIALS AND METHODS

Drug Selection:

In this dissertation, the *Padiga linga chenduram* preparation was collected from *Anuboga vaidya deva ragasiyam*, part:4, Page: 407, edition year: 1991

Ingredients:

- *padikaram* (Alum)
- *Lingam* (Cinnabar)

Collection and Authentication of Ingredients:

The raw drugs *Lingam* (Cinnabar), and *padikaram* (Alum) were purchased from authorized drug store in Nagarcoil at kanyakumari district. The raw materials were identified and authenticated by experts of PG Gunapadam department, Govt.Siddha Medical College, Palayamkottai, Tamilnadu.

After identification, the samples of raw materials had been conserved in the laboratory of the department for future reference.

Purification of Ingredients:

One of the most important tasks is removal of the toxic substances, which is called purification (*Suddhi*) of raw materials by *Siddhars*. Otherwise it may result in toxicity. *Suddhi* contributes the following changes in the raw drug:

- Reduction in particle size
- Conjugation of trace elements
- Elimination of unwanted elements
- Formation of desirable compounds

Purification of *Lingam* (Cinnabar),

The mixture contains equal portion of lemon juice, cow's milk and aqeous extract of Acalypha (Kuppaimeni) and allowed to fuse cinnabar so as to get it in a consolidated potency state.

4.1.PREPARATION OF *PADIGA LINGA CHENDURAM*:

Materials Required:

Purified cinnabar thathu (*lingam)*		-	4.1g
Alum	(padikaram)	-	35g

Procedure:

Setp 1: Take a mudpot place it on the gas stove

Step 2 : Put the alum on to the mudpot and then gently increase heat and after few hours the alum becomes the puffed alum. At the time of puffing of alumen, the powered, cinnabar is sprinkled over the alum. After the puffing of alum is completed. Then it is grinded in the mortar into powdered form.

Form of the medicine	:	*Chenduram* (Powder)
Shelf –life	:	75 year
Indications	:	*Athisaaram* (dysentery),

Seetha baedi (Diarrhea with bloody flux), Perum kalichal (Persistent diarrhea).

4.2. STANDARDIZATION OF THE DRUG

4.2.1. STANDARDIZATION AS PER SIDDHA CLASSICAL LITERATURE

1. Colour:

Mostly *Chendurams* are pink and red in colour.

2. Floating on Water:

When a pinch of *chenduram* is sprinkled over the water in a glass container, it should partly floated & partly immersed.

3. Finger Print Test:

Well prepared *chenduram* should be very fine. When a pinch of *chenduram* is taken and rubbed in between the thumb and index finger. It should enter into the lines of the fingers. It confirmed the fineness of *chenduram.*

4. Lustre:

If any glowing particles are seen in the *chenduram*, it shows that the drug is not prepared properly and possesses unchanged substances like metals and other toxic substances. So, there should be no glowing particles present in the properly prepared *chenduram*.

5. Taste:

Properly prepared *chenduram* should be completely tasteless. If any taste is present in *chenduram,* it indicates the preparation was not well prepared. It needs another *pudam* (incineration) process.

6. If the *chenduram* is put into a crucible and heated in a blower or oven, it should not revert to its metallic state.

The final product is analysed as per *Siddha* classical standardization methods. Based on the results it was suitable for further analysis.

Standardization of *Padiga linga chenduram* By Using Modern Techniques:

Owing to longstanding and time proven uses of herbo-mineral drug along with their safety margins, World Health Organisation (WHO) has taken necessary steps to ensure quality control with modern techniques and application of suitable standards for this purpose.Standardization of drug helps to authenticate and determine its quality and efficiency. Standardization of herbo mineral drug is rooted in qualitative and quantitative analysis by means of physico-chemical properties and instrumental analysis.

- The physico-chemical analysis of *Padiga linga chenduram* was done in AravindhHerbalsLab (P)LTD
- The chemical finger print was engaged by using modern analytical technique Fourier Transform Infra-Red Spectroscopy (FTIR).
- The particle size and qualitative analysis of chemical elements of *Padiga linga chenduram* were also assessed by Scanning Electron Microscope .
- The quantitative and qualitative analysis of chemical elements was carried out by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

4.2.2. ORGANOLEPTIC CHARACTERS:

Organoleptic properties are the aspects of drug as experienced by the senses, including taste, sight, smell, and touch. Organoleptic evaluation is also called morphological or macro scopical evaluation. It is a technique of quantitative evaluation based on study of morphological and sensory profile i.e. by colour, odour, taste, and size.

4.2.3.PHYSICO CHEMICAL ANALYSIS

Ash Value:

The Ash yielded by an organic compound measures the amount of Inorganic matter present as impurity. Ash values are helpful in determining the quality and purity of crude drugs in the powder form. Ash determination judges the identity and cleanliness of drugs and provides information regarding adulteration with inorganic matter

Determination of Total Ash Value:

Total ash includes physiological ash, which is derived from the medicine and non-physiological, which is often from environment contaminations such as sand and soil.2 g of powdered drug was accurately weighed in a silica crucible which was previously ignited and weighed. The powdered drug was spread as a layer on the bottom of the crucible. It was then incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was then cooled and weighed. The total ash value is recorded.

Determination of Water Soluble Ash:

The ash obtained in the determination of total ash is boiled for 5minutes with 25ml water. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignited for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue are weighed until constant weight is attained for determination of weight of insoluble ash. The weight of the water soluble ash is determined by subtracting the weight of insoluble ash from the weight of total ash.

Determination of Acid Insoluble Ash:

Total ash content alone is not sufficient to reflect the quality, thus the acid insoluble ash of the drug is quite important. The ash obtained in the determination of total ash, was boiled with 25 ml 10%Hcl for 5 minutes and the insoluble ash was collected in a clean filter paper and washed with hot water. The insoluble ash was then transferred to a pre weighed silica crucible, ignited for 5 minutes at a temperature not exceeding 450^{0} C, and then it is cooled and weighed. The results are recorded.

Loss on Drying:

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. 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 drugs

Determination of Loss on Drying(Normal range: 5%-8%)

A glass stopper and a shallow weighing bottle were 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 desiccators before weighing. The percentage loss on drying is calculated. The acceptable range is 5%–8%.

Solubility:

A pinch of the sample was taken in a dry test tube and shaken well with distilled water.

A little amount of the sample is shaken well with con HCl and then Con.H2SO4. Test sample Solubility was observed.

PH Value:

Potentiometrically pH value was determined by a glass electrode and a suitable pH meter. The pH of the KSK tablet was written in results column.

Thin-Layer Chromatography (TLC):

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.

Identification can be effected by observation of spots of identical R_F value and about equal magnitude obtained, respectively, with an unknown and a reference sample

chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Uses of Thin-Layer Chromatography:

1. To determine the number of components in a mixture.
2. To determine the identity of two substances.
3. To monitor the progress of a reaction.
4. To determine the effectiveness of a purification.

Apparatus:

Flat glass plates of appropriate dimensions which allow the application at specified points of the necessary quantities of the solution being examined and appropriate reference solutions and which allow accommodation of the specified migration path-length. The plates are prepared as described below; alternatively, commercially prepared plates may be used.An aligning tray or a flat surface on which the plates can be aligned and rested when the coating substance is applied.

The adsorbent or coating substance consisting of finely divided adsorbent materials, normally 5 µm to 40 µm in diameter, is suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of Plaster of Paris (Hydrated Calcium Sulphate) or with any other suitable binders. The adsorbent may contain fluorescing material to help in visualising spots that absorb ultra-violet light.A spreader which, when moved over the glass plate, will apply a uniform layer of adsorbent of desired thickness over the entire surface of the plate.A storage rack is used to support the plates during drying and transportation.

A developing chamber can accommodate one or more plates and can be properly closed and sealed. The chamber is fitted with a plate support rack that supports the plates, back to back, with lid of the chamber in place.Graduated micro-pipettes capable of delivering microlitre quantities say 10 µl and less.

A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.An ultra-violet light, suitable for observation at short (254 nm) and long (365 nm) ultra-violet wavelengths.

Preparation of Plates:

The plates are prepared in the following manner, unless otherwise specified in the monograph. Prepare a suspension of the coating substance in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, spread a uniform layer of the suspension, 0.25 to 0.30 mm thick, on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 100º to 105º for at least 1 hour (except in the case of plates prepared with cellulose when heating for 10 minutes is normally sufficient) and allow cooling, protected from moisture. Store the plates protected from moisture and use within 3 days of preparation. At the time of use, dry the plates again, if necessary, as prescribed in the monographs.

Method:

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow it to stand for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate. Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the form of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow to stand at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualise as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray. For two-dimensional chromatography dry the plate after the first development and carry out the second development in a direction perpendicular to the first. When the method prescribed in the monograph specified ‘protected from light’ or ‘in subdued light’ it is intended that the entire procedure is carried out under these conditions.

Visualization:

The phrases *ultra-violet light (254 nm)* and *ultra-violet light (365 nm)* indicate that the plate should be examined under an ultra-violet light having a maximum output at about 254 or at about 365 nm, as the case may be.

The term *secondary spot* means any spot other than the principal spot. Similarly, a *secondary band* is any band other than the principal band.

Rf. Value:

Measure and record the distance of each spot from the point of its application and calculate Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

4.2.4. CHEMICAL ANALYSIS

In the qualitative analysis procedure, the chemical properties of an unknown substance are determined by systematically reacting the unknown with a number of different reagents. By predetermining what the particular reaction will produce if a specific ion is present, the ions that actually are in the solution can be identified.

Preparation of the Extract:

100 mg of Padiga linga chenduram is weighed accurately and placed into a clean beaker and added a few drops of conc.Hydrochloric acid and is evaporated well.After evaporation the content is cooled and added a few drops of conc.nitric acid and evaporated.After cooling, the content is dissolved in 20ml of distilled water.It is then transferred to 100ml volumetric flask and made-up to 100ml with distilled water and is mixed well.It is filtered and is used for analysis.

Test for Basic and Acidic Radicals:

1. **Test for Calcium:**

When 2ml of the above prepared extract is taken in a clean test tube. To this add 2 ml of 4% ammonium oxalate solution. No white precipitate is formed. It indicates the absence of calcium

2. **Test for Sulphate:**

2ml of the extract is added to 5% Barium chloride solution.A white precipitate is formed. It indicates the presence of sulphate.

3. **Test for Chloride:**

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

4. **Test for Carbonate:**

The substance is treated with concentrated Hcl. No Brisk effervescence is formed. It indicates the absence of carbonate

5.**Test for Starch:**

The extract is added with weak iodine solution. No blue colour is formed. It indicates the absence of starch

6. **Test for Ferric Iron:**

The extract is acidified with glacial acetic acid and potassium ferrocyanide. Blue colour is formed. It indicates the presence of Ferric Iron

7. **Test for Ferrous Iron:**

The extract is treated with concentrated nitric acid and ammonium thiocyanatesolution. Blood red colour is formed. It indicates the presence of Ferrous Iron.

8. **Test for Phosphate:**

The extract is treated with ammonium molybdate and concentrated nitric acid. No yellow precipitate is formed. It indicates the absence of Phosphate.

9.**Test for Albumin:**

The extract is treated with Esbach's reagent. No yellow precipitate is formed. It indicates the absence of Albumin.

10.**Test for Tannic Acid:**

The extract is treated with ferric chloride. No blue black precipitate is formed. It indicates the absence of Tannic Acid.

11.**Test for Unsaturated Compounds:**

Potassium permanganate solution is added to the extract. It does not get decolourised. It indicates the absence of Unsaturated Compounds.

12.**Test for The Reduicing Sugar:**

5ml of Benedict's qualitative solution is taken in a test tube and allowed to boil for 2 mins and add 8-10 drops of the extract and again boil it for 2 mins. No colour change occurs. It indicates the absence of Reduicing sugar.

13.**Test for Amino Acid:**

One or two drops of the extract is placed on a filter paper and dried well .After drying, 1% Ninnhydrin is sprayed over the same and dried well. No violet colour is formed. It indicates the absence of Amino acid.

14.**Test for Zinc:**

The extract is treated with potassium Ferro cyanide. No white precipitate is formed. It indicates the absence of Zinc.

15.**Test for Mercury**

The extract is treated with ammonia and boil (till the ammonia cases off) and then potassium Iodide (1% solution) is added. No scarlet precipitate is formed. It indicates the absence of Mercury

4.2.5.INSTRUMENTAL ANALYSIS

4.2.5.1.SCANNING ELECTRON MICROSCOPE (SEM)

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

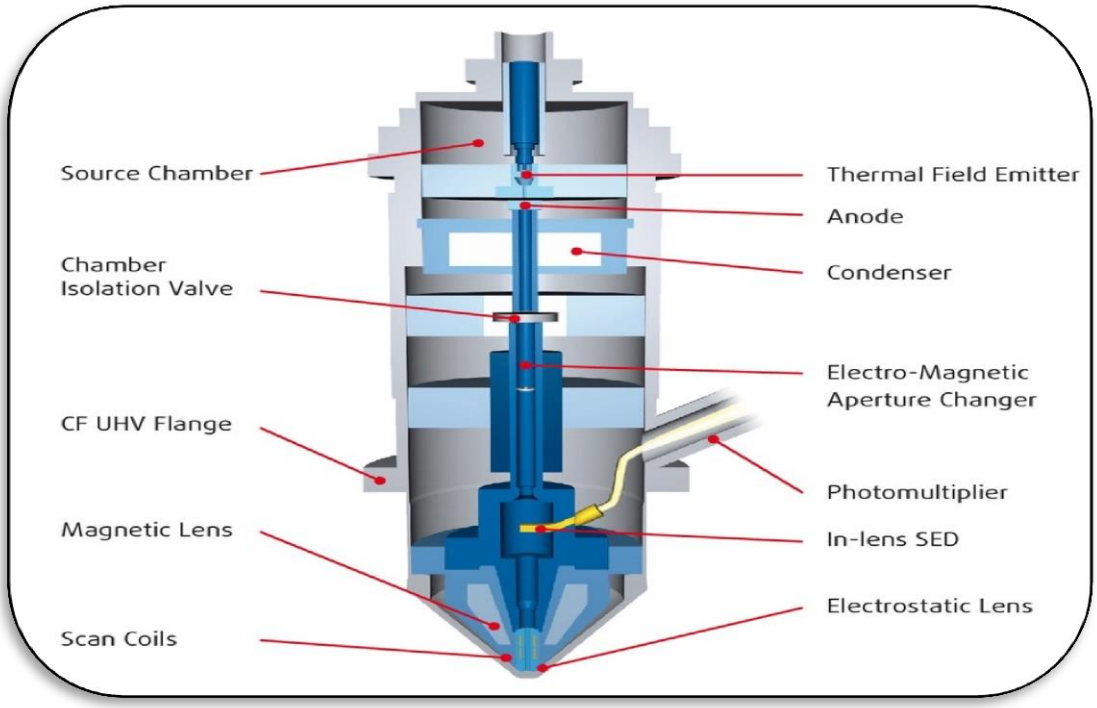
Introduction:

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.

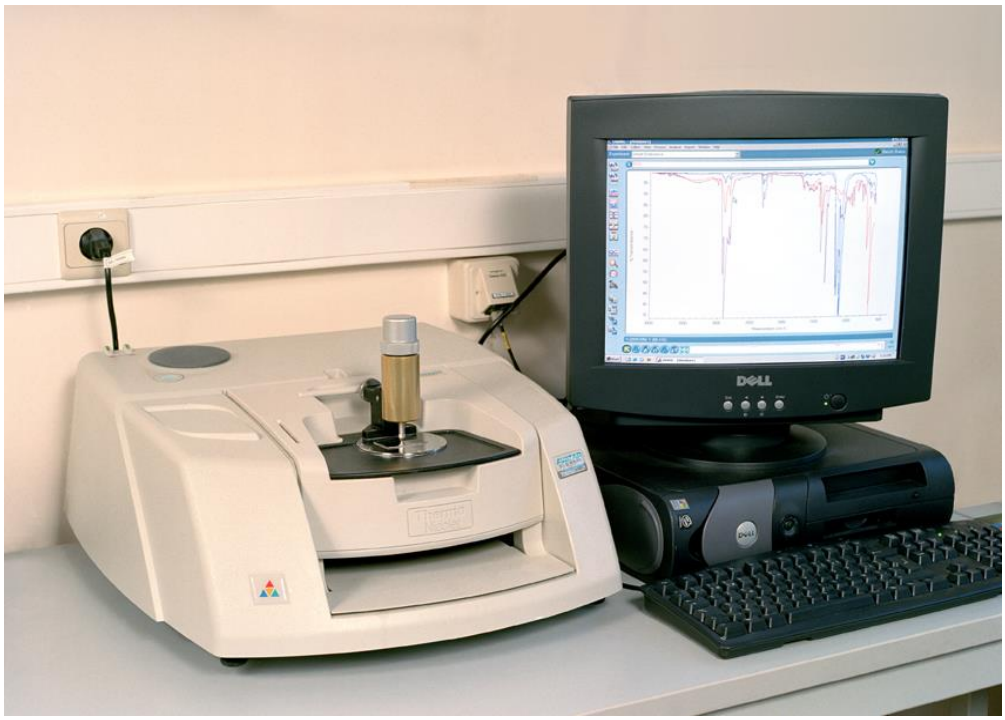
Applications:

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* mineral drug *PADIGALINGA CHENDURAM*. SEM results of *PADIGALINGA CHENDURAM* were represented in results section.

4.2.5.2.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:

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

- 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 Tris buffer.

- The washings took 24 h, which is the time of contact of the protein with the 2H20 medium 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 H_2O or $2H_2O$, respectively. FT-IR spectra were recorded by means of a Perkin-Elmer -Spectrum-1 FT-IR spectrometer using a deuteratedtriglycinesulfate detector.

- 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.

- 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

- The sample is grounded using an agate mortar and pestle to give a very fine powder.
- The finely powder sample is then mixed with about 100mg dried KBr salt.
- 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:

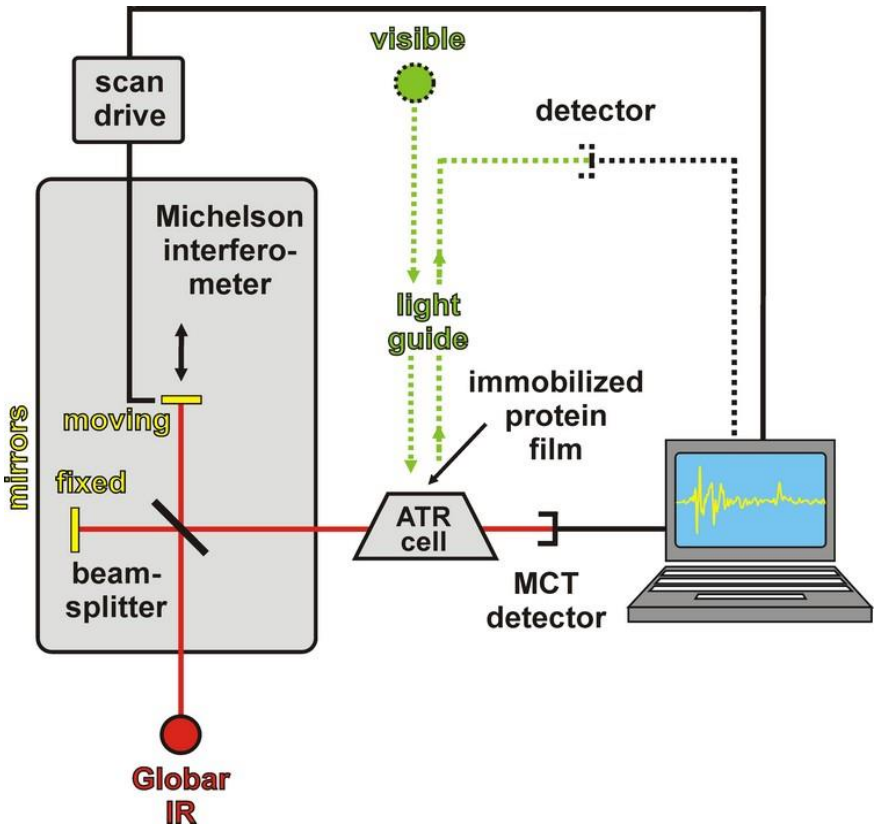
- The sample is ground using an agate mortar and pestle to give a very fine powder.
- A small amount is then mixed with nujol oil to give a paste and this paste is thenapplied between two sodium chloride plates.
- The plates are then placed in the instrument sample holder ready for scanning.

Liquids:

- Viscous liquids can be smeared in the cell and directly measured.
- 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.



Mechanism analyser

Analytical Capabilities:

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

4.2.5.3.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 "work coil" 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 arc 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 *PADIGA LINGA CHENDURAM.*

4.3.TOXICOLOGICAL STUDY

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

OBJECTIVES

The aim of this Study is to evaluate the toxicity of the test substance ***PADIGA 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,

Study Design and Controls:

1) Female Wister Rats in controlled age and body weight were selected.

2) ***PADIGA LINGA CHENDURAM*** was administered at **5 mg/kg, 50 mg/kg, 300 mg/kg, 1000 mg/kg, and 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

1.1. Supply

A total of 15 Female Wister Rats with an approximate age of 6 weeks and purchased from M/s.Venkateshwara Enterprises Pvt. Ltd, Bangalore. 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.

1.2. 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 6 mice 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.

2.1. 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.

3.1 Numbering and Identification

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

Table-2 Numbering and Identification

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

3.2 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.

Table-3 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

PADIGALINGACHENDURAM 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.

3.3 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.

3.4 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.

4 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

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

1. Objective

The objective of this '**Sub-Acute Toxicity Study of PADIGA 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.

2. Test Guideline Followed

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

3. Test Item Detail

Name: **PADIGALINGACHENDURAM**

4. 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 M/s. Sree Venkateshwara Enterprises Pvt. Ltd, Bangalore. 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.

5. Acclimatization

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

6. 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 6 male animals per group.

7. Numbering and Identification

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. CONTROL	**H,B,T,HB,NM (MALE)** **H,B,T,HB,NM (FEMALE)**
2. LOW DOSE OF PADIGALINGACHENDURAM 300mg/kg.	**H,B,T,HB,NM (MALE)** **H,B,T,HB,NM (FEMALE)**
3. MIDDLEDOSE OF PADIGALINGACHENDURAM 600mg/kg.	**H,B,T,HB,NM (MALE)** **H,B,T,HB,NM (FEMALE)**
4. HIGH DOSE OF PADIGALINGACHENDURAM 900mg/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:

Cage No	Group No	Animal Marking	Sex
1	**1. CONTROL**	**H,B,T,HB,NM** **H,B,T,HB, NM**	**Male** **Female**
2	**2. LOW DOSE OF PADIGALINGACHENDURAM 300mg/kg.**	**H,B,T,HB,NM** **H,B,T,HB, NM**	**Male** **Female**
3	**3. MIDDLEDOSE OF PADIGALINGACHENDURAM 600mg/kg.**	**H,B,T,HB,NM** **H,B,T,HB, NM**	**Male** **Female**
4	**4. HIGH DOSE OF PADIGALINGACHENDURAM 900mg/kg.**	**H,B,T,HB,NM** **H,B,T,HB ,NM**	**Male** **Female**

8.Husbandry

8.1 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 6 mice of the same sex and treatment group.

8.2 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.

8.3 Feed & feeding schedule

'Sai Durga Animal Feed, Bangalore. 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.

8.4 Water

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

8.5 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.

Table-2 Dose level

TEST GROUP	**DOSE TO ANIMALS (mg/kg body-weight/day)**	**NUMBER OFANIMALS**
Group-1	**1. CONTROL**	10 (5MALE and5 FEMALE)
Group-II	**2. LOW DOSE OF PC 300mg/kg.**	10(5MALE and 5 FEMALE)
Group-III	**3. MIDDLEDOSE OF PC 600mg/kg.**	10(5MALE and 5 FEMALE)
Group-IV	**4. HIGH DOSE OF PC 900mg/kg.**	10(5MALE 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

PADIGA 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 **PADIGA LINGA CHENDURAM** in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

8.6 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.

9. 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.

9.1. 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.

9.2. 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.

9.3. 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.

9.4 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 6 males 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

MATERIALS AND METHODS

ESTIMATION OF HEMATOLOGICAL PARAMETERS: [1]

Collection of blood for hematological studies

After the treatment period the animals were anaesthetized by ketamine hydrochloride and the blood was collected from Retro-orbital sinus by using capillary into a centrifugation tube which contains EDTA for haematological parameters The haematological parameters like RBC, WBC and Hb percentage, Differential cell count, MCV, MCHC, Hematocrit, MCH, platelet count were estimated by the following procedures.

- **ENUMERATION OF RED BLOOD CELLS:** [1] Ramnic 2007)

Reagents : RBC diluting fluid

Procedure:

Using a red blood cell pipette of haemocytometer, well mixed blood was drawn up to 0.5 mark and RBC diluting fluid was taken up to mark II. The fluid blood mixture was shaken and transferred onto the counting chamber. The cells were

allowed to settle to the bottom of the chamber for 2 min. See the fluid does not get dried. Using 45X or high power objective the RBC's were counted uniformly in the larger corner squares.

The cells were expressed as number of cells $x10^{12}/l$

- **ENUMERATION OF WBC: [2] John 1972)**

- **Reagents:**

Turk's fluid: Turk's fluid was prepared by mixing 2ml of acetic acid with 100 ml of distilled water. To this 10 drop of aqueous methylene blue 3 % w/v) was added. This solution haemolysis the red cells due to acidity so that counting of white cells becomes easy.

Procedure:

Using a white blood cell pipette of haemocytometer, well mixed blood was drawn up to 0.5 mark and WBC diluting fluid was taken up to mark II. The fluid blood mixture was shaken and transferred onto the counting chamber. The cells were allowed to settle to the bottom of the chamber for 2 min. See the fluid does not get dried.

Using 10X or low power objective the WBC's were counted uniformly in the larger corner squares.

The cells were expressed as number of cells/10mm.

- **DIFFERENTIAL LEUCOCYTE COUNT: [3] John 1972)**

Reagent:

Leishmann's stain: 150mg of powdered leishmann's stain was dissolved in 133ml of acetone free methanol.

Procedure:

A blood film stained with leishmann's stain was examined under oil immersion and the different types of WBCs were identified. The percentage distribution of these cells was then determined. Smears were made from anticoagulant blood specimens and stained with leishmann's stain. The slides were preserved for counting the number of lymphocytes and neutrophils, per 100 cells were noted.

From the different Leukocyte count and WBC count, absolute lymphocyte and neutrophil count were calculated.

$$\text{Absolute neutrophil count} = \frac{\text{Number of neutrophils}}{100} \times \text{TWBC}$$

$$\text{Absolute lymphocyte count} = \frac{\text{Number of lymphocytes}}{100} \times \text{TWBC}$$

J. C. Dacie and S. M. Lewis, Practical haematology, London: Churchill Livingstone, 1984, pp. 5.

Measurement of biochemical parameters estimation

Haemoglobin (Hb), was estimated using whole blood. Remaining parameters were measured in serum. All of the above biochemical parameters were estimated using semi-autoanalyzer (Photometer 5010 $_{V5+}$, Germany) with enzymatic kits procured from Piramal Healthcare limited, Lab Diagnostic Division, Mumbai, India.

Determination of aspartate aminotransferase (AST)

Aspartate aminotransferase, also known as Glutamate Oxaloacetate Transaminase (GOT) catalyses the transamination of L-aspartate and α keto glutarate to form oxaloacetate and L- glutamate. Oxaloacetate formed is coupled with 2,4-Dinitrophenyl hydrazine to form hydrazone, a brown coloured complex in alkaline medium which can be measured colorimetrically.

Reagents

Buffered aspartate (pH 7.4); 2,4- DNPH reagent; 4N sodium hydroxide; working pyruvate standard; solution I (prepared by diluting 1 ml of reagent 3 to 10 ml with purified water).

Procedure

Rietman and Frankle method was adopted for the estimation of SGOT. (Reitmann S, Frankel S, 1957. A colorimetric method for the determination of serum oxaloacetic and glutamic pyruvate transminases. American Journal of Clinical Pathology.28: 56-63.The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered aspartate was added into all the test tubes. Then 0.05 ml of serum was added to the test group tubes and 0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37^{o}C for 60 min, after which 0.25 ml each of 2,4- DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 2.5 ml of solution I was added to all test tubes. Mixed properly and optical density was measured in a spectrophotometer at 505 nm within 15 min.

The enzyme activity was calculated as:-

AST (GOT) activity in IU/L) = [(Absorbance of test - Absorbance of control)/ (Absorbance of standard - Absorbance of blank)] x concentration of the standard

Determination of alanine aminotransferase (ALT)

Alanine aminotransferase, also known as Glutathione Peroxidase (GPT) catalyses the transamination of L-alanine and α keto glutarate to form pyruvate and L- Glutamate. Pyruvate so formed is coupled with 2,4 – Dinitrophenyl hydrazine to form a corresponding hydrazone, a brown coloured complex in alkaline medium which can be measured colorimetrically.

Reagents

Buffered alanine (pH 7.4), 2,4–DNPH, 4N sodium hydroxide, working pyruvate standard, solution I (prepared by diluting 1 ml of reagent 3 to 10 ml with purified water).

Procedure

Rietman and Frankle method was dopted for the estimation of SGPT. The reaction systems used for this study included blank, standard, test (for each serum sample) and control (for each serum sample). 0.25 ml of buffered alanine was added into all the test tubes. This was followed by the addition of 0.05 ml of serum into the test group tubes and 0.05 ml of working pyruvate standard into the standard tubes. After proper mixing, all the tubes were kept for incubation at 37^{o}C for 60 minutes, after which 0.25 ml each of 2,4- DNPH reagent was added into all the tubes. Then, 0.05 ml of distilled water and 0.05 ml of each serum sample was added to the blank and the serum control tubes respectively. The mixture was allowed to stand at room temperature for 20 min. After incubation, 2.5 ml of solution **I** was added to all test tubes. Mixed properly and optical density was read against purified water in a spectrophotometer at 505 nm within 15 min.

The enzyme activity was calculated as:- ALT (GPT) activity in IU/L) = [(Absorbance of test - Absorbance of control)/ (Absorbance of standard - Absorbance of blank)] x concentration of the standard.

Determination of alkaline phosphatase (ALP)

Alkaline phoshatase from serum converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4-aminoantipyrine in presence of the oxidising agent potassium ferricyanide and forms an orange-red coloured complex, which can be measured spectrometrically. The color intensity is proportional to the enzyme activity.

Reagents:

Buffered substrate

Chromogen Reagent

Phenol Standard, 10 mg%

Procedure:

ALP was determined using the method of Kind (Kind PRM, King EJ, 1972. *In-vitro* determination of serum alkaline phosphatase. Journal of Clinical Pathology 7: 321-22\). The working solution was prepared by reconstituting one vial of buffered substrate with 2.2 ml of water. 0.5 ml of working buffered substrate and 1.5 ml of purified water was dispensed to blank, standard, control and test. Mixed well and incubated at 37^0C for 3 min. 0.05 ml each of serum and phenol standard were added to test and standard test tubes respectively. Mixed well and incubated for 15 min at 37^0C. Thereafter, 1 ml of chromogen reagent was added to all the test tubes. Then, added 0.05 ml of serum to control. Mixed well after addition of each reagent and the O.D of blank, standard, control and test were read against purified water at 510 nm.

Serum alkaline phosphatase activity in KA units was calculated as follows

[(O.D. Test-O.D. Control) / (O.D. Standard- O.D. Blank)] x 10

Determination of bilirubin

In toxic liver, bilirubin levels are elevated. Hyperbilirubinemia can result from impaired hepatic uptake of unconjugated bilirubin, such a situation can occur in generalized liver cell injury, certain drugs (e.g Rifampin and probenecid) interfere with the rat uptake of bilirubin by the liver cell and may produce a mild unconjugated hyperbilirubinemia. Bilirubin level rises in diseases of hepatocytes, obstruction to bilirubin excretion into duodenum, in haemolysis and defects of hepatic uptake and conjugation of Bilirubin pigment such as Gilbert's disease.

Elevation of total serum bilirubin may occur due to:

1.Excessive haemolysis or destruction of the red blood cells.Eg:Haemolytic disease of the new born.

2.Liver diseases.Eg.Hepatitis and cirrhosis.

3.Obstruction of the biliary tract.Eg.Gall stones.

The method is based on the reaction of Sulfonilic acid with sodium nitrite to form azobilirubin which has maximum absorbance at 546nm in the aqueous solution.

The intensity of the color Produced is directly proportional to the amount of direct or total bilurubin concentration present in the sample.

Reagents

1. Diazo A-(Reagent-R1) :Ready to use

2. Diazo B-(Reagent-R2):Ready to use

3. Bilirubin Activater :Ready to use

Procedure

Kind & King's method was followed for the estimation of Bilirubin. Five hundred µl of working reagent was added to 50 µl of rat serum & incubated for 5 min at 37°C. Absorbance was measured AT 546 NM in semi auto analyzer against the standard.

The Bilirubin content was calculated using the following equation:

Total bilirubin (mg/dt) = Abs of the sample blank x 15.

Estimation of Urea

Urea is the nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in blood are referred to as hyper uremia or azotemia.

Method

Estimation of urea was done by Urease-GLDH: enzymatic UV test.

Principle

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4 + 2HCO_3$$

$$\text{2- Oxoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GLDH}} \text{L- Glutamate} + NAD^+ + H_2O$$

Table 14. Reagents

R 1	TRIS pH 7.8	120 mmol/l
	2-Oxoglutarate	7 mmol/l
	ADP	0.6 mmol/l
	Urease	≥ 6 KU/l
	GLDH	≥ 1 KU/l
R 2	NADH	0.25 mmol
R 3	Standard	40 mg/dl

Procedure

a. Take 1000 µl of reagent-1 and 250 µl of reagent-2 in 5 ml test tube.
b. To this, add 10 µl of serum.
c. Mix well and immediately read the test sample at 340 nm Hg 334 nm Hg 365 nm optical path 1 cm against reagent blank (2-point kinetic).
d. And note down the value.

Normal range: 10 – 50 mg/dl.

ESTIMATION OF URIC ACID

Uric acid and its salts are end products of the purine metabolism. In gout the most common complication of hyperuricemia, ie. Increased serum levels of uric acid lead to formation of monosodium urate crystal around the joints.

Method

Enzymatic photometric test using TOOS (N ethyl- N (hydroxyl -3-sulfopropyl)-m- toluidin)

Principle

$$\text{Uric acid} + H_2O + O_2 \xrightarrow{\text{uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$\text{TOOS} + 4\text{ aminoantipyrine} + 2H_2O_2 \xrightarrow{\text{POD}} \text{Indamine} + 3H_2O$$

Table 15.reagents

R1	Phosphate buffer pH 7.0	100mmol/l
	TOOS	1mmol/l
	Ascorbate oxidase	≥1 KU/l
R2	Phosphate buffer pH 7.0	100mmol/l
	4- amino antipyrine	0.3mmol/l
	K_4 (Fe(CN)$_6$)	10µmol/l
	Peroxidase	≥1KU/l
	Uricase	≥50U/l

Procedure

a. Take 800µl of reagents -1 in a2ml centrifuge tube.

b. To this add 20µl of serum.

c. Mix well and incubate at 30°c for 5 minutes.

d. Then add 200µl of reagent2

e. Mix well incubate for 5min at 37°c

f. Measure the not down the values.

Normal range: 1.9-8.2mg/dl

ESTIMATION OF CREATININE:

Estimation of Creatinine by Jaffe Method (modified)

Principle:

Creatinine forms a coloured complex with picrate in alkaline medium.

The rate of formation of the complex is measured.

Reagents:

Reagent 1 Standard Creatinine (2mg/100ml)

Reagent 2 Picric acid solution

Reagent 3 sodium hydroxide solution

Procedure:

Take 500 µl of reagent -2 and 500 µl of reagent -3 in a 5ml test tube. To this add 100 µl of serum. Mix well and immediately read the test sample at Hg 492 nm 1cm light path and note down the values.

Normal range is 0.6 -1.1 mg/dl.

4.4.PHARMACOLOGICAL STUDY

4.4.1.ANTI-DAIRRHOEAL ACTIVITY

EFFECT OF *PADIGALINGACHENDURAM* ON CASTOR OIL - INDUCED SMALL INTESTINAL TRANSIT IN RATS

Diarrhea is one of the most common illnesses in all age groups. It is an uncomfortable condition that can have many causes, and vital gastroenteritis (stomach flu) is one of the most common causes of diarrhea. Diarrhea will normally heal itself within a few days. However, in some cases, diarrhea can lead to dehydration or be a sign of a more serious problem (Fontaine, 1988; Snyder and Merson, 1982).

Fontaine, O. (1988). Bacterial diarrhoea and treatment. Lancet. 1(8596): 1234 – 1235.

MATERIAL AND METHODS

ANIMALS:

Male Wister rats (140-180 and 180-200 g), were used in this study. All the animals were obtained from Animal house of the KMCH College of Pharmacy, Coimbatore. The animals were housed comfortably in a group of six in a single clean plastic cage with a metal frame lid on its top. They were housed under standard environmental conditions of temperature (24±1°C) and relative humidity of 30-70 %. A 12:12 h light dark cycle was followed. All animals had free access to water and standard pelletized laboratory animal diet ad libitum. All the experimental procedures and protocols used in this study were reviewed and approved via the Approval No. KMCRET/MD(S)/16/2016-2017 by the Institutional Animal Ethical Committee (IAEC) of KMCH College of Pharmacy, Coimbatore (685/PO/Re/S/2002/CPSCEA Dated 21st August 2002 constituted in accordance with the guidelines of the CPCSEA, Government of India.

CASTOR OIL-INDUCED DIARRHEA IN RATS

Male Wister rats were fasted 48 h, and water was given *ad libitum*. The water was withdrawn 1 h before starting the experiment. The experiment was performed according to the method of Venkatesan et al. (2005). Male Wister rats were given 0.5% carboxymethy cellulose (CMC) (orally) or atropine sulfate (intraperitoneally, i.p.). One hour later, they were given one milliliter castor oil orally and then weighed and placed individually in a transparent plastic box with absorbent paper underneath. Total amount of feces (in grams) after the castor oil administration was collected and weighed.

4.4.2.ANTI-PYRETIC ACTIVITY

EFFECT OF *PADIGALINGACHENDURAM* ON BREWER'S YEAST INDUCED PYREXIA IN RATS

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural function to create an environment where infectious agents or damaged tissues cannot survive. Normally, the infected or damaged tissue initiates the enhanced formation of pro inflammatory mediators (cytokines, such as interleukin 1β, α, β, And TNF- α), which increase the synthesis of prostaglandin (PgE2) near hypothalamic area and there by trigger the hypothalamus to elevate the body temperature.

PROCEDURE

Before yeast injection the basal rectal temperature of rats was recorded, Baseline body temperature was measured by inserting the digital rectal tele thermometer in to the anal cavity of the rat for about 2 mins. The steady temperature readings obtained were recorded as the pre temperature. After recording animals were given subcutaneous injection of 10 ml/ kg of 15 % w/v yeast suspended in 0.5 % w/v carboxymethyl cellulose solution for elevation of body temperature of rats. Rats were then returned to their home cages. 18hrs after yeast injection, rats with elevated body temperature was selected for grouping and the *PLC* and standard drug was suspended in CMC and administered by gastric tube.

Dosage schedule:

The required dose for mice/rat will be calculated by using the standard dose calculation procedure from recommended clinical dose.

Conversion formula:

human dose is 260mg,BD

Total clinical dose (a) x conversion factor (b) 0.018 = (c) per 200 gm of rat

260 mg x 2(a) x 0.018 (b) = 4.68 mg (c) /kg

4.6x1000/200 = 23mg/kg

Experimental Doses Calculated as per the standard procedures are:

S.No	Groups	Dose /kg, weight	Dose /200 gms. weight	Volume of administration
1	Vehicle Control	--	--	0.5 ml
2	Therapeutic Dose	23mg	4.6mg	0.5 ml
3	Average Dose	115mg	23mg	0.5 ml
4	High Dose	230mg	46mg	0.5 ml

4.4.3.IN-VITRO ANTI-SPASMODIC ACTIVITY OF PADIGA LINGA CHENDURAM ON EXCISED RAT ILEUM

ISOLATION OF RAT ILEUM:-

Rats were anesthetized and sacrificed by cervical displacement followed by exsanguinations. The ileum was dissected out, immersed in Tyrode'ssolutionand cleaned off the mesentery. Respective segments of 2-3cm long were mounted in a 25ml tissue organ bath, filled with a mixture of 95% O2 and 5% CO2 and maintained at 37 o C. The composition of Tyrode's solution (in mM for l lit) was 9 mg KCl, 0.1 mg NaCl, 0.1mg NaHCO3, 0.42mg NaH2PO4, 0.6 mg Glucose and pH value was 7.4.

ANTI-SPASMODIC ACTIVITY ASSAY PROCEDURE:-

1. Firstly concentration dependent responses of acetylcholine were recorded (with dose of 0.1ml, 0.2ml, 0.4ml, 0.8ml, 1.6ml, 3.2ml) using Sherrington's recording drum with a frontal writing lever. Contact time of 60 sec, and base line of 30sec time cycle were opted for proper recording of the responses in presence of plane Tyrode's solution as stock-I solution.
2. Then same concentration dependent responses of acetylcholine (Ach) using same procedure for a mixture of Tyrode's solution+ Lantana camara extract (with a concentration of 1mg/ml) as a stock-II solution were recorded.
3. Lastly the same concentration dependent responses of Ach for a mixture of Tyrode's solution+ Atropine (as a standard antispasmodic agent) as a stock-III solution were recorded.

4.5.MICROBIOLOGICAL ANALYSIS

ANTI-MICROBIAL ACTIVITIES BY WELL DIFFUSION METHOD

Aim:

The anti-microbial activity of padiga linga chenduram was adapted through well diffusion method (Agar diffusion testing)

Principle

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

Components of Muller Hinton agar medium:

Beef extract – 2gm/lit

Acid Hydrolysate of Casein – 17.5gm/lit

Starch – 1.5 gm/lit

Agar – 17 gm/lit

Distilled water – 1000 ml

PH -7.3±0.1 at 25^0 C

Procedure (Murray *et al.*, 1995)

Petriplates containing 20ml Muller Hinton medium were seeded with 24hr culture of bacterial strains. Wells were cut and 20 µl of the plant extracts (aqueous) were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around

the well (NCCLS, 1993). Streptomycin was used as a positive control. Standard for an In-vitro antimicrobial activity of padiga linga chenduram was screened against bacteria strains such as Klebsiella pneumonia, Streptococcus mutans,staphylococcus aureus, Escherichia coli, Pseudomonas aerugina, Enterococcus faecalis.

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR Manual of Clinical Microbiology, 6th Ed. ASM Press, Washington DC, 1995; 15-18.

5. RESULTS AND DISCUSSION

The Siddha drug *Padiga linga chenduram* had been subjected to various studies to establish the works of Siddhars to be true. Literary collections, physicochemical and Elemental analysis, toxicological study, pharmacological study and clinical study are done to prove the activity of *Padiga linga chenduram* in Anti diarrhoeal activity.

Lingam and padikaram, is the chief ingredient of the medicine *Padiga linga chenduram*, is indicated for *Kazhichal* in Siddha literature. The experimental analysis was done to standardize the *padiga linga chenduram* by its chemical compounds and particle size.

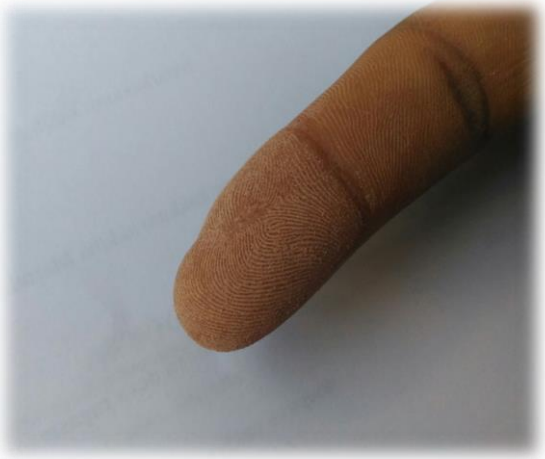
Siddha standardization methods

Siddhars used these following standardization methods to ensure the safety and efficacy of the chenduram. It shows the effectiveness of the drug.

Table No:1 Results of Siddha standardization

S.NO	Parameter	Results of ideal *chenduram*	Results of PC	Interpretation
1.	Colour	Pink colour or red colour, no shining	pink colour, no shining	One of the Ideal colours of chenduram.
2.	Floating on Water	Floats on water	Floats on water	Lightness of drug.
3.	Finger Print Test	Impinged in the furrow of fingers	Impinged in the furrow of fingers	Indicates fine particles of powder.
4.	Lustre	Lustreless	Lustreless	Change of specific metallic character of raw material after incineration
5.	Taste	No specific taste.	No specific taste.	Change of specific metallic character of raw material after incineration

Finger Print Test **Colour (pink)**



Floating on Water

PHYSIOCHEMICAL ANALYSIS OF PADIGA LINGA CHENDURAM

Organoleptic Characters:

Colour	:	Pink colour
Appearance	:	Powder
Touch	:	Nice
Smell	:	Odourless
Taste	:	Tasteless

Physico- chemical standards:

Loss of Drying at 105^0C	:	7.8%
Ash Content	:	19.4%
Ash insoluble Ash	:	7.5%
Ph	:	7.0
Nice	:	Fine powder
Water Floated Test	:	Partly Floated.

Microbial Limit test:

Total Viable aerobic count	:	1.2 x 10^4 col/g
Total Enterobacteriaceae	:	Nil
Total fungal count	:	Nil

Test for specific pathogens:

Salmonella sp	:	Nil
Staphylococcus aureus	:	Nil
E.coli	:	Nil
Pseudomonas aeruginosa	:	Nil

Interpretation:

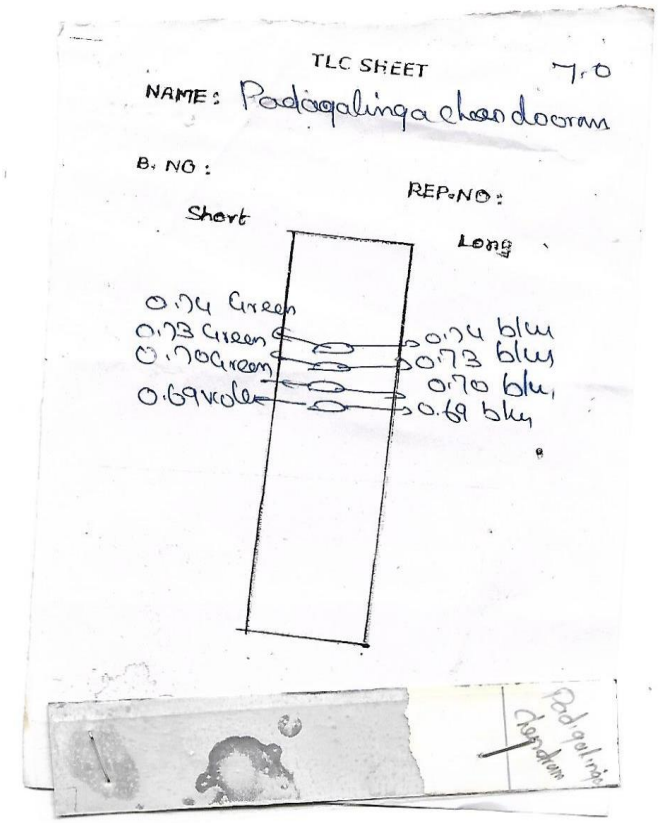
Loss of drying value is 7.8% which confirms that *padiga linga chenduram* has nil moisture content and hence is very stable.

Total ash content is 19.4% ensure that *padiga linga chenduram* does not contain much inorganic impurities.

As partical size is fine & as it floats of on water which is the desired properties of a chenduram, it is confirmed that it is well prepared.

Absence of Microbes ensures that it is free from contamination.

THIN LAYER CROMATOGRAPHY (TLC):



Interpretation:

Spots of green, orange colours were detected on the chromatoplates under UVlight. The R_f value of spots in the sample was approximately 0.74, 0.73, 0.70, 0.69, it indicates the presence of active compound in the sample which is responsible for the therapeutic effect.

BIO-CHEMICAL ANALYSIS OF "PADIGA LINGA CHENDURAM"

Table No:2 Preliminary test for basic and acidic radical:

S.no	Experiment	Inference
1.	Test for calcium:	Absent
2.	Test for sulphate:	**Present**
3.	Test for chloride:	**Present**
4.	Test for carbonate:	Absent
5.	Test for starch:	Absent
6.	Test for iron ferric:	**Present**
7.	Test for iron ferrous:	**Present**
8.	Test for phosphate:	Absent
9.	Test for albumin:	Absent
10.	Test for tannic acid:	Absent
11.	test for unsaturation:	Absent
12.	Test for the reducing sugar:	Absent
13.	test for amino acid:	Absent
14.	Test for zinc:	Absent

Interpretation:

- **Sulfur** is a very versatile molecule, because it can exit in several distinct oxidative states, ranging from +6 in sulfate radicle to -2 in hydrogen sulfide.

  Sulfur is a healing mineral and plays a role in metabolism.

- **Chloride:** It even helps in maintaining proper kidney function and assists in keeping fluid flow through tissues and blood vessels efficient.

Chloride, sodium, potassium regulate the acid-base balance of the body fluids. They regulate the water balance by maintaining the osmotic pressure of the body fluids.

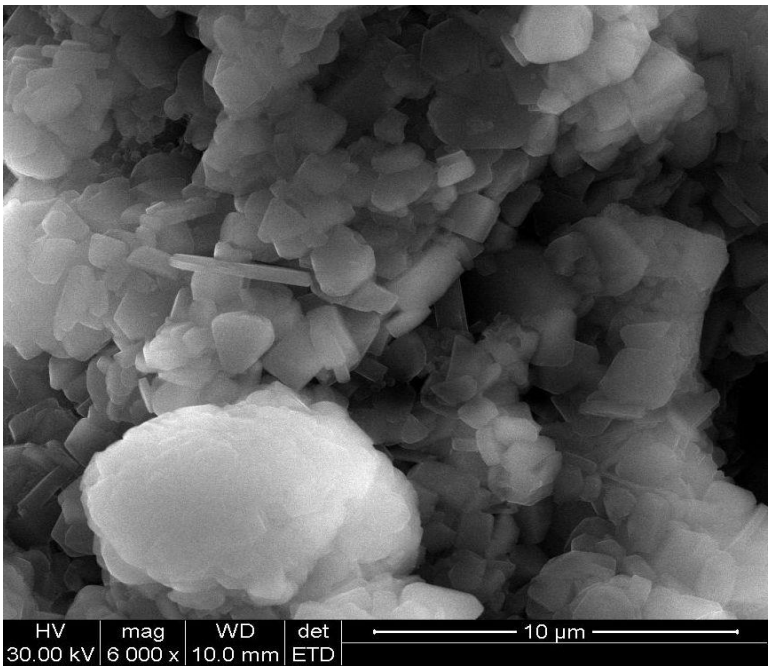
- **Iron** is a mineral. Most of the iron in the body is found in the hemoglobin of red blood cells and in the myoglobin of muscle cells. Iron is needed for transporting oxygen and carbon dioxide. It also has other important roles in the body.

  The heme containing enzymes such as catalase and peroxidase protect cell against potentially damaging highly reactive species.

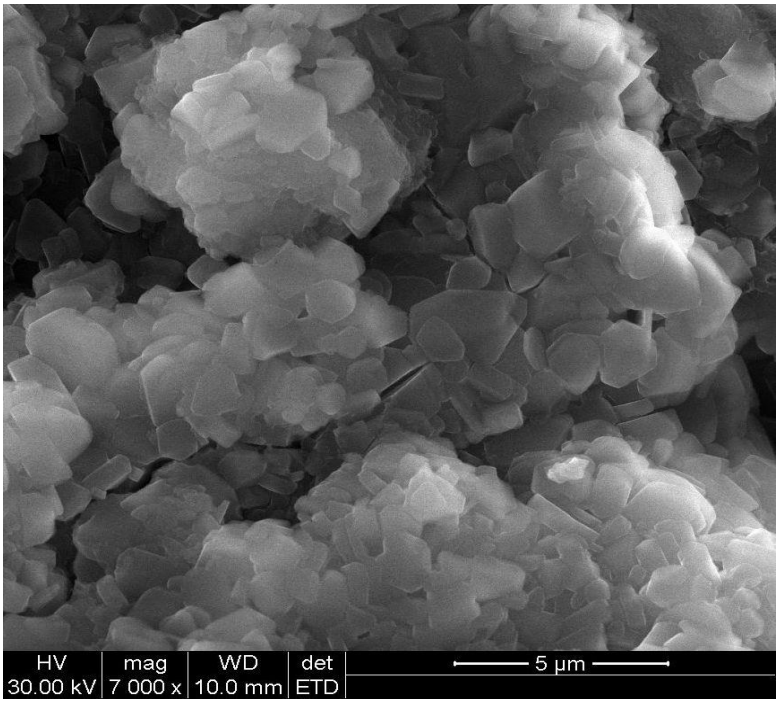
  Iron is essential for many numbers of biological functions such as growth, reproduction, healing and immune function.

  Iron deficiency may be determined by measurement of iron levels within the body, mainly serum ferritin levels, which may also help distinguish between iron deficiency anemia and anemia associated with chronic disease, such as chronic kidney disease (CKD).

SEM RESULT:



SEM picture 30,000 magnification



SEM picture 5,000 magnification

Interpretation:

The morphology of the *Padiga linga chenduram* sample 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. We have observed from SEM photographs that particles are rounded in shapes and sizes are in the range from 5µm to 10 µm. Although the particle sizes of different batches showed similarity, it seems that these particles are aggregates of much smaller particles. When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gives these particles a tendency to aggregate together to form larger particles. *Padiga 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.

Siddhars were the great scientist in ancient times. They used nano technology for the preparation of *chenduram, chenduram* to treat chronic diseases. Nano particles have beneficial properties that can be used to improve drug delivery system. Target cells take up these nano particles quickly .Because of their smaller size,lesser particles

enhance the bio absorption and bio availability resulting efficacy of the drug will be increased. Larger particles could not enter in to the target cell because of their size, resulting in excretion from the body. If a drug is cleared too quickly from the body, this could force a patient to use high dose, poor bio distribution is a problem that can affect normal tissue through wide spread distribution but the particles from drug delivery systems lower the volume of distribution and reduce the effect on non target tissue. Adjuvant and detoxification (Purification) is also important factors for drug transport.

FTIR RESULTS OF PADIGA LINGA CHENDURAM

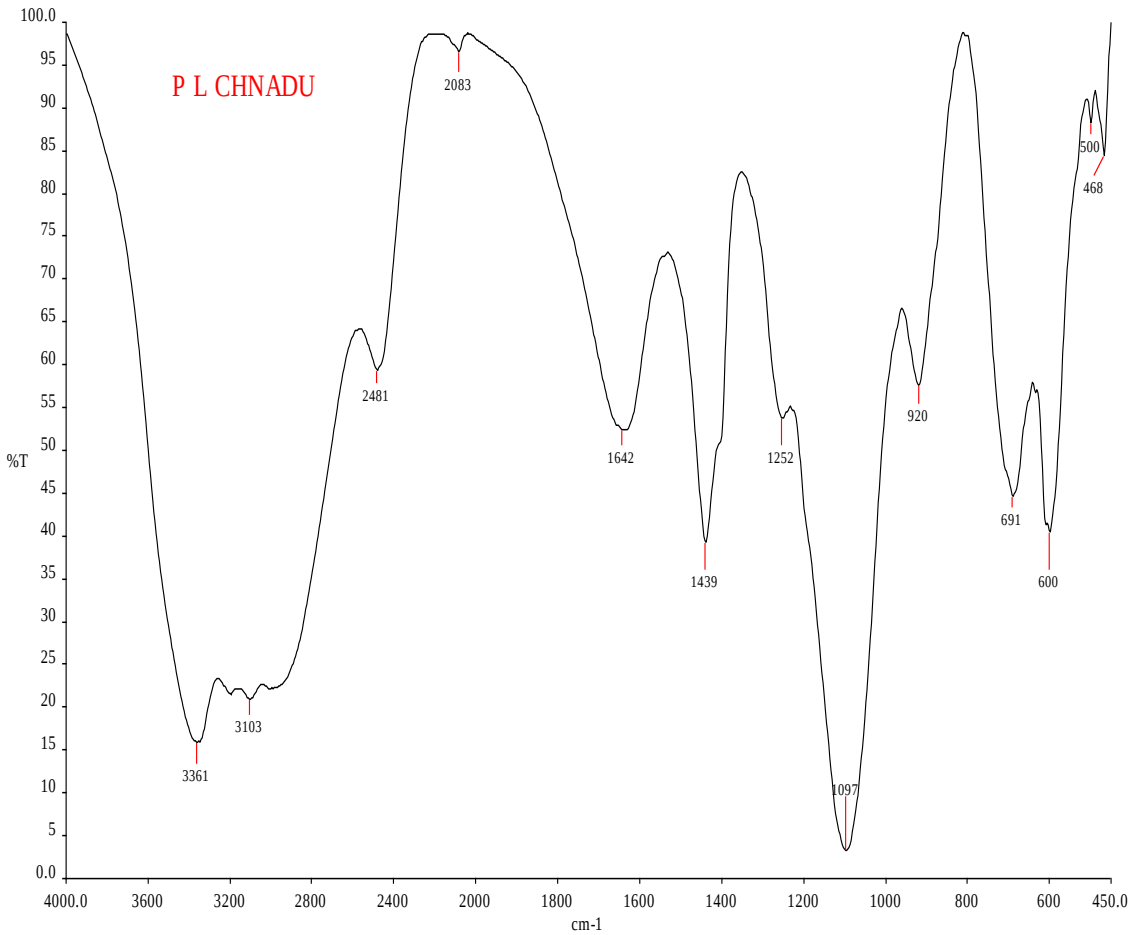


Table No.3 Interpretation

Absorption peak cm-1	Stretch	Functional Group
3500 - 3200 (s,b)	O-H stretch, H – bonded	Alcohols, phenols
3300 – 2500 (m)	O – H stretch	Carboxylic acids
1680 – 1640 (m)	-C = C -	Alkenes
1500 – 1400 (m)	C –C stretch (in – ring)	Aromatics
1320 – 1000 (s)	C – 0 stretch	Alcohols, carboxylic acids, esters, ethers
1000 – 650 (s)	=C – H bend	Alkynes
349 – 673	C-Br Stretch	Alkyl Halides

Interpretation:

In FTIR the wave numbers between 4000cm^{-1}-400cm^{-1} is known as functional group area.<400cm^{-1} wave number is known as finger print area. The corresponding absorption frequency by FTIR shows the presence of alcohols, phenols, alkenes, amines, aliphatic amines, alkyl halides.

It confirms that *Padiga linga chenduram* constitutes Alcoholic,Phenolic,, Alkanes, Aromatics, Carboxylic acids , Esters, Ethers Alkyne, Alkyl halides, 1^0,2^0 amines.

- OH group has higher potential towards inhibitory activity against microorganisms.
- Alcoholic group of act as antimicrobial activity.

phenols

- Phenols are also known as carbolic acid
- Phenolic groups has anti microbial, anti oxidant activities.
- Phenols as disinfectant prevent the development organisms.

Alkyl Halides:

- These are group of compounds derived from alkanes containing one or more halogens.
- Some are used as anesthetics and antiseptic agents. Some of them are used in medicine for the elimination of hook worms.

Alkynes:

- Alkyne is an unsaturated hydrocarbon containing one carbon –carbon triple bond,Some alkyne compounds which are physiologically acceptable salts are used as MCH antagonist .

Esters:

- Esters are organic compound formed when an acid combine with an alcohol and release water.

Ether:

- Ether is a class of organic compound characterized by an oxygen atom bonded to two alkyl or aryl group.

Carboxylic acid

- Carboxylic acid is class of organic compounds that are characterized by the presence of carboxyl group (-cooh) in them.
- They make up a series of fatty acids which are extremely good for human health. The omega-6 and omega-3 are essential fatty acids which are not produced by the body. They help in maintaining the cell membrane and control nutrient use along with metabolism.

ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy):

The drug *(Padiga linga chenduram)* sample was analysed by the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to detect the trace elements and other elements quantitatively. The result of ICP-OES is given on the

Table No: 4.

Sample ID	Elements Symbol	Wavelength(nm)	Concentration (A)P
1	Al	396.152	BDL
2	As	188.979	BDL
3	Cd	228.802	BDL
4	Cu	327.393	BDL
5	Hg	253.652	3.012mg/L
6	Ni	231.604	BDL
7	Pb	220.353	BDL
8	P	213.617	06.301mg/L
9	S	180.731	71.304mg/L

BDL:Below Detectable Limit (Normal – 1 ppm)

1% = 10000ppm,

1ppm = 1/1000000 or 1ppm = 0.0001%

The 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:

The result indicate that the formulation is extremely safe as it contains heavy metals within specified limits.

ICP-OES reveals high concentration of Hg(3.012mg/L), P(06.301mg/L), S(71.304mg/L), in Padiga linga chenduram. It also has physiologically important minerals like . In *Padiga ling a chenduram,* the heavy metals like As, Hg, Cu, Cd, Pb and trace element like Ni, Al, were below detectable level.The mercury found in small concentration, This reveals the safety of the drug.

- The main ingredient of the Test drug is (lingam) mercurial compounds, but the final product shows below detection limit of the minerals. This results shows Below detection limit(BDL) of As(arsenic) ,Hg(Mercury), Cd (Cadmium), Pb(Lead), Ni(Nikkal), Al(Aluminium), Cu (Copper).It is evident that the effectiveness of siddha medicine has been proved by the modern scientific way.
- **Sulfur** is used to kill parasites (organisms that live on other organisms) such as fleas and ticks.
- Sulfur is used to make proteins and nucleic acids, such as DNA. It also occurs in many essential enzymes. Enzymes are chemicals that make chemical reactions occur more quickly in cells. Humans usually have no problem getting enough sulfur in their diets.
- **Inorganic phosphorus** is critical for numerous normal physiologic functions including skeletal development, mineral metabolism, energy transfer through mitochondrial metabolism, cell membrane phospholipid content and function, cell signaling, and even platelet aggregation.
- Total adult body stores of phosphorus is approximately 700 g, of which 85% is contained in bone in the form of hydroxyapatite [$(Ca)_{10}(PO4)_6(OH)_2$]. Of the remaining, 14% is intracellular, and only 1% is extracellular

Effect of Acute Toxicity (14 Days) of *PADIGALINGACHENDURAM*

RESULT Table –1 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-5 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-6 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-7 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

INTERPRETATION

From acute toxicity study it was observed that the administration of ***PADIGALINGACHENDURAM*** at a dose of 2000mg/kg, to a rats. From acute toxicity study it was observed that the administration of ***PADIGALINGACHENDURAM*** 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 ***PADIGALINGACHENDURAM*** is 2000 mg/kg.

Discussion

PADIGALINGACHENDURAM 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 ***PADIGALINGACHENDURAM*** 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 ***PADIGALINGACHENDURAM*** on the growth rate. Body weight change in drug treated animals was found normal.

Summary & conclusion:

Summary:

The present study was conducted to know single dose toxicity of ***PADIGALINGACHENDURAM*** on female wister rats. The study was conducted using 15 female Wister rats. The female animals were selected for study of 8- 12 weeks old with weight range of within ± 20 % of mean body weight at the time of randomisation. The groups were numbered as group I, II, III, IV and V and dose with 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 2000mg/kg of ***PADIGALINGACHENDURAM***. The drug was administered by oral route single time and observed for 14 days. Daily the animals were observed for clinical signs and mortality. Body weight of all animals was recorded once in a week.

There were no physical and behavioral changes observed in albino mice of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats during 14 days.

Body weight of all animals did not reveal any significant change as compared to vehicle control group.

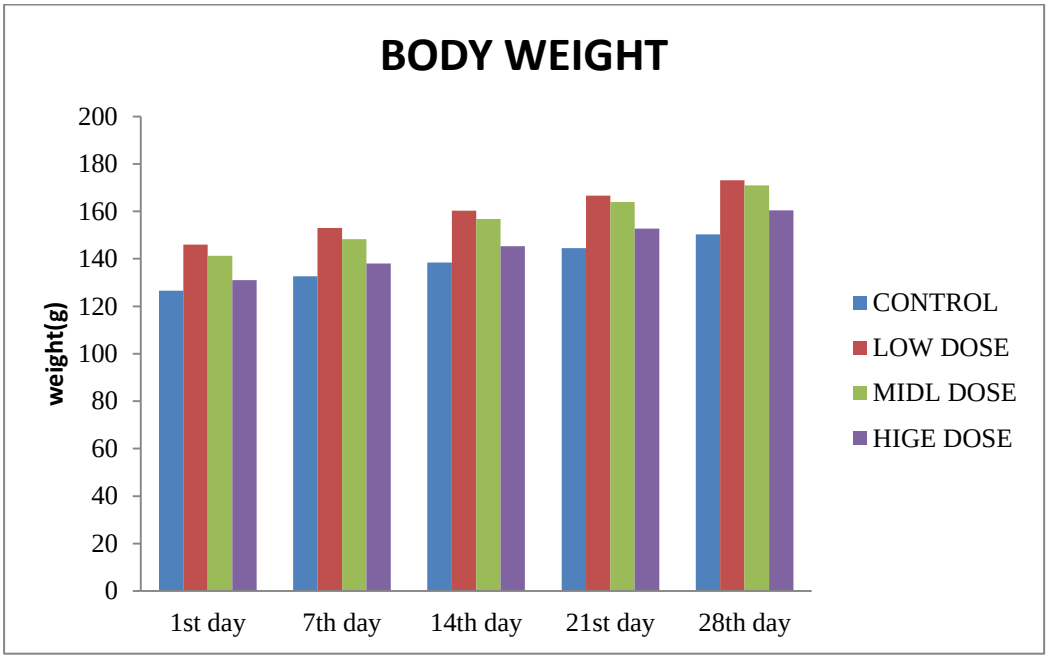
Food consumption of all group animals was normal.

Mortality was not observed in any treatment groups.

Conclusion:

The study shows that ***PADIGALINGACHENDURAM*** did not produce any toxic effect at dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats. So No-Observed-Adverse-Effect-Level (NOAEL) of ***PADIGALINGACHENDURAM*** is 2000 mg/kg.

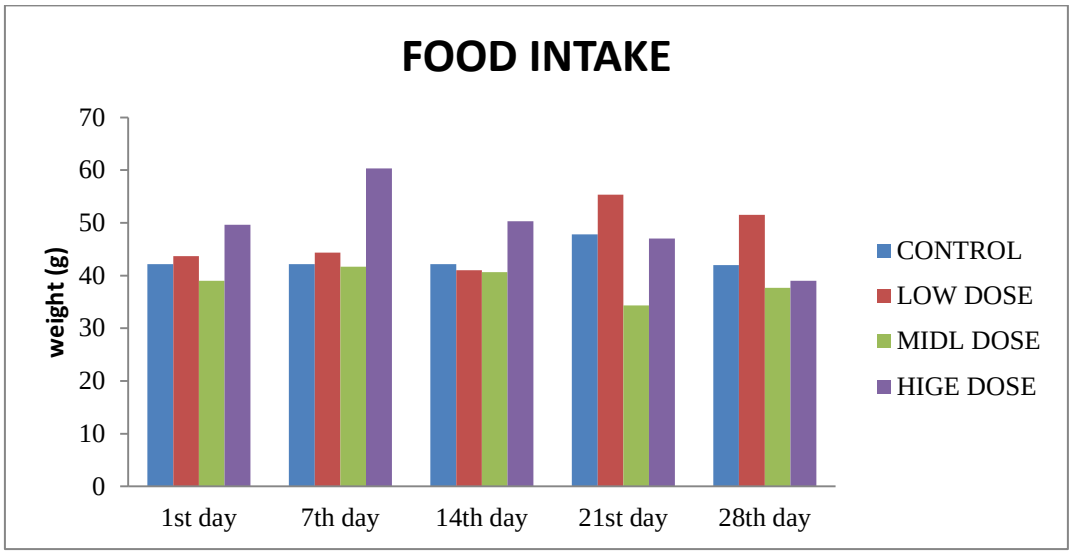
EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF PADIGA LINGA CHENDURAM ON BODY WEIGHT (PHYSICAL PARAMETER)



EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF PADIGA LINGA CHENDURAM ON FOOD INTAKE IN gms

GROUP	CONTROL	LOW DOSE	MIDEL DOSE	HIGE DOSE
1st day	126.667± 1.42984	146± 3.21455	141.833±6.88194	131±5.4037
7th day	132.667± 1.42984	153± 3.35659	148.833± 6.28976	138.167± 5.08866
14th day	138.5± 1.40831	160.333± 2.87131	156.833±5.67108	145.333±5.1099
21st day	144.5± 1.40831	166.667± 2.69155	164±5.39135	152.833±4.23018
28th day	150.333± 1.52023	173.167±2.80971	171±5.15752	160.5±3.63089

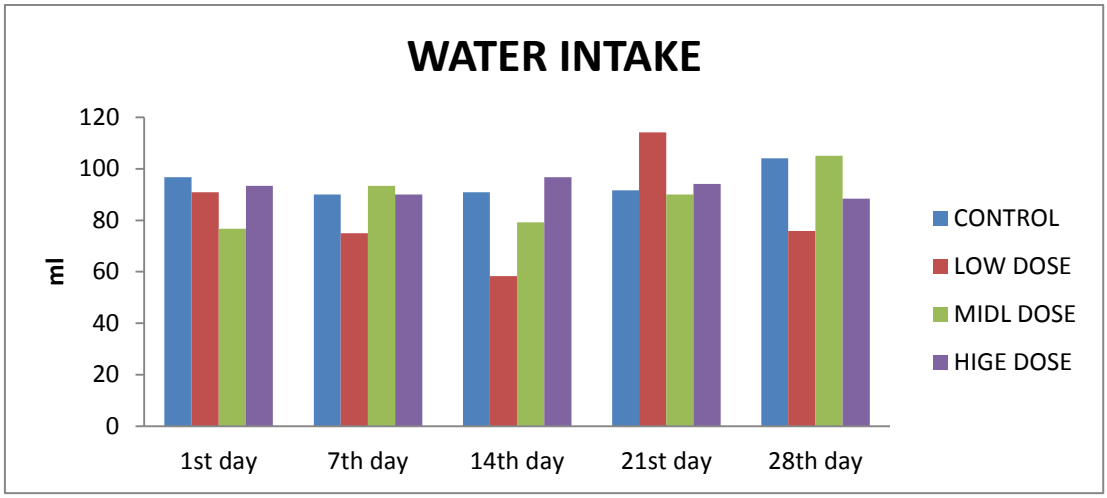
Values arae expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.



EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF PADIGA LINGA CHENDURAM ON WATER INTAKE IN ML

GROUP	CONTROL	LOW DOSE	MIDEL DOSE	HIGE DOSE
1st day	96.6667± 12.8236	90.8333± 12.0704	76.6667± 5.72519	93.3333± 14.1225
7th day	90±10.5672	75± 13.8444	93.3333±5.57773	90± 15.864
14th day	90.8333± 14.1667	58.3333± 6.91215	79.1667±8.20738	96.6667± 6.14636
21st day	91.6667±12.4944	114.167±10.6001	90±11.4746	94.1667± 11.2114
28th day	104.167±11.4322	75.8333±6.50854	105±12.6491	88.3333± 19.5221

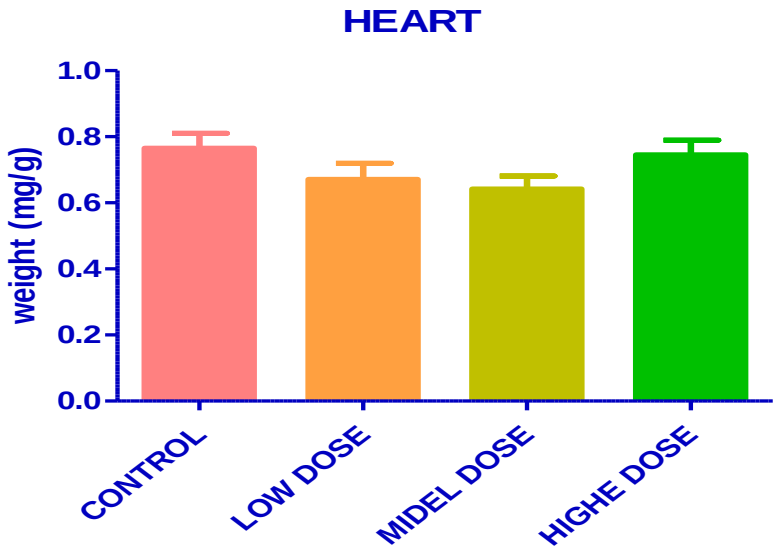
Values arae expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

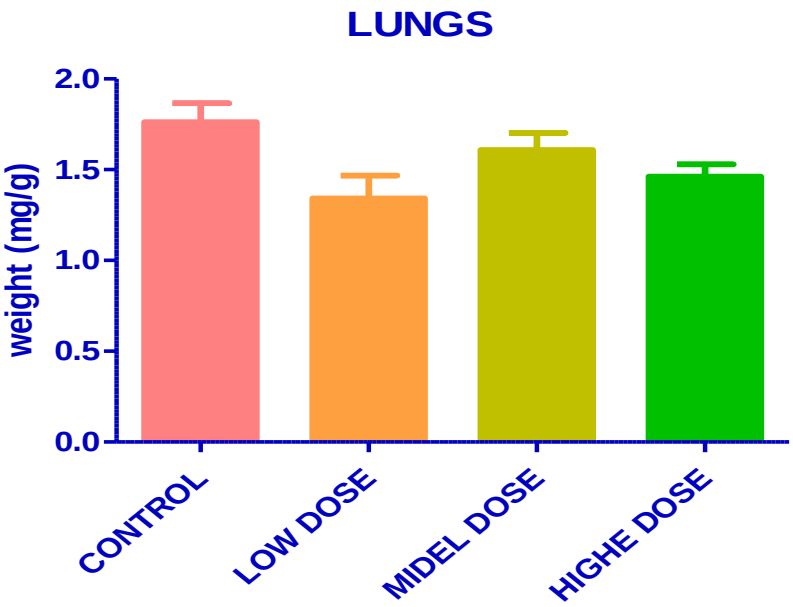


EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF PADIGALINGACHENDURAM ON ORGAN WEIGHT IN gms

GROUP		CONTROL	LOW DOSE	MIDEL DOSE	HIGE DOSE
BRAIN		1.464±0.1571	1.284±0.03355	1.549±0.05597	1.561±0.05641
HEART		0.765±0.04535	0.6713±0.04844	0.6423±0.03867	0.745±0.04461
LIVER		6.532±0.3209	5.77±0.3204	5.628±0.6815	5.836±0.5197
LUNGS		1.762±0.1037	1.342±0.1259	1.608±0.09397	1.463±0.06589
TESTIS		2.399±0.07283	2.533±0.1789	1.482±0.139	1.812±0.2581
UTRES		0.671±0.04838	0.4863±0.02489	0.501±0.03415	0.4547±0.04978
KIDNEY	L	0.7457±0.03883	0.6657±0.08325	0.5657±0.01725	0.4907±0.03692
	R	0.7267±0.05376	0.5863±0.02497	0.5303±0.03167	0.4667±0.03365

Values arae 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.






LIVER
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4
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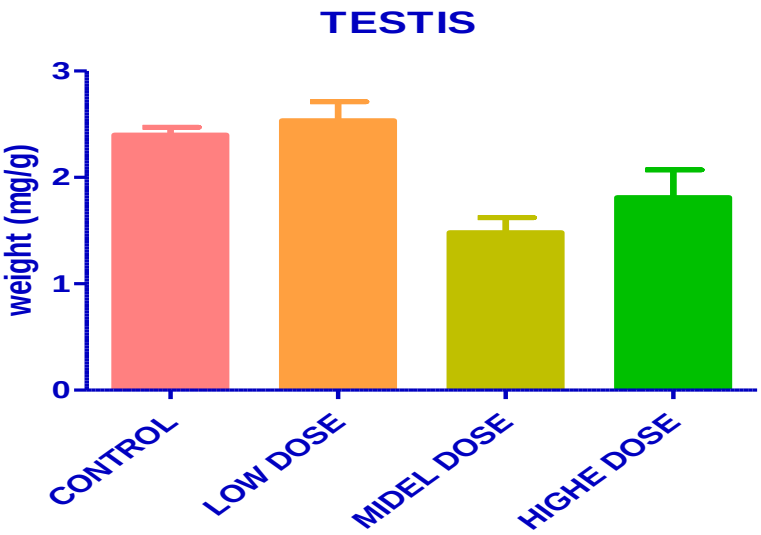
LOW DOSE
MIDEL DOSE
HIGHE DOSE


TESTIS
weight (mg/g)
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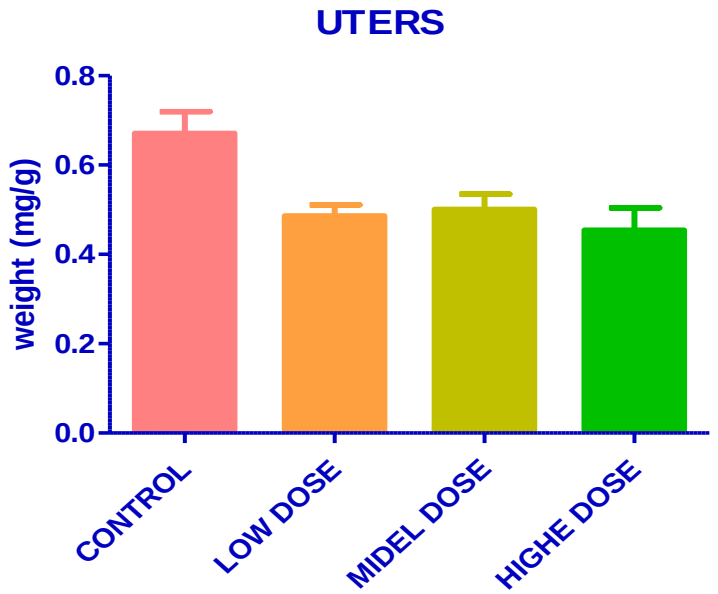
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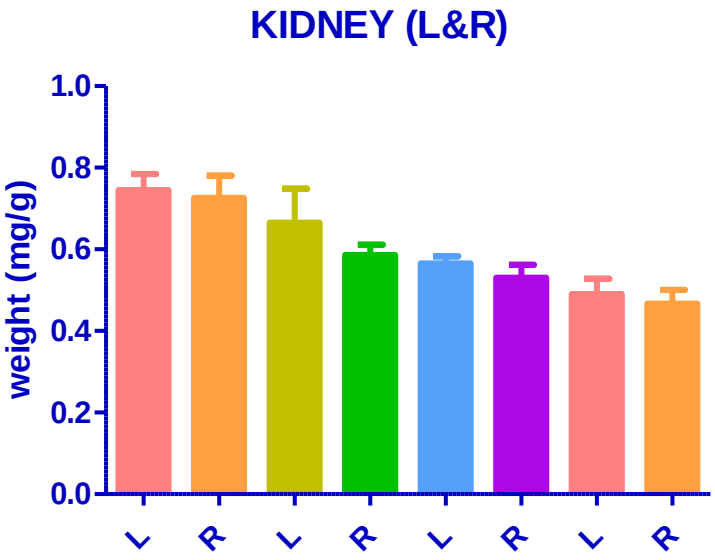
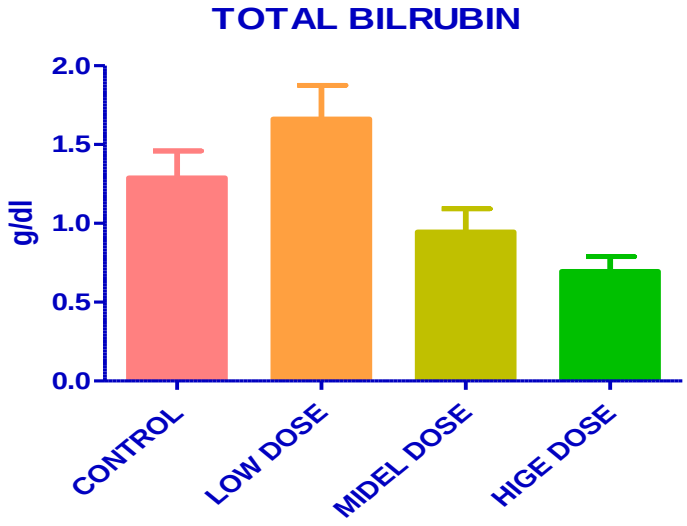


EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF PADIGALINGACHENDURAM ON BIOCHEMICAL PARAMETERS

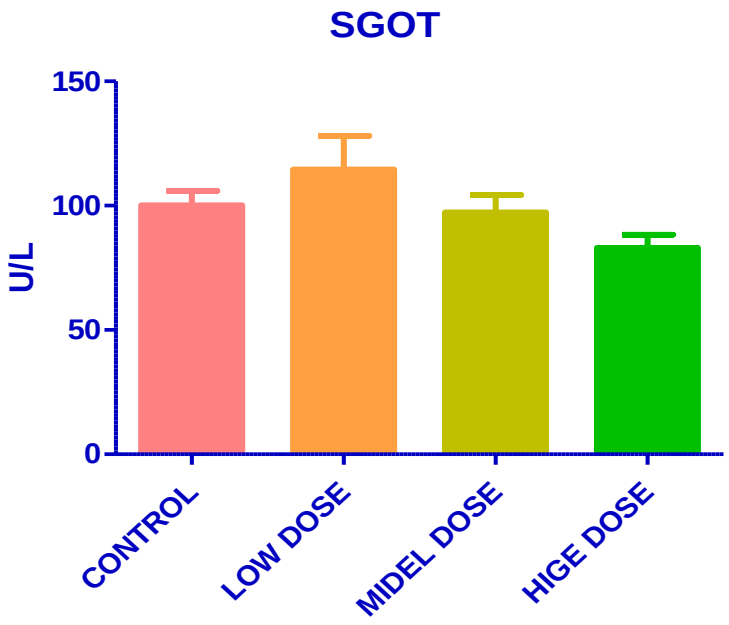
GROUP	CONTROL	LOW DOSE	MIDEL DOSE	HIGE DOSE
TOTAL BILURBINE (g/ml)	1.28867±0.169848	1.66333±0.212315	0.946667±0.145182	0.696667±0.0924362

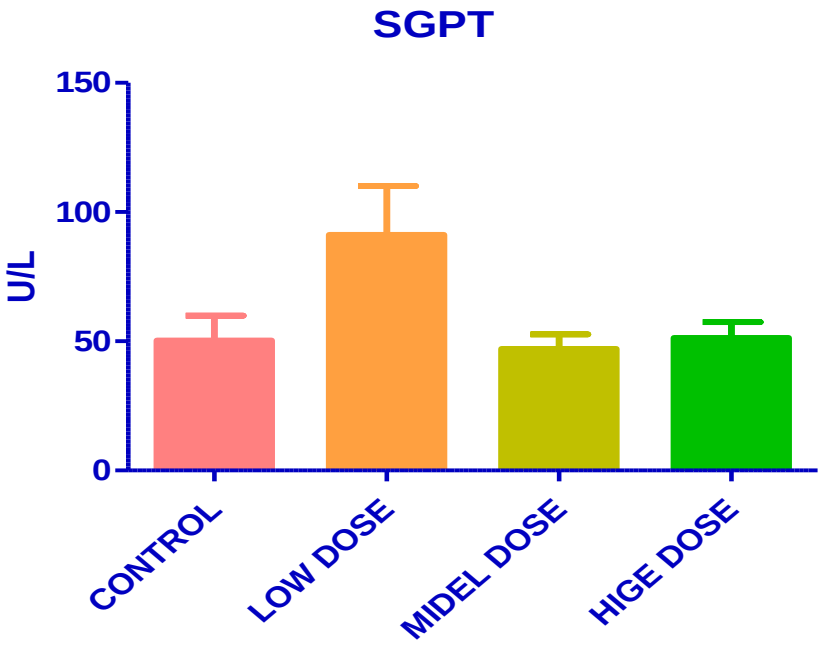


EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF PADIGALINGACHENDURAM ON BIOCHEMICAL PARAMETERS

GROUP	CONTROL	LOW DOSE	MIDEL DOSE	HIGE DOSE
SGOT (U/L)	100.133±5.78917	114.6±13.4538	97.3667±6.97838	83.1±5.19711
SGPT (U/L)	50.37±9.515	91.2±18.82	47±5.677	51.3±6.087
ALP (U/L)	184.3±41.41	161.9±14.66	156.2±37.88	180.4±16.87

Values arae 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.




SGPT
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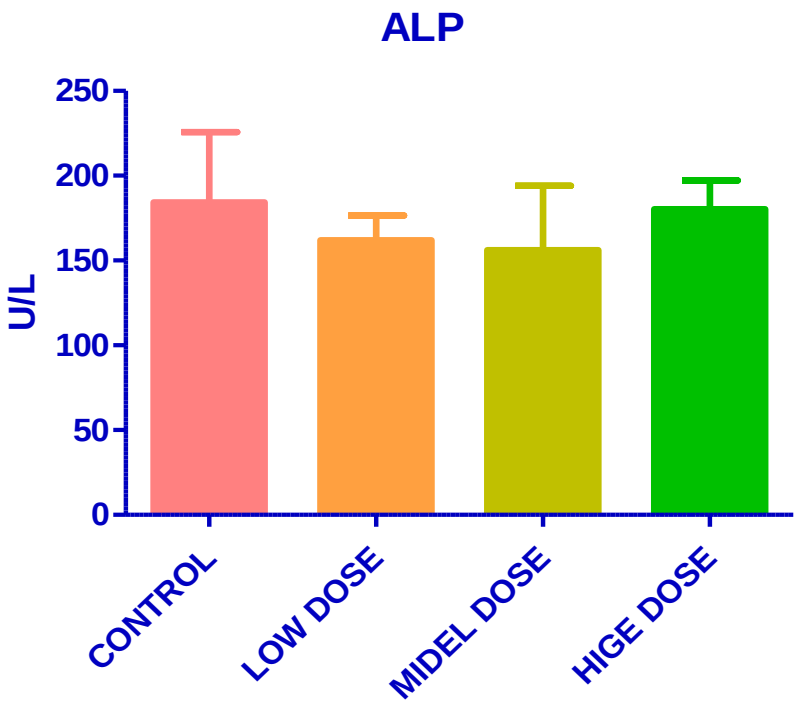
CONTROL
LOW DOSE
MIDEL DOSE
HIGE DOSE


ALP
250
200
150
100
50
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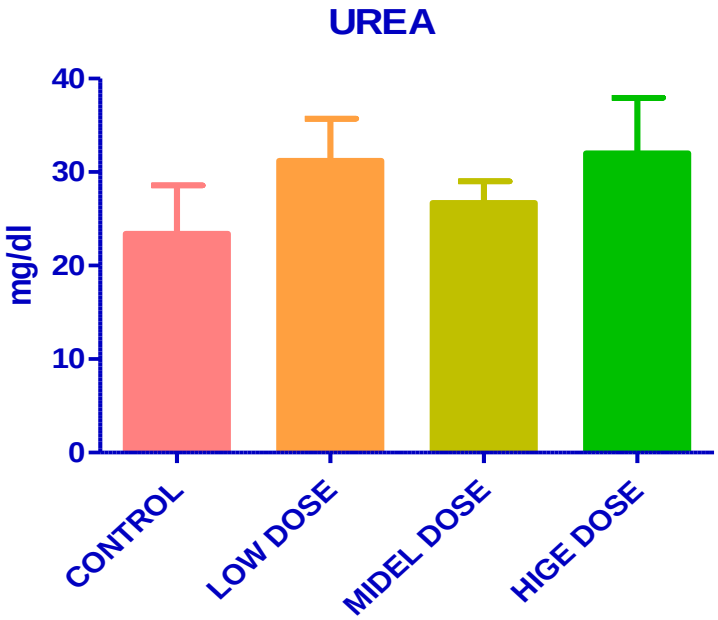
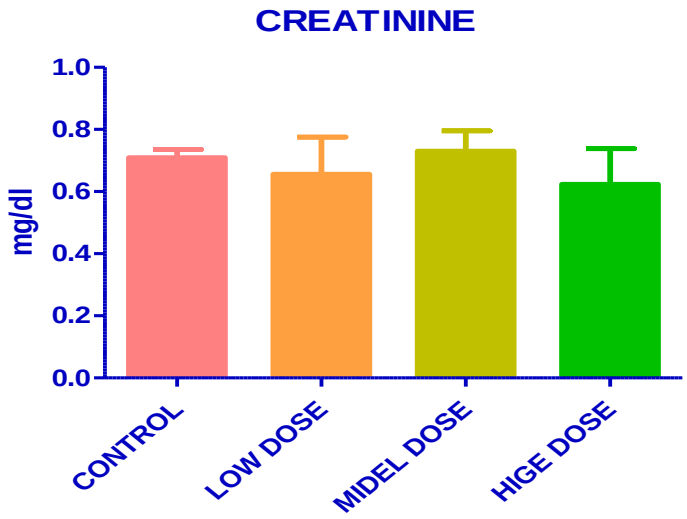
U/L
CONTROL
LOW DOSE
MIDEL DOSE
HIGE DOSE

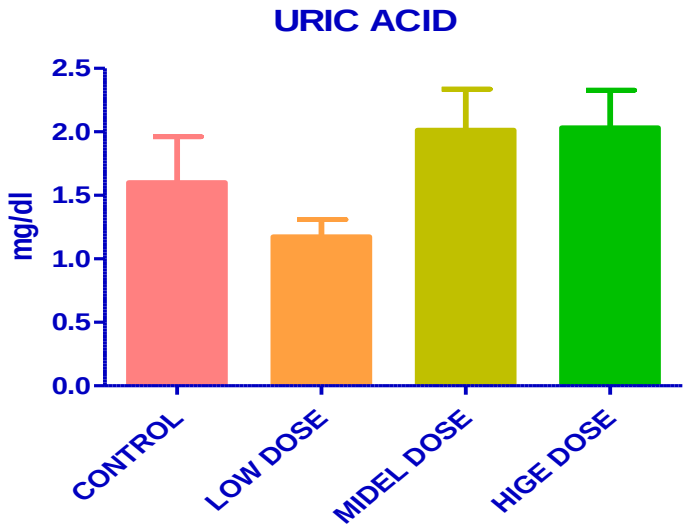
EFFECT OF SUB-ACUTE DOSES (28 DAYS) OF PADIGA LINGA CHENDURAM ON BIOCHEMICAL PARAMETERS

GROUP	CONTROL	LOW DOSE	MIDEL DOSE	HIGE DOSE
UREA (mg/ml)	23.43±5.143	31.23±4.471	26.7±2.309	32±5.965
URIC ACID (mg/ml)	0.71±0.02517	0.6567±0.1184	0.73±0.06506	0.6233±0.1146
CREATININE (mg/ml)	1.6±0.3612	1.173±0.1369	2.013±0.3238	2.033±0.2958

Values arae 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.







INTERPRETATION:

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 Table-1 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.4 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 (Table 4) 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 days 29 and recorded in Table 2 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.

Histopathology:

In histopathological examination, revealed normal architecture in comparison with control and treated animal.

DISCUSSION:

1) All the animals from control and all the treated dose groups up to 500 mg/kg survived throughout the dosing period of 28 days.

2) No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.

3) Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.

4) Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days

5) Haematological analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment.

6) Biochemical analysis conducted at the end of the dosing period on day 29 no abnormalities attributable to the treatment.

7) Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.

8) Histopathological examination revealed normal architecture in comparison with control and treated animal.

8.0 SUMMARY AND CONCLUSION:

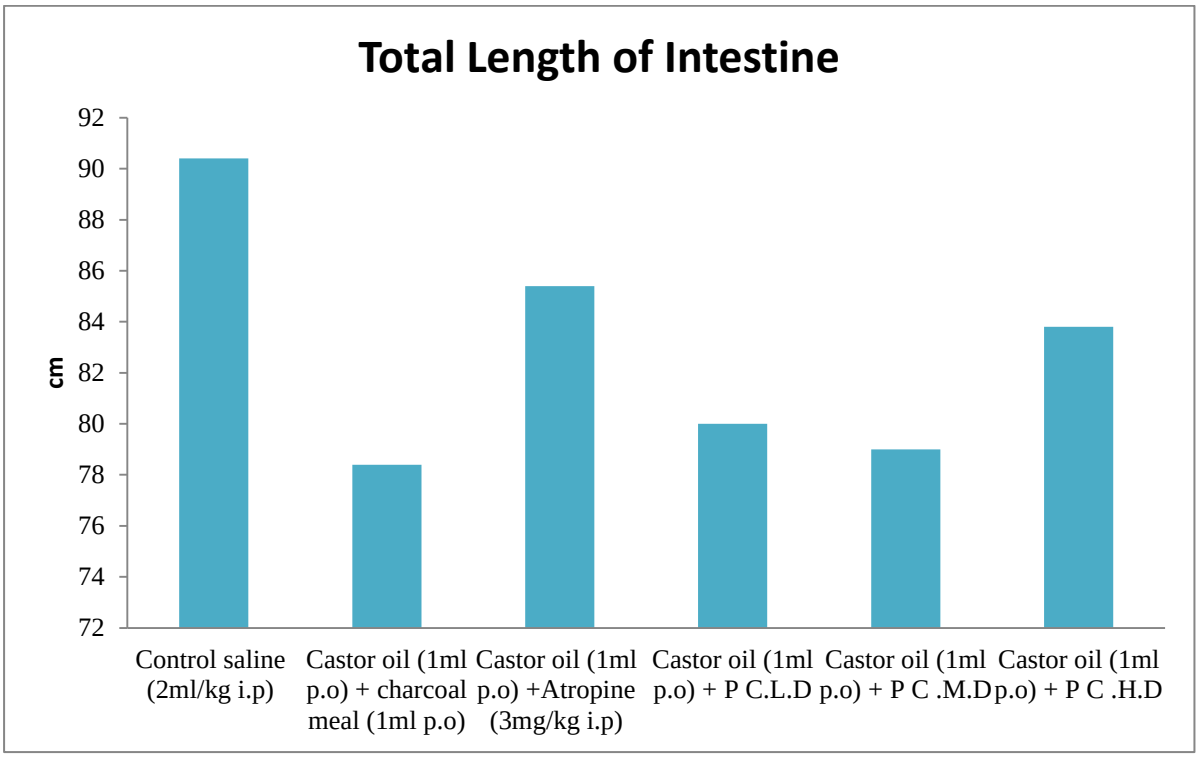
In conclusion ***PADIGALINGACHENDURAM*** can be considered safe, as it did not cause either any lethality or adverse changes with general behavior of rats and also there were no observable detrimental effects **(100 to 300 mg/kg body weight)** over a period of 28 days. Our results have demonstrated that the ***PADIGALINGACHENDURAM*** is relatively safe when administered orally in rats.

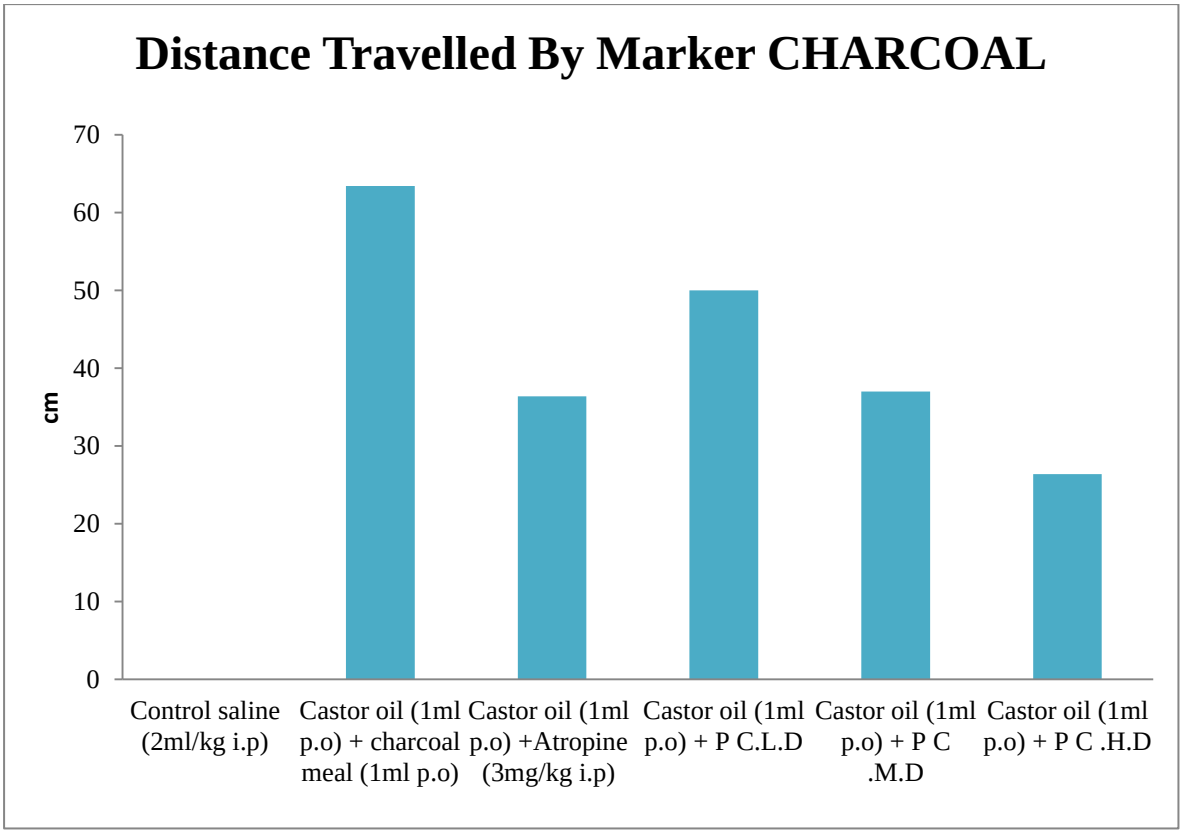
ANTI-DIARRHOAL ACTIVITY

EFFECT OF *PADIGA LINGA CHENDURAM* ON CASTOR OIL - INDUCED SMALL INTESTINAL TRANSIT IN RATS

GROUP	Total Length of Intestine	Distance Travelled By Marker CHARCOAL	**%Intestinal Transit**
Control saline (2ml/kg i.p)	90.4±1.3267	0±0	-----
Castor oil (1ml p.o) +charcoal meal (1ml p.o)	78.4±3.9192	63.4±2.99333	29.86 %
Castor oil (1ml p.o) +Atropine (3mg/kg i.p)	85.4±2.2271	36.4±2.27156	53.57 %
Castor oil (1ml p.o) + P C-4.6mg/kg	80±5.1865	50±2.91548	41.45 %
Castor oil (1ml p.o) + P C – 23 mg/kg	79±5.6921	37±3.97492	53.75 %
Castor oil (1ml p.o) + P C – 46mg/kg	83.8±2.9052	26.4±2.92575	66.58 %

Values arae 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.





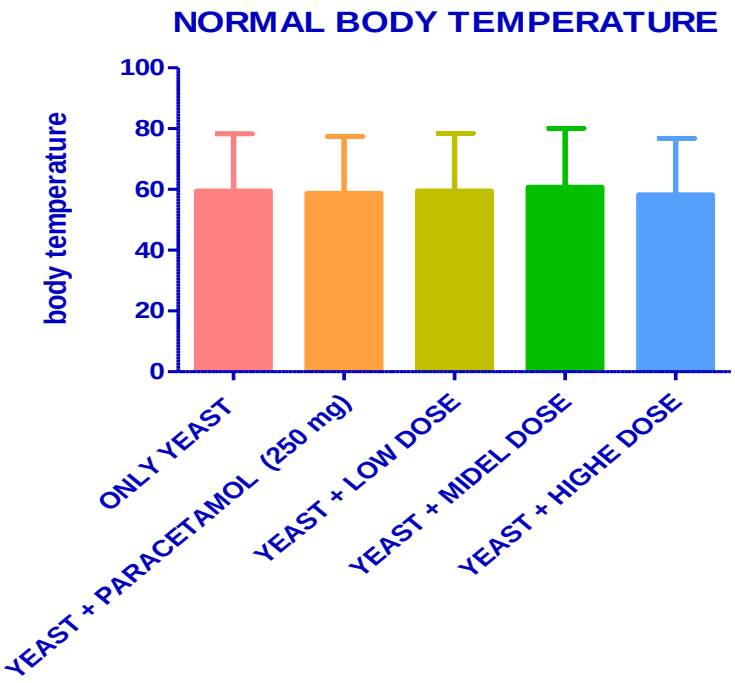
INTERPRETATION:

For diarrhoea atrophine is astaudurd drug while compare with padiga linga chenduram its shous significant activity for diarrhoea in high dosage.

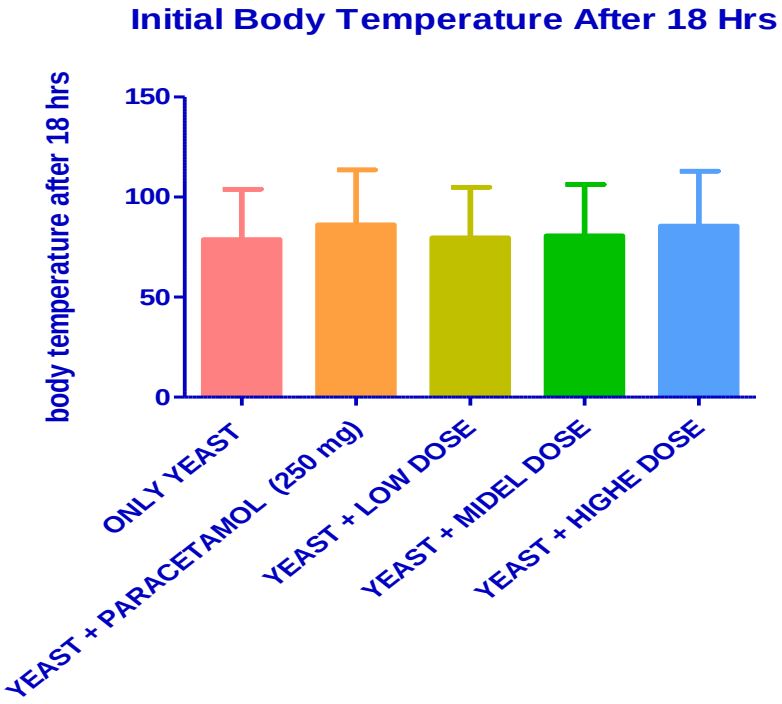
ANTI-PYRETIC

EFFECT OF *PADIGALINGACHENDURAM* ON BREWER'S YEAST INDUCED PYREXIA IN RATS

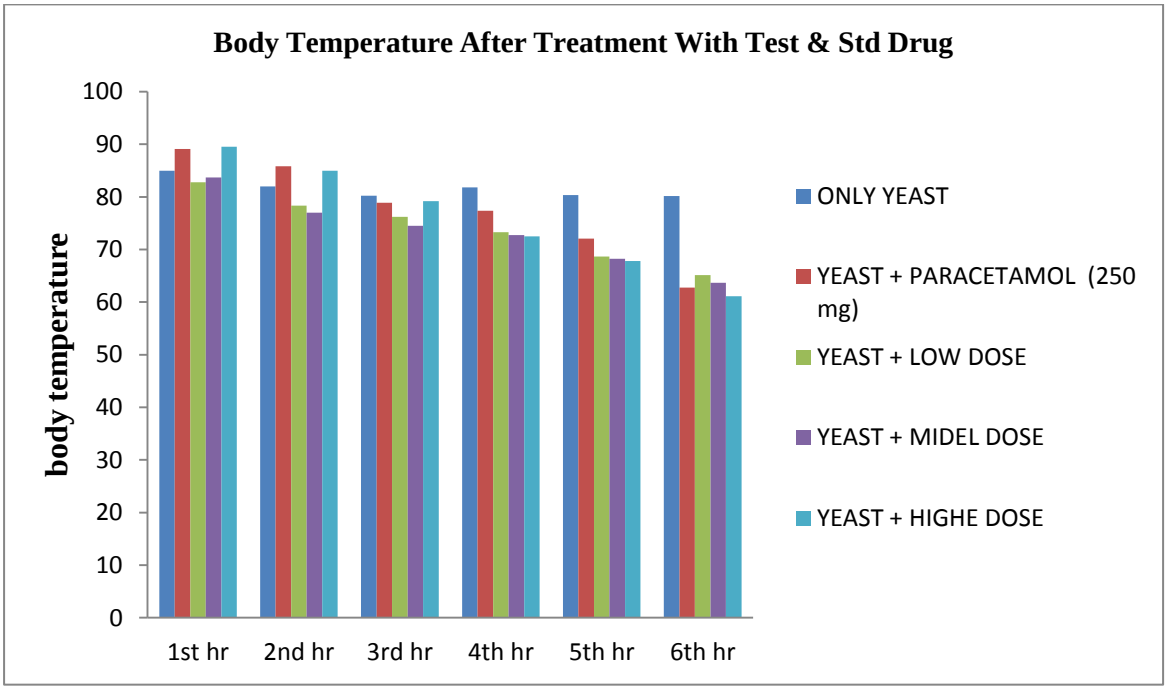
GPS	ONLY YEAST	YEAST + PARACETAMOL (250 mg)	YEAST + P.C 4.6mg/Kg	YEAST + P.C 23mg/Kg	YEAST + P.C 46mg/Kg
Initial Rectal Temperature 0 hr	59.5± 18.818	58.8333± 18.6341	59.5± 18.9275	60.8333± 19.3088	58.3333± 18.462
Rectal Temperature After induction with Yeast 18hr	78.8667± 25.0261	86.1833± 27.4031	79.6833± 25.2823	80.6667± 25.6461	85.5333± 27.4643
Rectal Temperature After Treated with Test and Standard 1st hr	84.9667± 26.9434	89.1± 28.3479	82.7833± 26.2959	83.6833± 26.6027	89.5167± 28.7086
2nd hr	81.9833± 25.9936	85.8167± 27.1763	78.3667± 24.8392	76.9833± 24.4682	84.9833± 27.1018
3rd hr	80.2333± 25.3779	78.8833± 25.006	76.2333± 24.1443	74.5± 23.6678	79.17± 25.2165
4th hr	81.8± 25.8891	77.35± 24.4648	73.3167± 23.234	72.7667± 23.1295	72.4667± 23.1825
5th hr	80.35± 25.4619	72.05± 22.805	68.6333± 21.7313	68.2167± 21.6276	67.8167± 21.5417
6th hr	80.1333± 25.3455	62.75± 19.8828	65.1± 20.5934	63.65± 20.1531	61.1± 19.3378



INITIAL BODY TEMPERATURE AFTER 18 HRS



EFFECT OF *PADIGALINGACHENDURAM* ON BREWER'S YEAST INDUCED PYREXIA IN RATS



INTERPRETATION:

Thus padiga linga chenduram its shows significant activity for fever.

ANTI-SPASMODIC ACTIVITY

Dose Response Relationship Observations of Acetylcholine

Si.No	Concentration/dose	Acetylcholine
		Response (cm)
1	0.1 ml	3.2 cm
2	0.2 ml	3.8 cm
3	0.4 ml	4.9 cm
4	0.8 ml	5.5 cm
5	1.6 ml	5.9 cm

Dose Response Relationship Observations of Atropine

Si.No	Concentration/dose	atropine
		Response (cm)
1	0.1 ml	-
2	0.2 ml	-
3	0.4 ml	-
4	0.8 ml	-
5	1.6 ml	-

Dose Response Relationship Observations of Acetylcholine and **padiga linga chenduram**

Si.No	Concentration/dose	Acetylcholine + **PC**
		Response (cm)
1	0.1 ml +0.1 ml	2.8 cm
2	0.2 ml +0.2 ml	3.2 cm
3	0.4 ml +0.4 ml	3.7 cm
4	0.8 ml +0.8 ml	4.2cm
5	1.6 ml + 1.6 ml	4.9 cm

Comparative Dose Response of Ach and Ach followed by **padigalingachenduram**

Si No	Treatment	Dose(ml)	response	% of response
1	Acetylcholine	0.1 ml	3.2 cm	
2		0.2 ml	3.8 cm	
3		0.4 ml	4.9 cm	
4		0.8 ml	5.5 cm	
5		1.6 ml	5.9 cm	
6	Acetylcholine + padigalingachenduram	0.1 ml +0.1 ml	2.8 cm	12.5 %
7		0.2 ml +0.2 ml	3.2 cm	15.78 %
8		0.4 ml +0.4 ml	3.7 cm	24.48 %
9		0.8 ml +0.8 ml	4.2 cm	23.63 %
10		1.6 ml + 1.6 ml	4.9 cm	16.94 %

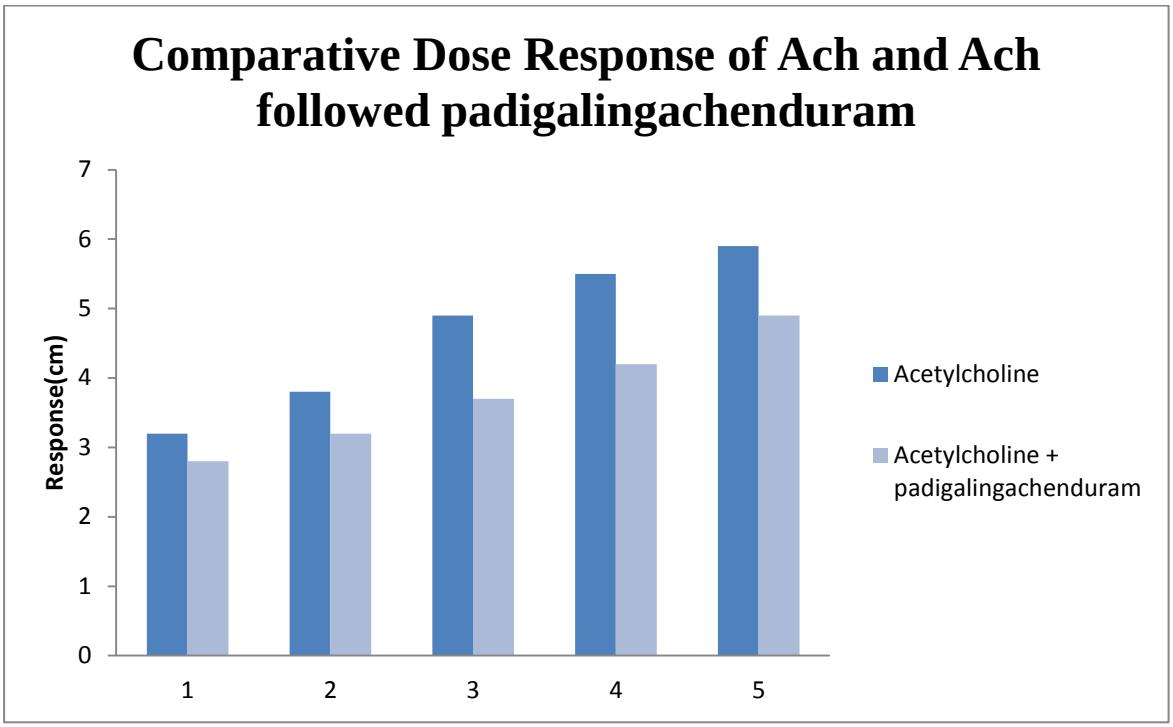
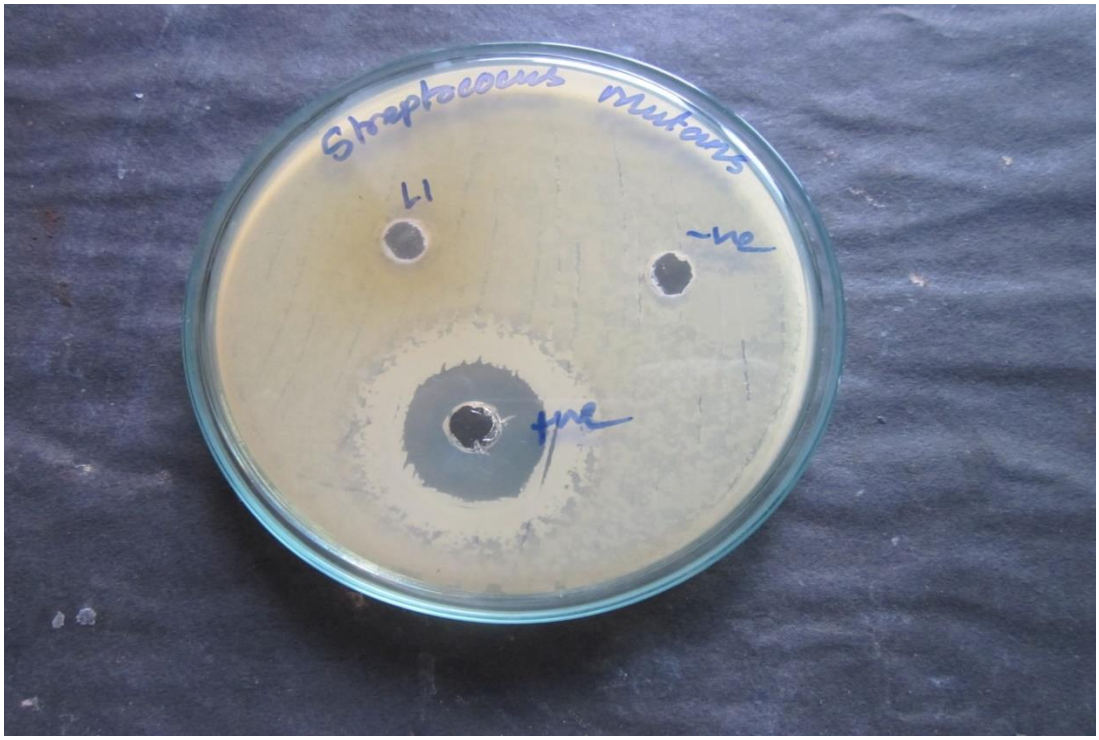


Fig: Comparative dose response relationship of Acetylcholine and **Padiga linga chenduram** on excised rat ileum.

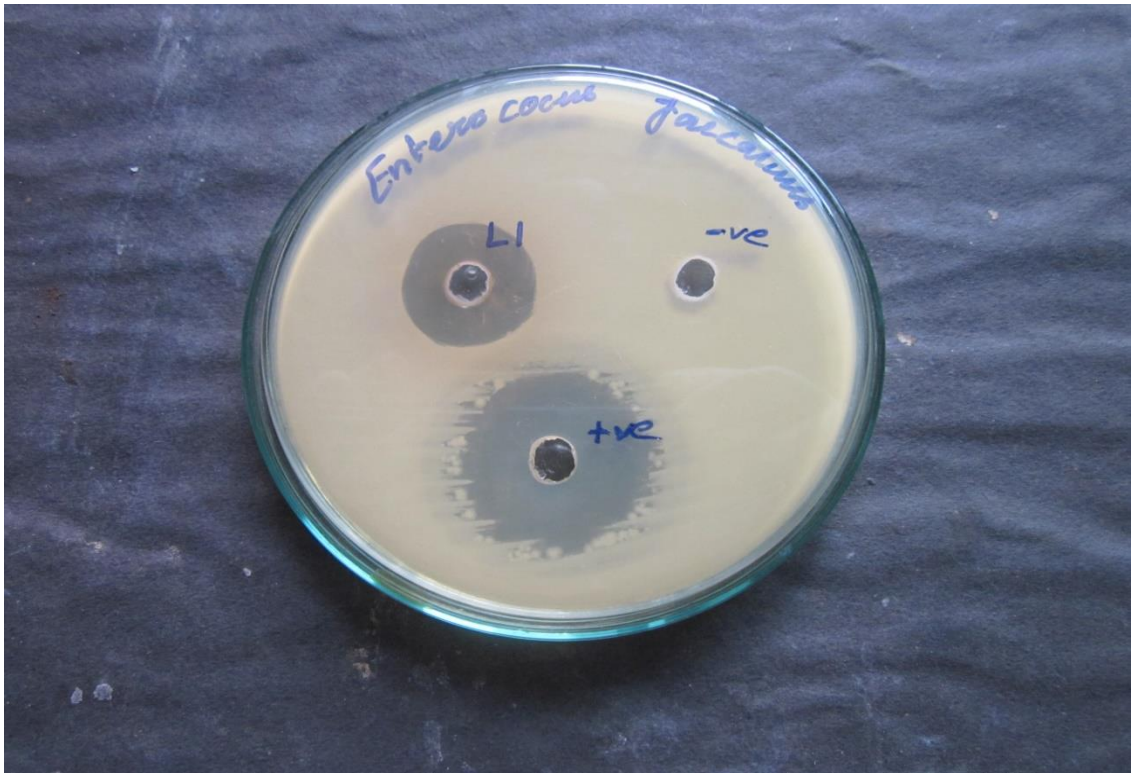
INTERPRETATION:

This revealed that **padiga linga chenduram** possess a high degree of spasmolytic (anti-spasmodic) activity by blocking cholinergic receptors.

MICROBIOLOGY RESULT

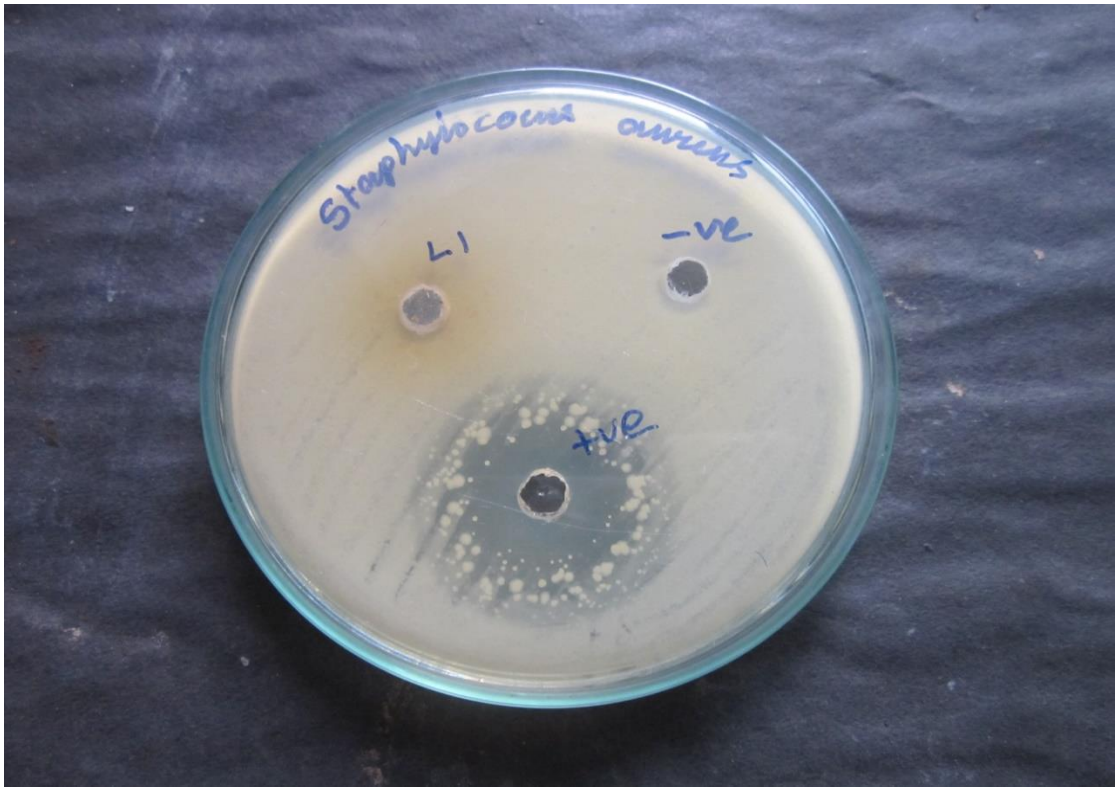


Streptococcus mutant

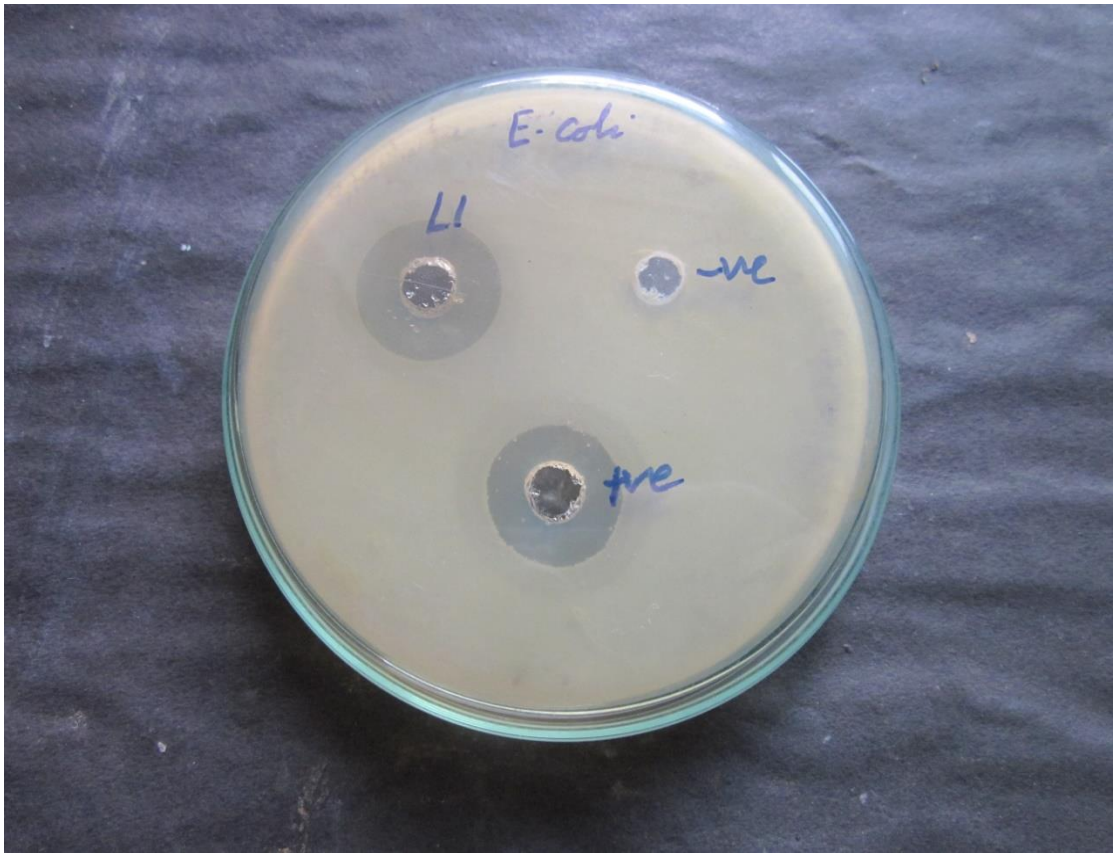


Enterococcus faecalis

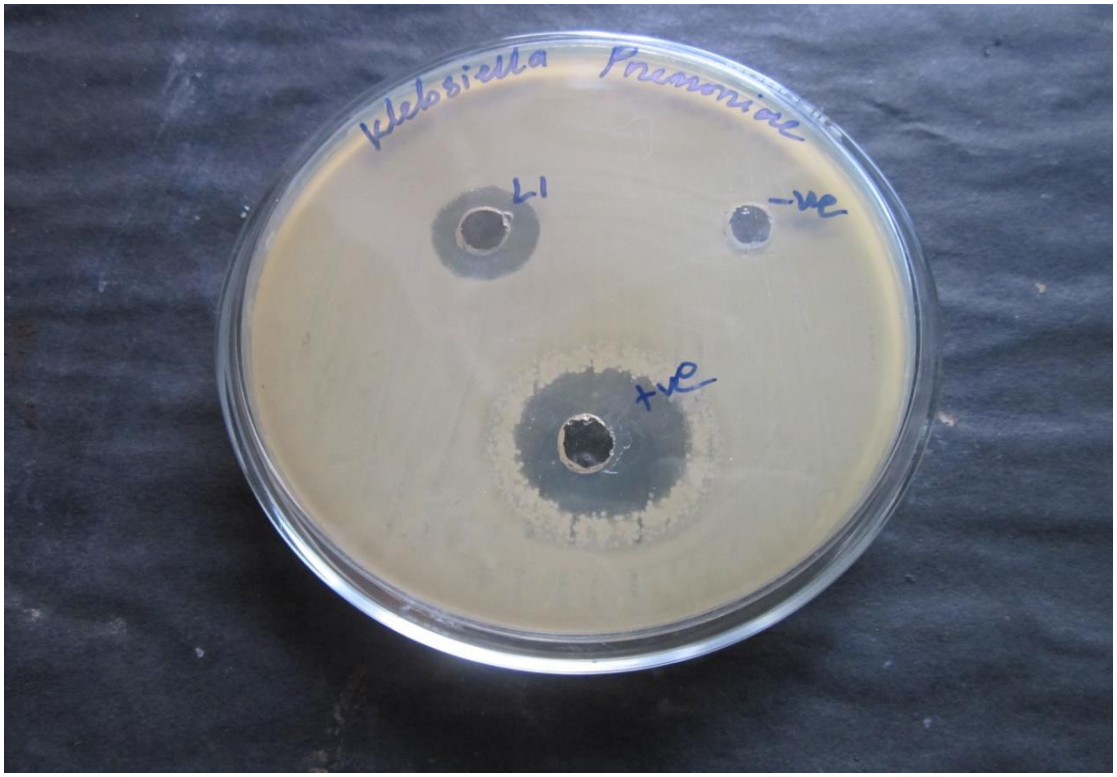
Pseudomonas aeruginosa



Staphylococcus aureus



E.coli



Klebsiella pnemoniae

BACTERIA	SAMPLE	POSITIVE CONTROL (Streptomycin)	NEGATIVE CONTROL
E.coli	21mm	18mm	NZ
Klebsiella pnemoniae	17mm	21mm	NZ
Staphylococcus aureus	NZ	35mm	NZ
Streptococcus mutant	NZ	21mm	NZ
Enterococcus faecalis	21mm	30mm	NZ
Pseudomonas aeruginosa	16mm	24mm	NZ

NZ – NO ZONE FORMATION

* - Diameter of inhibition zone less than 4 mm is represented as "–".

14 mm-Low Sensitivity:15mm-Moderate:above 16mm-Highly sensitive

INTERPRETATION:

1.Escherchia.coli- Highly sensitive in 4 mg/disc

2.Enterococcus faecalis- Highly sensitive in 4 mg/disc

3. Streptococcus mutant - Low sensitive in 4 mg/disc

4. Pseudomonas aeruginosa- Highly sensitive in 4mg/disc.

5.Staphylococcusaureus-low sensitive in 4mg/disc

6. Klebsiella pnemoniae - Highly sensitive in 4mg/disc

6.SUMMARY

- The trial drug *padiga linga chenduram* was selected from the text *ANUBOGA VAIDYA DEVA RAGASHIYAM*, Page: 407, Edition-1 for the validation of safety, efficacy and its potency in Anti-diarrhoeal activity, Anti-pyretic and anti-spasmodic activities.

- The raw drugs *padikaram, lingam* were purchased from authorized drug store in Nagarcoil at kanyakumari district. The raw material was identified and authenticated by experts of PG Gunapadam department, Govt.Siddha Medical College, Palayamkottai, Tamilnadu.

- The trial drug was prepared as per the standard operating procedure. Various literary collections of *Siddha* and Modern science about the ingredients of the drug supported the fact that *padiga linga chenduram* has anti-diarrhoeal activity.

- The taste of patikaram in Astringent. This Astringent acts with *pitha veerium* according to pancha poothic theory astringent taste is a combination of primitive elements prithivi and vayu. The prithivi pootha have a character of produce dryness. These three characters very mach usefulmto treat the diarrhoea by making formed stools.

- As per the original character of lingam can cure diarrhoea, as said in the quote **"பேதிசுரஞசந்நி பெருவிரணநீரொடு"……** when both patikaram and lingam get combines means there is a good result for diarrhoea. By this "PADIKALINGAM CHENDURAM" that treat "DIARRHOEA"

- The results of Physico-chemical analysis confirm that the chenduram had fine particle with pink colour and low moisture content and did not contain free metals or microbes.Thus ensuring its safety and efficacy.

- Chemical analysis revealed the presence of sulfate, chloride, ferrous iron, ferric iron.

- **Sulfur** is just now becoming more widely appreciated as a really critical nutrient, without which many other things don't work properly, and most people are probably not getting enough sulfur from their diet anymore. For example, sulfur plays a critical role in detoxification, and also in inflammatory conditions. For detoxification, sulfur is part of one of the most important antioxidants that your body produces: glutathione. Without sulfur, glutathione cannot work.

- sulfur plays an important role in the production of glutathione—one of the most important antioxidants that your body produces. Glutathione also serves important functions for detoxification. Without sulfur, glutathione cannot work. So, while not an antioxidant by itself, part of MSM's action is to improve your body's ability to make its own antioxidants.
- A person who does not get enough sulfur in his or her diet develops certain health problems. These include itchy and flaking skin and improper development of hair and nails. Under very unusual conditions, a lack of sulfur can lead to death. Such conditions would be very rare, however. Eggs and meats are especially rich in sulfur.

chloride

- Some chloride-containing minerals include the chlorides of sodium (halite or NaCl), potassium (sylvite or KCl), and magnesium (bischofite), hydrated $MgCl_2$. Called serum chloride, the concentration of chloride in the blood is regulated by the kidneys. A chloride ion is a structural component of some proteins, e.g., it is present in the amylase enzyme. So chloride is maintend by fluid and electrolyte balance help of the diarrhoeal

- In industrial application, iron(III) chloride is used insewage treatment and drinking water production
- SEM photographs show that the particles are spherical in shapes and sizes are in the range from 0.5 micron to 1microns .The size of the particles enables better absorption.Hence the bioavailability is increased and even a minimal dose yields better results.

FT-IR

- Ester C is non acidic and gentle on the stomach
- Phosphorous directly clears up indigestion, constipation, diarrhea, and generally tones up the digestive system for regular, healthy bowel movements. This increases the health of the digestive system, as well as that of the kidneys, since the toxins are being eliminated from the body, rather than recycling through the kidneys and stressing that system.

- The acute study shows that ***PADIGALINGACHENDURAM*** did not produce any toxic effect at dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 2000mg/kg to rats. So No-Observed-Adverse-Effect-Level (NOAEL) of ***PADIGALINGACHENDURAM*** is 2000 mg/kg.

Acute Result:

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

SUB-ACUTE RESULTS:

1) All the animals from control and all the treated dose groups up to 500 mg/kg survived throughout the dosing period of 28 days.

2) No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.

3) Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.

4) Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days

5) Haematological analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment.

6) Biochemical analysis conducted at the end of the dosing period on day 29 no abnormalities attributable to the treatment.

7) Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.

8) Histopathological examination revealed normal architecture in comparison with control and treated animal.

- Anti – diarrhoeal activity is carried out in rats by using charcoal meal method. The test drug padiga linga chenduram has significant Anti-diarrhoeal activity, which reveals the effectiveness of padiga linga chenduram in treating diarrhoea.

- Anti-pyretic activity of test drug padiga linga chenduram carried out by using yeast-induced method. The drug padiga linga chenduram showed potent anti-pyretic activity.

- From all observations and results obtained for the present study it was concluded that padiga linga chenduram.,(Ghaneri) exhibits promising anti-spasmodic activity. Also when compared with a standard anti-spasmodic agent (atropine), it was found that padiga linga chenduram has comparatively less potent spasmolytic activity than atropine.

- Anti-microbial study of the test drug padiga linga chenduram carried out by disc diffusion method. It observed that padiga linga chenduram is sensitive to Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella pnemoniae, E.coli. These padiga linga chenduram has significant anti-bacterial activity.

- Thus the drug padiga linga chenduram found to be effective and safe for kazhitchal on basis of Anti-diarrhoeal, Anti-pyretic, Antispasmodic and Anti-microbial action.

7. CONCLUSION

Padiga linga chenduram was selected for the elaborate study of its efficacy on Kazhitchal (diarrhoea).

From the literature review, Physico-chemical, Pharmacological, Microbiological, Biochemical, Instrumental analysis, It has been concluded that Padiga linga chenduram has got a good Anti-diarrhoeal, Anti-pyretic, and Antispasmodic hence effective for diarrhoea.

8. FUTURE SCOPE OF STUDY

Having made up of nano particles, *padiga linga chenduram* holds extraordinary promise for the prevention and treatment of *kazhitchal*. This study directs future research in separating the compound responsible for this activity. It will open a new era of Receptor Targeted Treatment in the management of *kazhitchal*. Thus the ancient wisdom of *Siddhars* will remains as one important source of future medicine and therapeutics.

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GOVT. SIDDHA MEDICAL COLLEGE

PALAYAMKOTTAI

SCREENING COMMITTEE

Candidate Reg. No: 3213120009

Department: GUNAPADAM

This is to certify that the dissertation topic PADIGHA LINGHA CHENDURAM ANTI DIARROEAL, ANTI PYRETIC & ANTISPASMODIC ACTIVITES has been approved by the screening committee.

Branch	Department	Name	Signature
1	Pothu Maruthuvam	Dr.S.Aathi Narayanan MD(S).,	
2	Gunapadam	Dr.M.Ravi Chandran MD(S).	
3	Sirappu Maruthuvam	Dr.S.Kaniraja MD(S).,	1.7.15
4	Kuzhanthai Maruthuvam	Dr.D.K.Soundararajan MD(S).,	1/7/15
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F.No.GSMC/5676/P&D/Res/IEC/2014 Date: 16.07.2015

CERTIFICATE OF APPROVAL

Address of Ethical Committee	Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu, India. Pincode: 627002.
Principal Investigator	Dr.F.Sophia anthuvanth MD(s)- II year, Department of Gunapadam , Reg. No.: 321312009.
Guide	Dr. M.RAVICHANDRAN MD(s), ph.D H.O.D., Department of Gunapadam Govt. Siddha Medical College and Hospital, Palayamkottai, Tirunelveli District.
Dissertation Topic	PRE CLINICAL EVALUATION OF SIDDHA MINERAL FORMULATION **'PADIGALINGA CHENDURAM'** FOR ITS ANTI DIARRHOEAL, ANTI PYRETIC & ANTI SPASMODIC ACTIVITIES.
Documents Filed	1) Protocol
Clinical / Non Clinical Trial Protocol	Non Clinical Trial Protocol
Informed Consent Document	NA
Any other Documents	NA
Date of IEC Approval & its Number	GSMC-II-IEC/2015-Br.-II/09/16.07.2015

We approve the trial to be conducted in its presented form.

The Institutional Ethical Committee expects to be informed about the process report to be submitted to the IEC atleast annually of the study, any changes in the protocol and submission of final report.

16/7/2015

Chairman
(Prof. Dr. M. Logamanian)

16/7/15

Member Secretary
(Prof. Dr. S. Soundararajan)

AUTHENTICATION CERTIFICATE

Date: 08.07.2015

Certified that the following raw drugs, mineral submitted for identification by Dr.F.Sophia anthuvanth, PG Department Of Gunapadam, Govt.Siddha Medical College, Palayamkottai, are identified as

1. Lingam (Cinnabar)
2. Padikaram (Alum)

Dr.A.KINGSLY,M.D(S).,
Lecturer,
H.O.D Department of Gunapadam
Govt.Siddha medical college, Palayamkottai.

KMCH COLLEGE OF PHARMACY – COIMBATORE

IAEC - CERTIFICATE

This is to certificate that the project title PRECLINICAL STUDY OF SIDDHA DRUG PADIGALINGA CHENDURAM FOR ITS ANTI-DIARRHOEAL, ANTISPASMODIC, ANTI PYRETIC ACTIVITIES has been approved by the IAEC/KMCRET/MD(S)/16/2016-2017.

Name of the Chairman / Member Secretary IAEC:

Signature with Date

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Chairman / Member Secretary of IAEC

Name of the CPCSEA Nominee

CPCSEA Nominee
(V. VINAYAK KULKARNI)

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by office).

The Tamil Nadu Dr.M.G.R. Medical University

#69, Anna salai, Guindy, Chennai-600 032.

This certificate is awarded to

Dr./~~Mr.~~/~~Ms.~~ F. SOPHIA ANTHUVANTH

for participating as ~~Resource~~ ~~Person~~ / Delegate in the Sixteenth Workshop on

"Research Methodology & Biostatistics"

for AYUSH Post Graduates & Researchers

Organised by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University from 04.08.2014 to 08.08.2014

Dr. N. KABILAN M.D. (Siddha)
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23rd January 2016

CERTIFICATE

Certified that Prof ./Dr./Mr./Ms./Mrs.....F. SOPHIA ANTHUVANTH.....

*Participated in the National Level Seminar on "**Analytical and Bio - Analytical Techniques in Plant Research**" as a Delegate and Presented a Poster Entitled.....*

organised by S.A. Raja Pharmacy College and sponsored by Association of Pharmaceutical Teachers of India, Tamil Nadu Branch.

Dr.S.A. Jacob Raja	**Dr.N.Balakrishnan**	**Dr.R.Xavier Arulappa**	**Dr.K. L.Senthil Kumar**
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GOVERNMENT SIDDHA MEDICAL COLLEGE
PALAYAMKOTTAI

CME

Conducted by

POST GRADUATE DEPARTMENT OF POTHU MARUTHUVAM

Certificate

This is to certify that Dr. SOPHIA ANTHUVANTH. F

of GUNAPADAM *department has participated in the*

Continue Medical Education Programme on ***Renal Diseases***

held at ***Government Siddha Medical College Palayamkottai*** *On 08 - 06 - 2016 Wednesday*

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