

SAFETY AND PHARMACOLOGICAL PROFILE OF THIRIKADUGU DRAVAGAM

The dissertation Submitted by

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Introduction

1. INTRODUCTION

The siddha system date backs to 5000 B.C Profounded by saint Agathiyar and his clan numbering 18 such siddhars. This system is an amalgam of Tamil literature, culture, tradition, Health and many such living forms of 64 types.

The National Institute of Siddha was started on 30.9.2004 and the department of Gunapadam is also functioning on this date. So Many researches have been carried out for animal experimentation and drug profile.

The Present Study About The Safety and Pharmacological Profile Of *Thirikadugu Dravagam* On Animal Models.

The experiment was permitted by IAEC approval constituted by KK College of pharmacy, Gerugambakkam, and the study is met with the relevant OECD Guidelines & CPCSEA norms also.

The standard operative procedure was followed and the drug labeling was done.

Review of the Ingridients, Chemical analysis, Phytochemical analysis, shows sufficient Molecules of therapeutic benefits for the safety and efficacy of the drug *Thirikadugu Dravagam*.

Analytical study includes, Organoleptic character, physical characterization, Elemental analysis, Gas chromatography(GC-MS) all these shows the drug is safe.

Therapeutic dose of *Thirikadugu dravagam* is 5ml Bid. As per OECD guideline three dose levels were selected for the study. They are low dose (X), mid dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose (10ml) and the body surface area of the rat (0.018). i.e X dose is 0.9 ml/kg, 5X dose is 5ml /kg,10X dose 10 ml/kg.

The routine Hematological and Biochemical parameters were observed for sub-acute and sub-chronic study. The was no significant change in Hematological and Biochemical parameters. Histopathology studies did not reveal any abnormal macroscopic changes.

The pharmacological Activity bronchodilator, Anti-histaminic and Anti-inflammatory activity shows satisfied results. There is no toxicity on administration.

Thirikadugu dravagam seems to be safe on animal experimentation, Studies may carried out further for clinical evaluation.

Aim and objectives

Materials
And
Methods

3. MATERIALS AND METHOD

STANDARD OPEATIVE PROCEDURE:

Drug selection

In this research work *Thirikadu dravagam*, a poly herbal formulation have been selected as a trial drug from the Siddha literature “*Yaakobu vaithiya chinthamani 700*” Third edition-1992, Written by : S.P.Ramachandiran

Ingredients

<i>CHUKKU</i>	- <i>Zinngiber officinale</i>	8 palam (280 g)
<i>MILAGU</i>	- <i>Piper nigrum</i>	8 palam (280 g)
<i>THIPILLI</i>	- <i>Piper longum</i>	8 palam (280 g)
<i>ELAM</i>	- <i>Eletaria cardamomum</i>	8 palam (280 g)
<i>SEERAGAM</i>	- <i>Cuminum cyminum</i>	8 palam (280 g)
<i>OMAM</i>	- <i>Carum copticum</i>	8 palam (280)

Source of collection

Drugs were purchased from authorized raw drug shop in Parris, Chennai.

Identification and Authentication of the drug

All the plant materials were identified and authenticated by the Botanist, Department of Gunapadam, National Institute of Siddha.

Dravagam

Dravagam or Theeneer is the distilled essence, which contains the volatile constituents of the drugs used in the preparation, in a medium of water and is equivalent to “aqua” or water of the western pharmacopoeia.

PURIFICATION PROCESS

Purification of *chukku*

Chukku was purified by removing the outer layer and soaking in the limestone water for three hours.

Purification of *Milagu*

Milagu was purified by soaking it in *karisalai* juice for 7 times, then it was dried.

Purification of *Thipili*

Thipili was purified by soaking it in *kodiveli* decoction for three hours and then it was dried.

Purification of *Elam*

Elam was purified by little fried.

Purification of *seeragam*

Seeragam was purified by soaking it in limestone water for three hours and then it was dried.

Purification of *Omam*

Omam was purified by soaking it in limestone water for three hours and then it was fried

INGREDIENTS OF THIRIKADUGU DRAVAGAM

Chukku



Milagu



Thipilli



Elam



Seeragam



Omam



Method of preparation

Raw drugs were purified as per Siddha text, and make them into coarse powder individually, and mixed together and transfer the powder into vessel.

Purified powder was soaked in water for three days and then subjected into distillation process by vaaluka yanthiram.

Finally dravagam was collected and stored in a container.



Labeling

Name of the preparation : *THIRIKADUGU DRAVAGAM*

Date of preparation : 8/6/2015 and 15/2/2016

Dose : 5ml

Adjuvant / vehicle : water

Indication : *kasam, kshayam, ushnavaya*

Date of expiry : 1 year from date of preparation

Therapeutic administration of drug

Form of drug : Liquid form

Route of administration : oral

Review of literature

Review of literature

Siddha aspect

4.1 REVIEW OF LITERATURE

Gunapadam review

சுக்கு

வேறு பெயர்கள் : அருக்கன், அதகன் ,ஆர்த்ரகம், உபகுல்லம், உலர்ந்த இஞ்சி, சௌபர்ணம், சுண்டி சொண்டி, கடுபத்திரம்.

பயன்படும் உறுப்பு : கிழங்கு

சுவை : கார்ப்பு

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை

வெப்பமுண்டாக்கி

பசித்தீதுண்டி

அகட்டுவாய்வகற்றி

பொதுகுணம்

சூலைமந்தம் நெஞ்செரிப்பு,தோடமப் பம்மழலை

மூலம் இரைப்பிருமல் மூக்குநீர்- வாலகம்

தோடமதி சாரத் தொடர்வாத குன்மநீர்த்

தோட ஆமம்போக்குஞ் சுக்கு.

(அகத்தியர் குணவாகடம்)

சுக்கினால் செரியாமை, இரைப்பு, இருமல், வயிற்றுப்பிசம் போம்.

சுக்கு சேரும் மருந்துகள்

வார்பத்தியாதி சூரணம்

சிவதை சூரணம்

மிளகு

வேறு பெயர்கள் : மலையாளி, சருமபந்தம், கோளகம், குறுமிளகு

பயன்படும் உறுப்பு : விதை,கொடி

சுவை : கைப்பு,கார்ப்பு

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை

அகட்டுவாய்வகற்றி

முறைவெப்பகற்றி

வெப்பமுண்டாக்கி

பொதுகுணம்

சீதசுரம் பாண்டு சிலேதம் கிராணி குன்மம்

வாதம் அருசி பித்தம், மாமூலம்- ஓதுசந்நி

யாசமபஸ் மாரம் அடன் மேகம் காசமிவை

நாசங் கறி மிளகினால்

(அகத்தியர் குணவாகடம்)

மிளகினால் இருமல், கபக்குற்றங்கள் போம்.

மிளகு சேரும் மருந்துகள்

சுவாசகுடோரி சூரணம்

கண்டங்கத்தரி சூரணம்

திப்பிலி

வேறு பெயர்கள் : ஆர்கதி, காமன், குடாரி, கோழையறுக்கி, கணம், அம்பு, ஆதிமருந்து

பயன்படும் உறுப்பு : காய், அரிசி

சுவை : இனிப்பு

தன்மை : தட்பம்

பிரிவு : இனிப்பு

செய்கை

அகட்டுவாய்வகற்றி

வெப்பமுண்டாக்கி

பொதுகுணம்

இருமல் குன்மம் இரைப்பு கயப்பிணி

ஈளை பாண்டு சந்யாசம் அரோசகம்

பொருமல் ஊதை சிரப்பிணி மூர்ச்சைநோய்

(அகத்தியர் குணவாகடம்)

ஈளை, இருமல், இரைப்பு, கடுமையான ஐயப்பிணிகளை அகற்றி, உடற்கு வன்மையை அளித்திடும்.

திப்பிலி சேரும் மருந்துகள்

திப்பிலியாதி சுரணம்

சுரபுங்க வில்வாத இளகம்

ஏலம்

வேறு பெயர்கள் : ஆஞ்சி,கோரங்கம், துடி

சுவை : கார்ப்பு

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை

அகட்டுவாய்வகற்றி

வெப்பமுண்டாக்கி

பசித்தீதூண்டி

பொது குணம்

பண்டை வெக்கை விதாகநோய் காசமும்

பாமுஞ் சோமப் பிணிவந்து நட்டமும்

அண்டை யீளைவன் பித்தம் இவைக்கெல்லாம்

ஆல மாங்கமழ் ஏல மருந்தே.

(தேரன் குணவாகடம்)

இது தொண்டை நோய்களையும், இருமல், நெஞ்சின் கோழைகட்டு இவற்றை போக்கும்

ஏலம் சேரும் மருந்துகள்

கண்டாத்திரி இளகம்

கற்பூராதி சூரணம்

சீரகம்

வேறு பெயர்கள் : ஆசை, சீரி, உபகும்பபீசம், துத்தசாம்பலம், நற்சீரி, பித்தநாசினி,

மேத்தியம், பேசனகுடோரி

பயன்படும் உறுப்பு : விதை

சுவை : கார்ப்பு,இனிப்பு

தன்மை : தட்பம்

பிரிவு : இனிப்பு

செய்கை

அகட்டுவாய்வகற்றி

வெப்பமுண்டாக்கி

பசித்தீதூண்டி

துவர்ப்பி

பொதுகுணம்

பித்தமெனும் மந்திரியைப் பின்னப் படுத்தியவன்

சத்துருவை யுந்துறந்து சாதித்து- மத்தனெனும்

ராசனையு மீவென்று நண்பைப் பலபடுத்தி

போசனகு டோரிசெயும் போர்.

(தேரன் வெண்பா)

இதனால் இரைப்பு போம்.

சீரகம் சேரும் மருந்துகள்

இலவங்காதிச் சூரணம்

திப்பிலி நெய்

ஓமம்

வேறு பெயர்கள் : அசமோதம், தீப்பியம்

பயன்படும் உறுப்பு : விதை

சுவை : கார்ப்பு,

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை

அகட்டுவாய்வகற்றி

வெப்பமுண்டாக்கி

பசித்தீதூண்டி

உரமாக்கி

பொதுகுணம்

சீதசுரம் காசம் செரியாமந் தம்பொருமல்

பேதியிரைச் சல்கடுப்பு பேராமம்- ஒதிருமல்

பல்லொடுபல் மூலம் பகமிவைநோ யென்செயுமோ

சொல்லொடுபோம் ஓமனெச் சொல்

(அகத்தியர் குணவாகடம்)

இதனால் ஐயசுரம், இருமல், இரைப்பு, போம்

ஓமம் சேரும் மருந்துகள்

சங்சீவி தீநீர்

சித்தாதி இளகம்

Review of literature

Botanical aspect

4.2 LITERATURE REVIEW

Botanical review

CHUKKU

Botanical name : *Zingiber officinale*

Family : Zingiberaceae

Vernacular name

English : Ginger

Hindi : Adrak

Tamil : *chukku*

Parts used

Rhizome

Organoleptic Characters

Odour : Aromatic

Taste : spicy, pungent, hot, biting

Action

Carminative

Stomachic

Stimulant

Digestive

Chemical constituents

- α -curcumene
- β -phellandrene,
- calamene

Phytochemical property

The dried rhizome of ginger contains 1-4% volatile oils, these are the aromatic principle include, zingiberene, bisabolene.

Medicinal uses

- The herb is an excellent remedy for strengthening and healing the respiratory system fighting often colds and flu, removing congestion, soothing sore throats.
- It is recommended during pregnancy for treating morning sickness and digestive problem.
- Useful for Belching, Laryngitis and Rheumatoid arthritis.

Lateral Articles

- A study done in France the researchers found that (6) gingerol, a major constituent of ginger was sufficient to support Eosinophilia.
- This suggested that ginger could suppress TH2-mediated immune response and might thus provide a possible therapeutic application in “Allergic asthma”
- A study in Canada shows that ginger inhibits airway contraction and associated Ca^{2+} signalling possibly via blockage of plasma membrane Ca^{2+} channels, thus reiterating its effectiveness in treating respiratory illness.

Safety Evaluation of Trikatu

Acute and sub-acute toxicity study of Trikatu, a generic herbal formulation of Indian system of medicine was carried out in Charles foster rats for safety profiling. In acute toxicity 2,000mg/kg once was well tolerated by experimental animals and no changes were observed in mortality, morbidity, gross pathology, gain in weight, haematological and biochemical parameters.

MILAGU

Botanical name : *Piper nigrum*

Family : Piperaceae

Vernacular name

Tamil : *Milagu*

English : Black pepper

Hindi : kalimirch

Part used : Fruits

Organoleptic characters

Taste : Bitter, hot

Nature : Hot

Division : Hot

Action

Carminative, Digestive, Stomachic

Chemical constituents

Seed : Amidepipericide , alkaloid piperine , oleoresin, volatile oils

Stem : piperine , β - sitostrol ,sesquisabinene , Hentriacontane

Medicinal Uses

- Black pepper is an best anti-dote.
- Useful in Asthma, Bronchitis, Epilepsy.

Lateral articles : Oral administration of piperine in different propotion of mice suppressed and reduce the infiltration of eosinophilia, hyper responsiveness and inflammation due to the suppression of the production of histamine interleukin-5, immunoglobulin E and interleukin.

THIPPILI

Botanical name : *Piper longum*

Family : Piperaceae

Vernacular name

English : Indian long pepper

Hindi : Pipli

Tamil : *Thipilli*

Parts used : Roots and Fruits

Organoleptic characters

Taste : Sweet

Nature : Hot

Division : Sweet

Action

Carminative, Expectorant, Digestive

Chemical constituent

- piperine
- piperlongumine
- pipartine

Medicinal uses

Piper longum is most commonly used for its benefits in respiratory and digestive systems. Decoction of immature fruits and roots is used in chronic bronchitis and asthma.

In Vivo and in vitro Anti-asthmatic studies of Piper : The extract of piper longum 100µg/ml significantly inhibited the histamine induced Broncho constriction of isolated guinea pig ileum.

ELAM

Botanical name : *Elettariacardamomum*

Family : Zingiberaceae

Vernacular name

Tamil : elakkaay

Hindi : elachi

Part used : Seed

Organoleptic characters

Taste : Hot

Nature : Hot

Division : Hot

Action

Carminative, Stomachi, Stimulant

Chemical constituent

- Oleum
- Cardamoni
- Sabinene
- d – α -terpineol
- Methyl eugenol.

Medicinal uses

Seeds are used to prevent Halitosis , indigestion , and nausea

It is also used to common cold,cough,bronchitis, fever, inflammation of mouth and pharynx.

SEERAGAM

Botanical name : *cuminum cyminum*

Family : Apiaceae

Vernacular name

Telugu : jilaka

Hindi : shajjira

Tamil : *Seeragam*

Part used : (Seeds) fruit

Organoleptic characters

Taste : Hot, sweet

Nature : cold

Division : sweet

Action

Carminative, Astringent, Stomachic, Anti- spasmodic

Chemical constituents

- Pinene
- Sabinene
- Myrcene

Medicinal uses

It is very useful in digestive disorders like biliousness, morning sickness.

Lateral article

A recent study showed bronchodilator effect in guinea pig tracheal chain using the oil of *cuminum cyminum*.

OMAM

Botanical name : *Carum copticum*

Family : Apiaceae

Vernacular name

English : The bishops weed

Hindi : Ajvayam

Part used : Seeds

Organoleptic characters

Taste : Hot, bitter, pungent

Nature : Hot

Division : Hot

Action

Stomachic, Anti-spasmodic, Carminative

Chemical constituents

- Thymol
- Terpene
- Carvacol

Medicinal uses

- It is used for therapeutic effect including bloating, fatigue, diarrhea, abdominal tumors, and respiratory distress.
- It has benefits such as anti-fungal, antioxidant, anti-parasitic, and hypo-lipidemic effect.
- **Lateral articles** ; *Carum copticum* has a relative bronchodilator effect on asthmatic airways which was compared to theophylline.

Pharmaceutical review

4.3 PHARMACEUTICAL REVIEW

Dravagam or Theeneer is the distilled essence, which contains the volatile constituents of the drugs used in the preparation, in a medium of water and is equivalent to “ aqua” or water of the western pharmacopoeia.

The term Dravagam or Theeneer denotes the acceptable aromatic nature of the drug and indicates that it is in the Aqueous state.

Preparation

The preparation of Dravagam or Theeneer involves the efficient distillation of water soaked coarse powders of the drugs. The volatile principles which are evolved admixture with water vapor from the still are condensed and taken.

Equipment required

Bottle of large capacity of handle the distillate.

An assembly of apparatus as described below or modifications thereof for distillation

Process of preparation

The drugs are crushed into a coarse powder and soaked in the prescribed quantity of water, for about a day or at least over night and then charged into the still, along with the water.

The lid is tightly set in and around to prevent leakage of vapors. For the purpose of sealing a cloth ribbon with a paste of black gram is used. Heat is applied to the drug mixture and distillate is collected in bottles, and then the whole condensate from one charging is thoroughly mixed, because the portions that had condensed later would be a weaker in concentration. A Continuous water current should be maintained in the condenser.

Storage

Dravagam or Theeneer should be stored in air tight containers of glass. If kept open, the volatile active principles will be lost by evaporation. Dravagam or Theeneer is administered along with an equal volume of water.

Analytical study

5 . ANALYTICAL STUDY OF *THIRIKADUGU DRAVAGAM*

Analytical study of the drug brings the validation to be used as a medicine by subjecting the drug to many analysis and determining the quality and effectiveness. Analytical study includes such as organoleptic characterization, Physical characteristic, TLC and HPTLC Finger print analysis also to assess the active principles and element present in the drug. Thus standardization brings the efficacy and potency of the drug.

As per AYUSH Protocol for standardization, the following parameters were evaluated.

- Organoleptic characters
- Physical characterization
- Chemical analysis
- TLC&HPTLC Finger print analysis
- Elemental analysis (ICP-OES)
- Gas –Chromotography (GS-MC)

5.1 Organoleptic character of Thirikadugu Dravagam

Colour : The medicine was taken in watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.

Odour : The medicine was smell individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste : Small amount of *Thirikadugu dravagam* was kept over the tip of the tongue

5.2 Physical properties

i) Determination of PH

Five grams of Thirikadhugu dravagam was weighed accurately and placed in clear 100ml beaker. Then 50 ml distilled was added to it and dissolve well. After 30 min it was then applied into Ph metera as standard buffer solution of 4.0, 7.0,9.2. Repeated the test four and average was recorded.

Chemical analysis

5.2 CHEMICAL ANALYSIS OF *THIRIKADUGU DRAVAGAM*

The chemical analysis of *Thirikadugu dravagam* was carried out in Bio-chemistry lab, National institute of siddha.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Physical Appearance of extract	Pale yellow in colour	
2.	Test for Silicate a. A 2ml of the sample was shaken well with distilled water.	Completely soluble	Absence of Silicate
3.	Action of Heat: A 2ml of the sample was taken in a dry test tube and heated gently at first and then strong.	No White fumes evolved. No brown fumes evolved.	Absence of Carbonate Absence of Nitrate.
4.	Flame Test: A 2ml of the sample was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.	No bluish green flame	Absence of copper
5.	Ash Test: A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No Appearance of yellow colour flame	Absence of sodium

Preparation of Extract:

5ml of sample was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 10 minutes. Then it is cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation is used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it.

S.N	EXPERIMENT	OBSERVATION	INFERENCE
	I. Test For Acid Radicals		
1.	Test For Sulphate: a.2ml of the above prepared extract was taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	No cloudy appearance	Absence of Sulphate
2.	Test For Chloride: 2ml of the above prepared extracts was added with 2ml of dil-HCl is added until the effervescence ceases off..	Cloudy appearance present.	Presence of Chloride
3.	Test For Phosphate: 2ml of the extract were treated with 2ml of dil.ammoniummolybdate solution and 2ml of con.HNO ₃	No cloudy yellow appearance present	Absence of Phosphate
4.	Test For Carbonate: 2ml of the extract was treated with 2mldil. Magnesium sulphate solution.	No cloudy appearance.	Absence of carbonate

5	Test For Nitrate: 1gm of the extract was heated with copper turning and concentrated H ₂ SO ₄ and viewed the test tube vertically down.	No Brown gas is evolved	Absence of nitrate
6.	Test For Sulphide: 1gm of the extract was treated with 2ml of con. HCL	No rotten egg smelling gas is evolved	Absence of sulphide
7.	Test For Fluoride & Oxalate: 2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil. calcium chloride solution and heated.	No cloudy appearance.	Absence of fluoride and oxalate
8.	Test For Nitrite: 3drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution is placed.	No characteristic changes	Absence of nitrite
9.	Test For Borate: 2 Pinches (50mg) of the extract was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	No Appearance of bluish green colour	Absence of borate
II. Test For Basic Radicals			
1.	Test For Lead: 2ml of the extract was added with 2ml of dil.potassium iodine solution.	No Yellow precipitate is obtained	Absence of lead

2.	Test For Copper: a. One pinch (25mg) of extract was made into paste with con. HCl in a watch glass and introduced into the non-luminous part of the flame.	No blue colour precipitate	Absence of copper
3.	Test For Aluminium: To the 2ml of extract dil. sodium hydroxide was added in 5 drops to excess.	No characteristic changes	Absence of Aluminium.
4.	Test For Iron: a. To the 2ml of extract add 2ml of dil. ammonium solution	No Red colour appeared	Absence of Iron
5.	Test For Zinc: To 2ml of the extract dil. sodium hydroxide solution was added in 5 drops to excess and dil. ammonium chloride is added.	No White precipitate is formed	Absence of Zinc
6.	Test For Calcium: 2ml of the extract was added with 2ml of 4% dil. ammonium oxalate solution	Cloudy appearance and white precipitate obtained	presence of calcium
7.	Test For Magnesium: To 2ml of extract dil. sodium hydroxide solution was added in drops to excess.	No White precipitate is obtained	Absence of magnesium
8.	Test For Ammonium: To 2ml of extract 1 ml of Nessler's reagent and excess of dil. sodium hydroxide solution are added.	No Brown colour appeared	Absence of ammonium

9.	Test For Potassium: A pinch (25mg) of extract was treated of with 2ml of dil.sodium nitrite solution and then treated with 2ml of dil.cobalt nitrate in 30% dil.glacial acetic acid.	No Yellow precipitate is obtained	Absence of potassium
10.	Test For Sodium: 2 pinches (50mg) of the extract is made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved.	Absence of sodium
11.	Test For Mercury: 2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.	No Yellow precipitate is obtained	Absence of Mercury
12.	Test For Arsenic: 2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.	No Brownish red precipitate is obtained	Absence of arsenic
III. Miscellaneous			
1.	Test For Starch: 2ml of extract was treated with weak dil.Iodine solution	No Blue colour developed	Absence of starch
2.	Test For Reducing Sugar: 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes are noted.	No Brick red colour is developed	Absence of reducing sugar

3.	<p>Test For The Alkaloids:</p> <p>a) 2ml of the extract was treated with 2ml of dil.potassium iodide solution.</p> <p>b) 2ml of the extract was treated with 2ml of dil. picric acid.</p> <p>c) 2ml of the extract was treated with 2ml of dil. phosphotungstic acid.</p>	Yellow colour developed	Presence of Alkaloid
4.	<p>Test For Tannic Acid:</p> <p>2ml of extract was treated with 2ml of dil.ferric chloride solution</p>	No Blue-black precipitate is obtained	Absence of Tannic acid
5.	<p>Test For Unsaturated Compound:</p> <p>To the 2ml of extract 2ml of dil.Potassium permanganate solution is added.</p>	Potassium permanganate is not decolourised	Absence of unsaturated compound
6.	<p>Test For Amino Acid:</p> <p>2 drops of the extract was placed on a filter paper and dried well. 20ml of Burette reagent is added.</p>	No violet colour	Absence of amino acid
7.	<p>Test For Type Of Compound:</p> <p>2ml of the extract was treated with 2 ml of dil.ferric chloride solution.</p>	<p>No green and red colour</p> <p>No Violet colour developed</p> <p>No Blue colour developed.</p>	<p>Absence of quinolepinephr inepyrocatecho antipyrine Aliphatic amino acid and meconic acid. Apomorphine salicylate and Resorcinol are absent Morphine,Phenol cresol and hydrouinone</p>

5.4 TLC/HPTLC FINGER PRINT ANALYSIS

Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound.

TLC/HPTLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. TLC/HPTLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC/HPTLC is to obtain well defined, well separated spots.

Retention Factor

After a separation is complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (R_f) which is equal to the distance migrated over the total distance covered by the solvent. The R_f formula is

R_f = distance traveled by sample /distance traveled by solvent The R_f value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions. The compound with the larger R_f value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower R_f value

TLC and HPTLC Methodology

10ml of the given sample soaked in 10ml of 95%ethyl alcohol kept it overnight. Then 40ml of ethyl acetate added. Ethyl acetate layer was separated and dried over anhydrous sodium sulphate, filtered and concentrated to 5ml at room temperature. 45 µl,50 µl of the above solution were applied on Merck Aluminium plate pre-coated with silica gel 60 F₂₅₄ of thickness using ATS-IV. The plate was developed in Toluene: Ethyl acetate (1:2). The plate was dried and visualized in UV 254 nm and UV 366nm and photos were taken. The plate was scanned at 254 nm before dipping. Then the plate was dipped in vanillin – sulphuric acid and heated at 105°C till the Colour of the spots appeared and photos were taken.

4.5 ELEMENTAL ANALYSIS

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSIONS SPECTROMETRY

ICP-OES is a trace level elemental analysis that uses the emission spectra of a sample to identify and qualify the elements present. The experimental procedure was done at SAIF, IIT Madras.chennai-36.

Introduction

The element composition of a sample is often an important part of the information needed to assess its properties. Hence there is a need for scientific instrumentation like ICP-OES which plays a pivotal role in the determination of these elements. ICP-OES is widely employed for the estimation of metals and metalloids at trace, minor and major concentration.

Principle

In this technique, the high temperature plasma source atomizes the sample and excites the atoms resulting in emission of photons. The atoms of each element in the sample emit specific wavelength of light. The emission spectrum from the plasma is dispersed by an optical spectrometer, so that intensity of the individual wavelength can be measured. The number of photons emitted is directly proportional to the concentration of the element. The photons may be detected either sequentially or simultaneously. Quantitative analysis is achieved by measuring the intensity of these specific wavelength and after performing the calibration using known standards.

Identifying the presence of emission at the wavelength characteristic of the element of interest obtaining quantitative information i.e how much of an element is in sample can be accomplished using plots of emission intensity versus concentration called calibration curves.

ICP-OES Operating conditions

Rf frequency	40 M Hz
Range	165-782nm
Detection time	up to ppm level using SCD detector

Sample preparation – Microwave Digestion

- Weight 0.25 g of test sample and transfer into a liner provided with instrument.
- Slowly add 9ml of Nitric acid or Sulphuric acid such that no piece of sample sticks on the slide.
- Mix thoroughly and allow reacting for few minutes.
- Place the liner in the vessel jacket.
- Close the screw cap hand- tight in clockwise direction.
- Seal the vessel and placed in the rotor fixed in microwave.
- Set temperature to 180°C for 5 minutes, hold at 180°C for least 10 minutes.
Allow the vessels to cool down to a vessel interior temperature below 60°C and to a vessel surface temperature (IR) below 50°C before removing the rotor.
- The digested sample was made upto 100ml with Millipore water.
- If visible insoluble particles exist, solution could be filtered through whatmann filter paper.
- Transfer the digested solution into plastic containers and label them properly.



RESULTS

The analytical results of Elemental analysis in Thirikadugu dravagam using ICP-OES are showed in table 5.5

5.6 GAS CHROMATOGRAPHY : (GC-MS)

Gas chromatography is a chromatographic separation technique based on the difference in the distribution of species between two non- miscible phase in which the mobile phase is a carrier gas moving through or passing the stationary phase contained in a column. It is applicable to substances or their derivatives, which are volatilized under the temperatures employed.

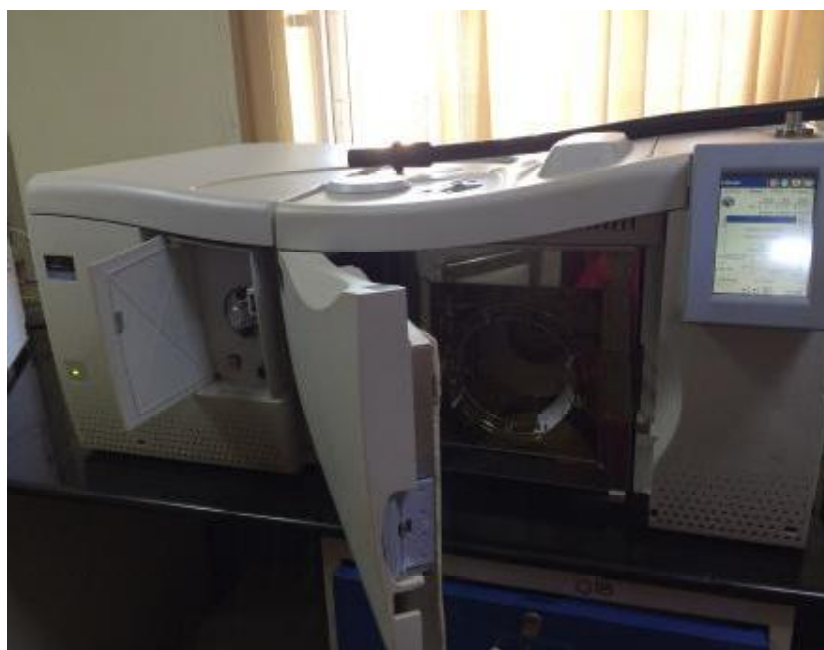
GC is based on mechanism of adsorption, mass distribution or size exclusion.

METHOD

Equilibrate the column, the injector and the detector at the temperatures and the gas flow rates specified in the monograph until a stable baseline is achieved. Prepare the test solution (S) and the reference solution (S) as prescribed. The solution must be free from solid particles.

GC-MS SPECTROMETER

The JEOL GCMATE II GC-MS with Data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization (CI) .



Applications

- Structural elucidation of organic compounds.
- Mechanistic study of fragmentation process under mass spectrometric condition.
- Molar mass and structural analysis of small biomolecules.

Instrument name	:	JEOL GC MATE II
Front inlet temp	:	220 degree c
Column	:	HP 5 Ms
Carrier gas	:	high pure helium
Flow rate	:	1 ml /min
Oven temp	:	50 to 250 @ 10 deg / min
Ion chamber tem	:	250 deg c
GC interface temp	:	250 deg c
Mass analyzer	:	quadrupole with double focusing mass analyzer,
Detector	:	Photon multiplier tube
Scam	:	50 to 600 amu 70ev electron impact ionization

Toxicity studies

6. TOXICOLOGICAL EVALUATION OF *THIRIKADUGU DRAVAGAM* ON WISTAR ALBINO RATS

Plan work :

Safety is a fundamental principle in the provision of traditional medicines and herbal products for health care and a critical.

Component of quality control. OECD guidelines provide practical and technical guidance for monitoring the safety of traditional medicines within Pharmacovigilance systems. The safety monitoring of traditional medicines is compared and contrasted with that of other Medicines, currently undertaken in the context of the WHO International Drug perspective.

Scope of work:

Monitoring Programme. While there are regulatory and cultural differences in the preparation and use of different types of medicines, they are all equally important from a pharmacovigilance

Assurance of safety, quality and efficacy of Indian System of Medicines (ISM) is the key issue that needs to be addressed while conducting toxicity studies. It is an essential step, which will strengthen the acceptance of Siddha medicines by scientific community. Information of toxicity and adverse effects of these formulations are lacking. Some of the formulations are proved to be effective in various animal studies and many more are yet to be tested.

Hence, the present study was carried out to evaluate the Preclinical toxicity studies of *THIRIKADUGU DRAVAGAM* in animal models.

The following studies were carried out on *THIRIKADUGU DRAVAGAM*

- Acute Oral toxicity – OECD 423
- Repeated dose 28 Days Oral Toxicity Study – OECD 407
- Repeated dose 90 Days Oral Toxicity study-OECD 408

**ACUTE AND SUBACUTE TOXICITY STUDIES OF THIRIKADUGU
DRAVAGAM ON WISTAR ALBINO RATS**

ACUTE ORAL TOXICITY-EXPERIMENT PROCEDURE:

Introduction:

Acute toxicity studies were carried out according to the OECD (Organization of Economic Co-operation and Development) guidelines 423. Healthy female wistar Albino rats, weighing 150–200 g, were selected and oral administration of the single doses of *Thirikadugu Dravagam* with water.

All the protocols and the experiments conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and supervision of experiments on animals. Animal experimentation and protocol were approved by Institutional animal ethical committee. **(KKCP/2015/029)**

Animals and dose levels:

Healthy young adult animals of commonly used laboratory used. Three and 12 weeks old and its weight should fall in an interval within ± 20 % of the mean animals weight of any were used for each step. Each animal, at the commencement of its dosing, should be between 8 previously dosed animals.

The dose level used as the starting dose was selected from fixed levels 10ml/kg body weight. Dose for animal is freshly prepared shortly prior to administration. The available information suggest that highest starting dose level 10ml/kg body weight, so the trial or limit test was conducted. The time interval between treatment groups is determined by the onset, duration, and severity of toxic signs.

Administration of doses:

Thirikadugu Dravagam was administered as a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance

administration An oral (p.o) dose of 1mL/kg bw, 5ml/kg bw and 10ml/kg bw was administered step by step according to the guidelines. The general behaviors of the rats were continuously monitored for 1 hr after dosing, periodically during the first 24 h (with special attention given during the first 4 hours and then daily thereafter, for a total of 14days.

Changes in the normal psychomotor activity and external morphology and their body weights were monitored periodically before dosing and the time at which signs of toxicity or mortality were recorded.

The visual observations included skin changes, morbidity, aggressiveness, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water 12 hr prior to the administration of the test substance. Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

OBSERVATIONS:

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal. Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somato motor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded.

*Repeated dose 28 days oral
toxicity*

**REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF *THIRIKADUGU*
DRAVAGAM ON WISTAR ALBINO RATS**

(OECD – 407 guidelines)

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into 3 groups of 10 animals (5 male and 5 female). Group I served as control (distilled water at the dose of 10ml/kg bw) and Group-II and III were treated with *Thirikadugu Dravagam* at the dose of 5ml and 10ml/kg bw for 28 days. The toxic symptoms such as signs of toxicity, mortality and body weight changes were monitored. Rats were anesthetized with ether at the end of the treatment period. All rats were sacrificed after the blood collection.

Test Substance	: <i>Thirikadugu Dravagam</i>
Animal Source	: Animal house of King Institute of Preventive Medicine
Animals	: Male and Female Wistar Albino Rats
Age	: More than 8 weeks
Acclimatization	: Seven days prior to dosing.
Veterinary examination	: Prior to and at the end of the acclimatization period.
Identification of animals	: By cage number and individual marking on fur.
Diet	: Pelleted feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	: Aqua guard portable water in polypropylene bottles <i>ad libitum</i> .
Housing & Environment	: The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	: Between 20 & 24°C,
Relative humidity	: Between 30% and 70%,
Dark and light cycle	: Each of 12 hours.
Duration of the study	: 28 days

Justification for Dose Selection:

The results of acute toxicity studies in rats indicated that *Thirikadugu Dravagam* was non toxic and no behavioral changes were observed up to the dose level of 10ml/kg body weight in acute treatment.

As per OECD guideline three dose levels were selected for the study. They are low dose (X), mid dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose (10ml) and the body surface area of the rat (0.018). i.e X dose is 0.9 ml/kg, 5X dose is 5ml /kg, 10X dose 10 ml/kg

Preparation and administration of dose:

Thirikadugu Dravagam was aseptically administered to animals at the dose levels of 5ml and 10ml/kg bw. The test substance was freshly used from the drug container. The drug was administered for a period of 28 days. The control animals were administered water (10ml/kg bw) only. Administration was by oral (gavage), once daily for 28 consecutive days.

METHODOLOGY**Randomization, Numbering and Grouping of Animals:**

Ten Rats (Five Male and Five Female) in each group randomly divided into three groups for dosing up to 28 days. Animal's acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliporous and non-pregnant.

OBSERVATIONS:

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight:

Weight of each rat was recorded on day 0 and at 5 days intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated.

Food and water Consumption:

The quantity of food consumed by groups consisting of ten animals of for different doses was recorded at weekly interval. Food consumed per animal was calculated for control and the treated dose groups.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study

TERMINAL STUDIES:**Laboratory Investigations:**

Following laboratory investigations were carried out on day 29 in animals' fasted over-night. On 29th day, the animals were fasted for approximately 18 h, then slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations:

Blood samples of control and experimental rats were analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Mean corpuscular volume (MCV) and packed cell volume (PCV). From the estimated values of RBC count (millions/mm³) and PCV (volumes percent), mean corpuscular volume (MCV) was calculated.

Biochemical Investigations:

Serum and Urine was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for Protein, Bilirubin, Urea, Creatinine, Triglyceride, Cholesterol and Glucose levels was carried using standard methods. Activities of glutamate Oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), Glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and Alkaline phosphatase were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on day 29. Necropsy of all animals were carried out and the weights of the organs including Liver, Kidneys, Brain, Heart, and Lungs were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}} \times 100$$

Histopathology:

Histopathological investigation of the vital organs were done. The organ pieces (3-5µm thick) were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing melted paraffin at 50°C and then in a cubical block of paraffin made by the “L” moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

The organs included Brain, Heart, Kidneys, Liver and Lungs of the animals were preserved they were subjected to histopathological examination.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, and hematology and blood chemistry were subjected to One-way Anova.

*Repeated dose 90 days oral
toxicity*

**REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY OF
THIRIKADUGU DRAVAGAM ON WISTAR ALBINO RATS**

Test Substance	:	<i>THIRIKADUGU DRAVAGAM</i>
Animal Source	:	King institute of technology, Guindy.
Animals	:	Wister Albino Rats (Male-12 and Female-12)
Age	:	6-8 weeks
Body Weight	:	150-200gm.
Acclimatization	:	Seven days prior to dosing.
Veterinary examination	:	Prior and at the end of the acclimatization period.
Identification of animals	:	By cage number, animal number and individual marking by using picric acid
Diet	:	Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	:	Aqua guard portable water in polypropylene bottles.
Housing & Environment	:	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	:	between 22°C±3°C.
Relative humidity	:	between 30% and 70%,
Air changes	:	10 to 15 per hour
Dark and light cycle	:	12:12 hours.
Duration of the study	:	90 Days.
IAEC	:	NIS/IAEC-1/2016/04

Methodology:**Randomization, Numbering and Grouping of Animals:**

24 Wistar Albino Rats (12M + 12F) were selected and divided into 4 groups. Each group consist of 6 animals (Male 3- and Female-3). First group treated as a control and other three group were treated with test drug (low, mid, high) for 90 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Justification for Dose Selection:

As per OECD guideline three dose levels were selected for the study. They were low dose (X), mid dose (5X), high dose (10X). X was calculated by multiplying the therapeutic dose (10 ml) and the body surface area of the rat (0.018).i.e X dose was 1ml/kg , 5X dose was 5ml,10X dose was 10ml/kg.

Preparation and Administration of Dose:

Thirikadugu dravagam was suspended with distilled water. It was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 90 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 90 consecutive days.

Observations:

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight:

Weight of each rat was recorded on day 0, at 15 days intervals throughout the course of study.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study.

Necropsy:

All the animals were sacrificed by excessive anesthesia on day 91. Necropsy of all animals was carried out.

Laboratory Investigations:

Following laboratory investigations were carried out on day 91 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Haematological Investigations:

Haematological parameters were determined using Haematology analyzer.

Biochemical Investigations:

Biochemical parameters were determined using auto-analyzer.

Histopathology:

Control and highest dose group animals were initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group was examined. Organs were collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6 μ m sections and were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption and hematology and blood chemistry were subjected to One-way ANOVA followed by dunnet test using a computer software programme -INSTAT-V3 version

*Pharmacological
Studies*

7.1 PHARMACOLOGICAL STUDY OF THIRIKADUGU DRAVAGAM

BRONCHO-DILATOR ACTIVITY

Aim:

To evaluate Bronchodilator activity using Guinea pig by Isolated tracheal chain preparation method.

Procedure:

All the animals were fed with standard diet and water ad-libitum and maintained under standard laboratory conditions. Guinea pigs of either sex, weighing 250-300 g were sacrificed by cervical dislocation and carotid bleeding. The trachea was dissected out and transferred to a dish containing kreb's solution (composition (g/l): NaCl (6.8), KCl (0.35), CaCl₂ (0.28), MgSo₄7H₂O (0.25), NaHCO₃ (2.1), KH₂PO₄ (0.16) and glucose (2.0)) and cut transversely between the segments of the cartilage so as to give a number of rings of the trachea. About 5-6 rings these were tied to form a chain of approximately 4-5 cm length, which was in kreb's solution, contained in an organ bath maintained at 37°C and continuously aerated with carbogen (95% O₂+5% CO₂). One end of the tracheal chain was attached to a tissue holder at the base of organ bath and the other end to a frontal lever; the responses were recorded on a slow moving kymograph. The suspended tracheal was allowed to stabilize for at least 30 minutes. During stabilization, the bath was supplied with fresh kreb's solution ones per every 15 minutes. Then cumulative concentration response to histamine in the absence and presence of *Thirikadugu dravagam* were recorded with a slow moving (0.25 mm/sec) kymograph.

Anti-histaminic activity

Aim

To evaluate Anti-histaminic activity of Thirikadugu dravagam using Guinea pig in vivo.

*** KK. College of Pharamacy

Procedure:

Overnight fasted guinea pigs were divided into 4 groups each containing 6 animal.

- Group 1 was treated as control,
- Group 2 received standard drug chlorpheniramine maleate (2 mg/kg).
- Groups 3, 4 *Thirikadugu dravagam* (1 and 2ml/kg).

All the doses were given orally once a day for 7 days. Prior to drug treatment each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The pre convulsive dyspnea (PCD) was determined from the time of exposure to onset of convulsions. As soon as the PCD were noted, the animal were removed from the chamber and placed in fresh air. Group 2 received Chlorpheniramine maleate. Group 3 and 4 received *thirikadugu dravagam* at the dose of 1ml and 2ml/kg bw respectively. These animals were again subjected to histamine aerosol after 1hr of drug administration and PCD was determined.

ANTI-INFLAMMATORY**Aim:**

To Evaluate the anti-inflammatory activity of Thirikadugu dravagam on wistar albino rats by carageenan induced paw edema.

Procedure:

(*Thirikadugu Dravagam* (1 and 2ml/kg) or indomethacin (10 mg/kg) was administered orally to four groups each group containing 6 animals. Acute inflammation was induced half an hour after above treatment by sub-planter injection of 0.1 ml freshly prepared 1% suspension of carrageenan in right hind paw in rats (Winter et al., 1962) The paw volume was measured initially and then at 1, 2, 3 and 4 h after the carrageenan injection by using plethysmographic method of Harris and Spencer 1962).

Results

RESULTS

In My research, studies like Organoleptic characters, physical characters, chemical analysis, phytochemical analysis, Elemental analysis, Gas chromatography, Toxicity and pharmacological studies have been carried to know the potency and efficacy of the drug *Thirikadugu dravagam*.

- Botanical aspect explains the active principle and medicinal uses of the plants.
- Gunapadam review brings the effectiveness of the drug in treating Bronchial ashtma..

The pharmacological review explains about the methodology of Bronchodilator , Anti-histaminic activity and the drugs used.

Analytical study of the drug is more essential to derive the efficacy, potency of the drug by analysing it by various studies. Following are the results of physicochemical , Phytochemical analysis, estimation of basic and acidic radicals, Elemental analysis, GS-MC have been done and tabulated.

Toxicological results of the drug and pharmacological activity of the drug were derived. Its result has been tabulated and interpretation was made below. Thus it is to give a complete justification to bring the effectiveness of the trial drug *Thirikadugu dravagam*.

5.1 ORGANOLEPTIC CHARACTERS

Table : 1 Organoleptic characters of Thirikadugu dravagam

S.NO	Parameters	Results
1	Colour	pale yellow
2	Odour	Aromatic odour
3	Taste	pungent
4	State of matter	liquid

5.2 PHYSICAL CHARACTERIZATION

Table 2 Physical characterization of Thirikadugu dravagam

S.NO	Parameters	Results
1	pH	4.65
2	Specific Gravity	0.9992
3	Volatile Matter	Negligible

5.3 CHEMICAL ANALYSIS

Table: 3 Result of Acid radical study:

S.NO	Parameter	Result
1	Test for Sulphate	Negative
2	Test for Chloride	Positive
3	Test For Phosphate	Negative
4	Test For Carbonate	Negative
5	Test For Nitrate	Negative
6	Test for Sulphide	Negative
7	Test For Fluoride & oxalate	Negative
8	Test For Nitrite	Negative
9	Test For Borax	Negative

Interpretation

The acidic radicals test shows the presence of **Chloride**

Table 4 5.3 b : Results of basic radicals studies.

S.NO	Parameter	Result
1	Test for Lead	Negative
2	Test for Copper	Negative
3	Test For Aluminium	Negative
4	Test For Iron	Negative
5	Test For Zinc	Negative
6	Test for Calcium	Positive
7	Test For Magnesium	Negative
8	Test For Ammonium	Negative
9	Test For Potassium	Negative
10	Test For Sodium	Negative
11	Test For Mercury	Negative
12	Test For Arsenic	Negative

Interpretation

The basic radical test shows the presence of, **calcium** and absence of heavy metals such as lead, arsenic and mercury.

5.4 TLC AND HPTLC -Table 5

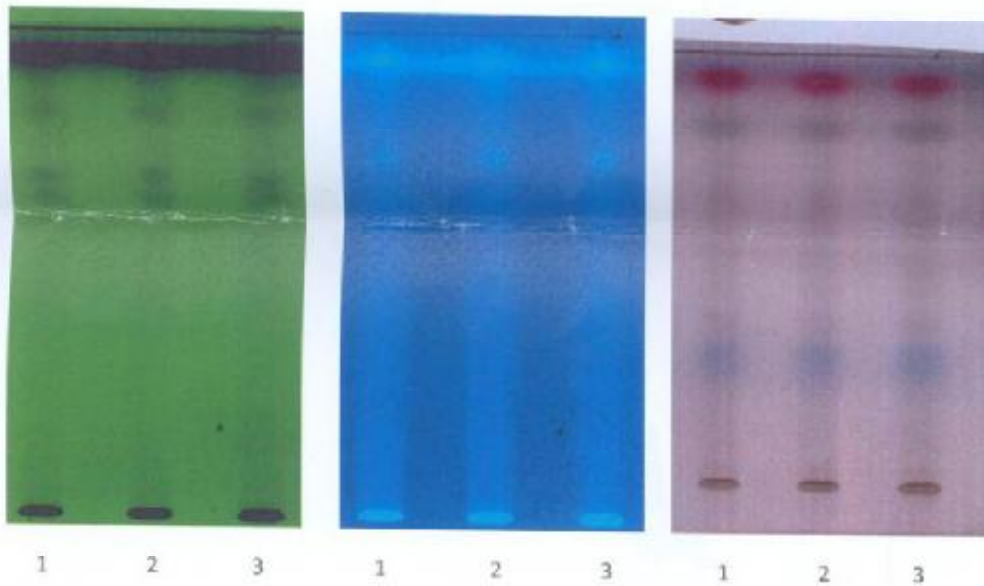
s.no	254 nm		366nm		Dipped in vanillin-Sulphuric acid	
	Colour	Rf	Colour	Rf	Colour	Rf
1	Green	0.66	Blue	0.76	Blue	0.29
2	Green	0.70	Blue	0.94	Grey	0.84
3	Green	0.83			Pink	0.95

TLC PHOTODOCUMENTATION OF DTL SAMPLE 1510356

UV 254 nm

UV 366 nm

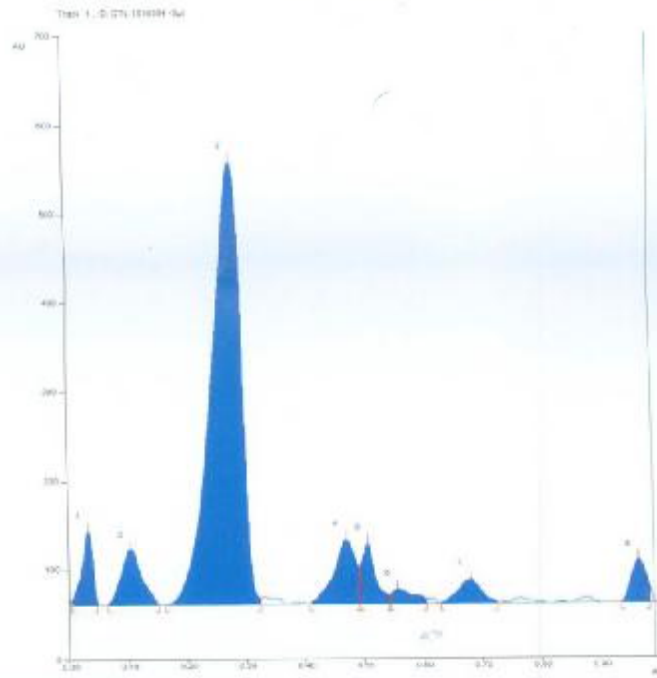
DERVATISED WITH VANILLIN
SULPHURIC ACID



Track 1 - 45 μ l , Track -2 - 50 μ l, TRACK 3 - 50 μ l

SOLVENT SYSTEM : TOLUENE : ETHYL ACETATE : 1 : 2

HPTLC finger print profile of DTL 1:10354 sample (Ethanol extract) - 5µl- 254nm



Track 1, D:DTL 1510354-5µl

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.01 RT	5.2 AU	0.01 RT	85.4 AU	9.50 %	0.06 RT	1.7 AU	1219.0 AU	4.22 %	unknown *
2	0.07 RT	0.2 AU	0.11 RT	82.0 AU	7.67 %	0.18 RT	0.0 AU	1795.3 AU	6.01 %	unknown *
3	0.16 RT	3.1 AU	0.20 RT	495.1 AU	98.88 %	0.32 RT	8.5 AU	30275.1 AU	70.16 %	unknown *
4	0.41 RT	0.0 AU	0.47 RT	22.7 AU	0.35 %	0.49 RT	10.3 AU	2160.6 AU	7.44 %	unknown *
5	0.49 RT	40.4 AU	0.51 RT	60.5 AU	7.81 %	0.54 RT	9.3 AU	1230.7 AU	4.22 %	unknown *
6	0.55 RT	9.8 AU	0.56 RT	15.4 AU	1.07 %	0.61 RT	4.9 AU	477.9 AU	1.55 %	unknown *
7	0.68 RT	0.0 AU	0.68 RT	27.3 AU	3.11 %	0.75 RT	0.0 AU	940.9 AU	2.91 %	unknown *
8	0.92 RT	0.1 AU	0.96 RT	48.1 AU	5.48 %	0.96 RT	16.1 AU	1766.6 AU	5.95 %	unknown *

5.5 ELEMENTAL ANALYSIS

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES)

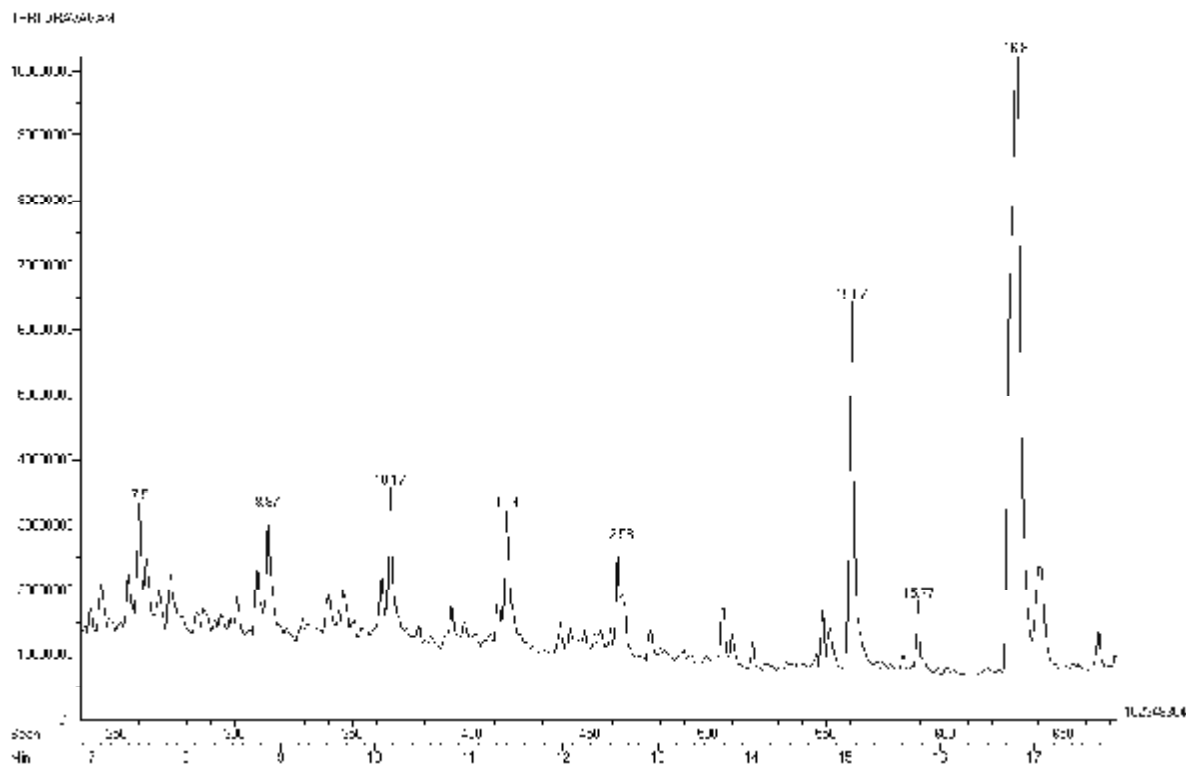
Table : 6

S.NO	Elements	wavelength in nm	Thirikadugu dravagam
1	Arsenic	As 188.979	BDL
2	calcium	Ca 315.807	15.860 mg/L
3	cadmium	Cd 228.802	BDL
4	copper	Cu 327.393	BDL
5	Mercury	Hg 253.652	BDL
6	Potassium	K 766.491	03.821mg/L
7	Sodium	Na 589.592	02.520mg/L
8	Nickle	Ni 231.604	BDL
9	Lead	Pb 220.353	BDL
10	Phosphorous	P 213.617	26.358mg/L

BDL - Below Detection limit

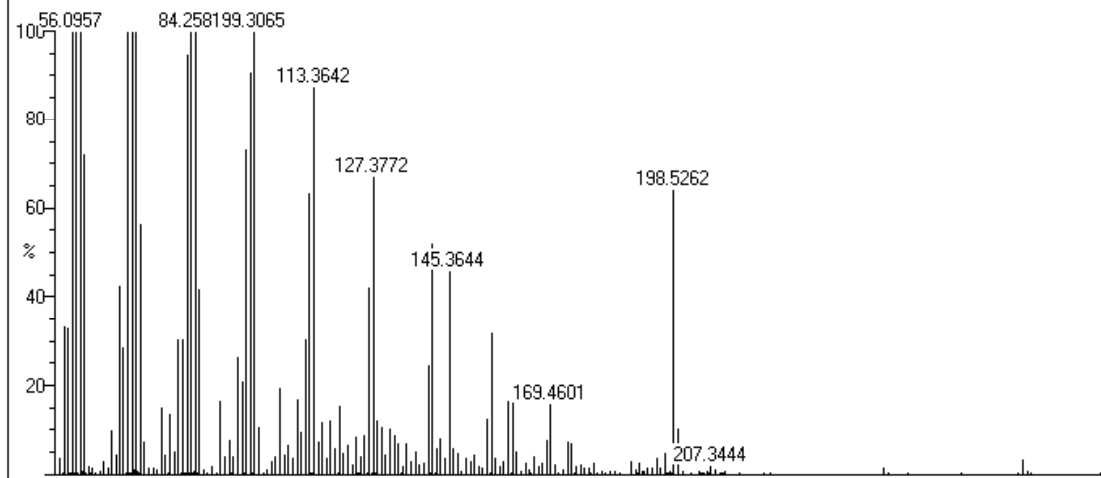
The results shows quantitative analysis of the elements present in *Thirikadugu dravagam*. The heavy metals were found to be within normal limits. The presence of other elements shows the therapeutic value of *Thirikadugu dravagam*. Hence the drug is consider as safe dug.

5.6 GAS CHROMATOGRAPHY(GS-MC)
Gas chromatograph of Thirikadugu dravagam

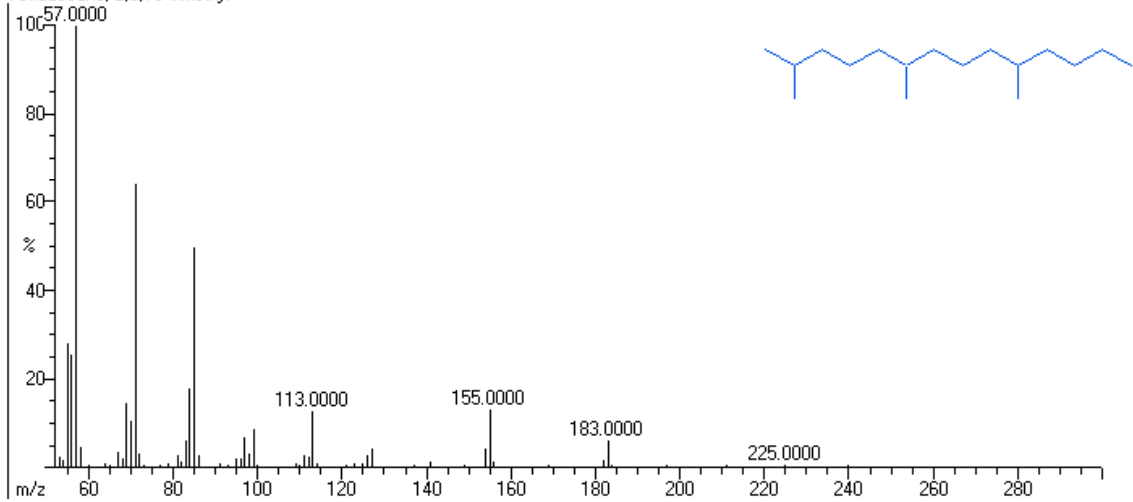


THRI DRAVAGAM

Scan: 314 TIC=32290288 Base=100%FS #ions=1435 RT=8.87

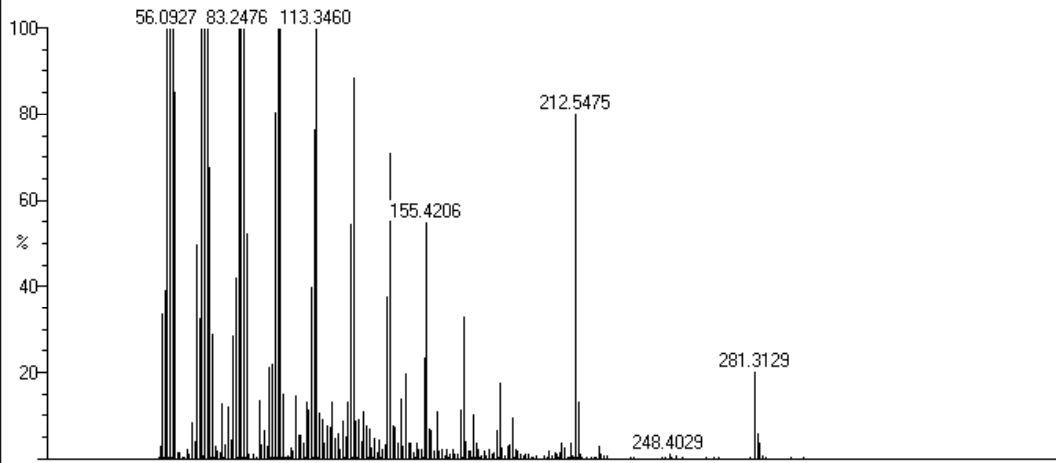


NIST MS 1 of 100 (14905-56- #ions=112
Tetradecane, 2,6,10-trimethyl-

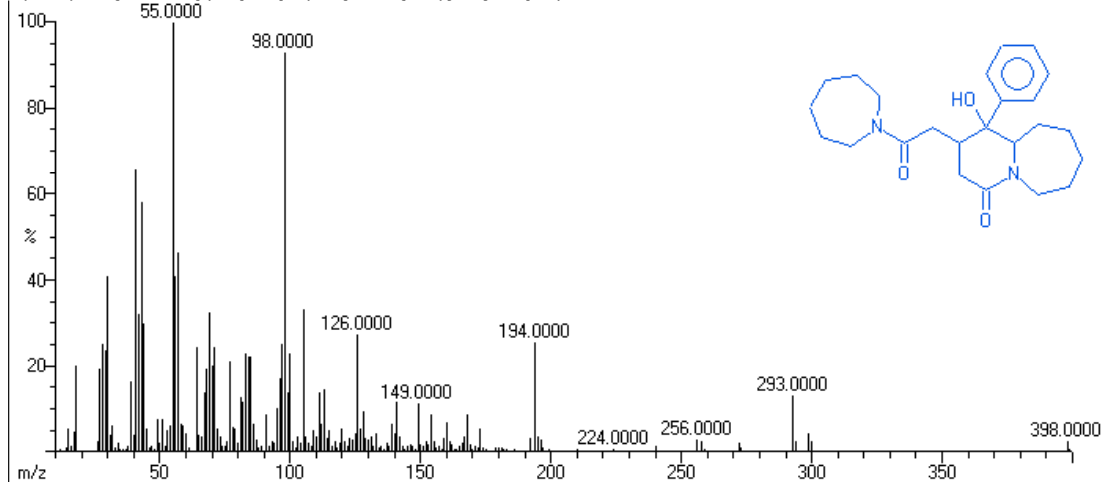


THRI DRAVAGAM

Scan: 366 TIC=35772128 Base=100%FS #Ions=1546 RT=10.17

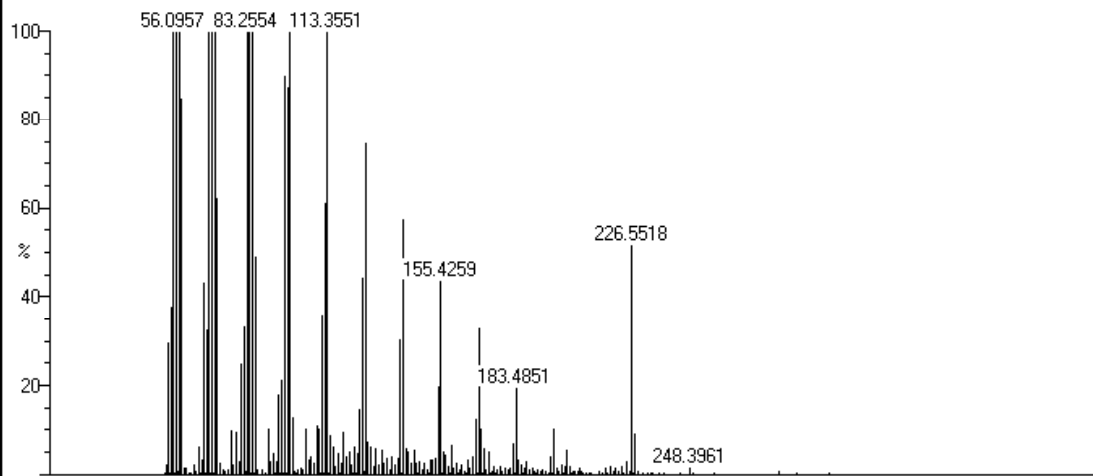


NIST MS 2 of 100 (DB# 17541 #Ions=255)
2-(2-Azepan-1-yl-2-oxoethyl)-1-hydroxy-1-phenyl-octahydro-pyrido[1,2-a]azepin-4-one

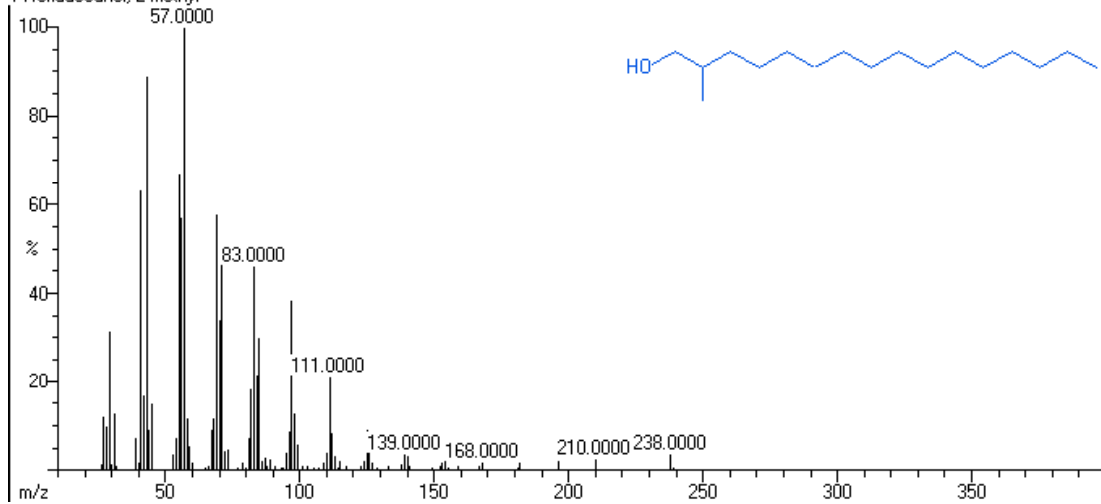


THRI DRAVAGAM

Scan: 415 TIC=32240928 Base=100%FS #ions=1474 RT=11.4

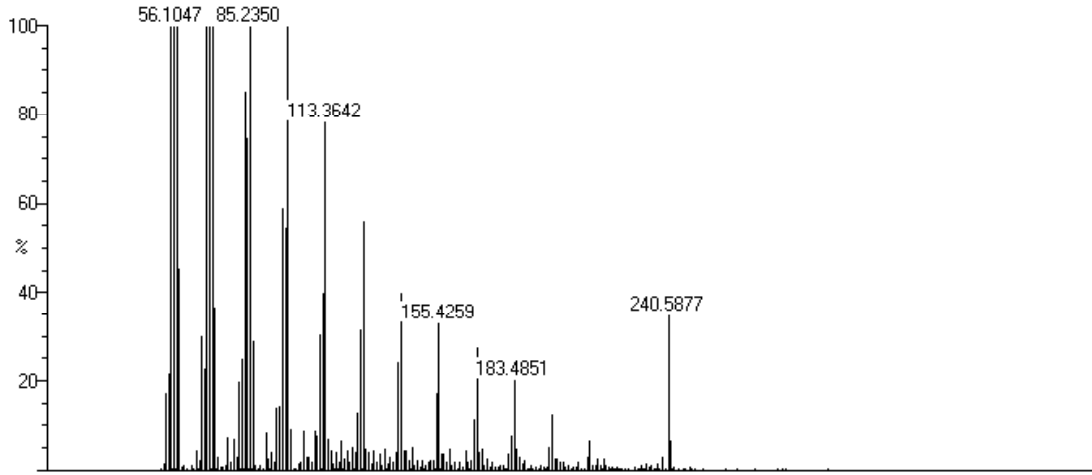


NIST MS 2 of 100 (2490-48-4 #ions=216
1-Hexadecanol, 2-methyl-

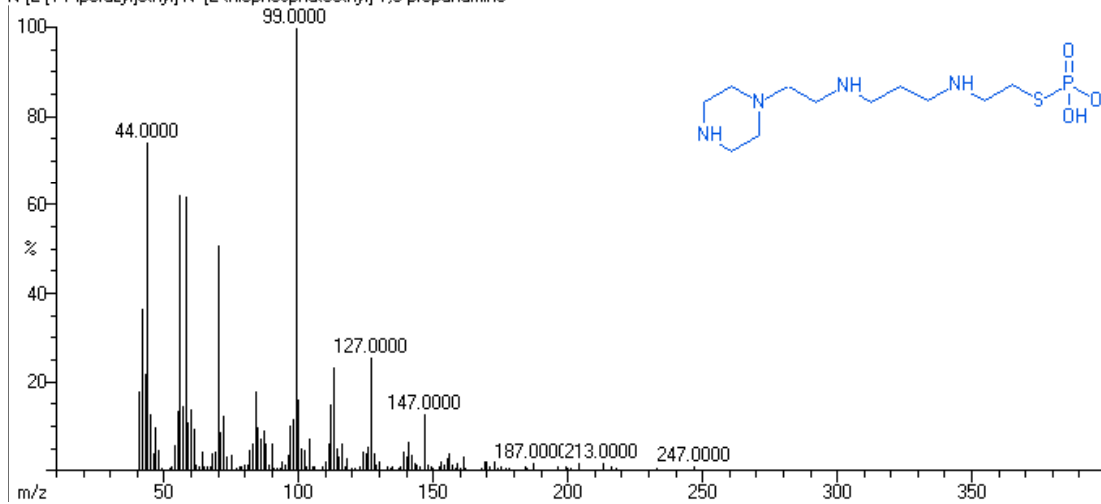


THRI DRAVAGAM

Scan: 462 TIC=27293904 Base=100%FS #ions=1486 RT=12.58

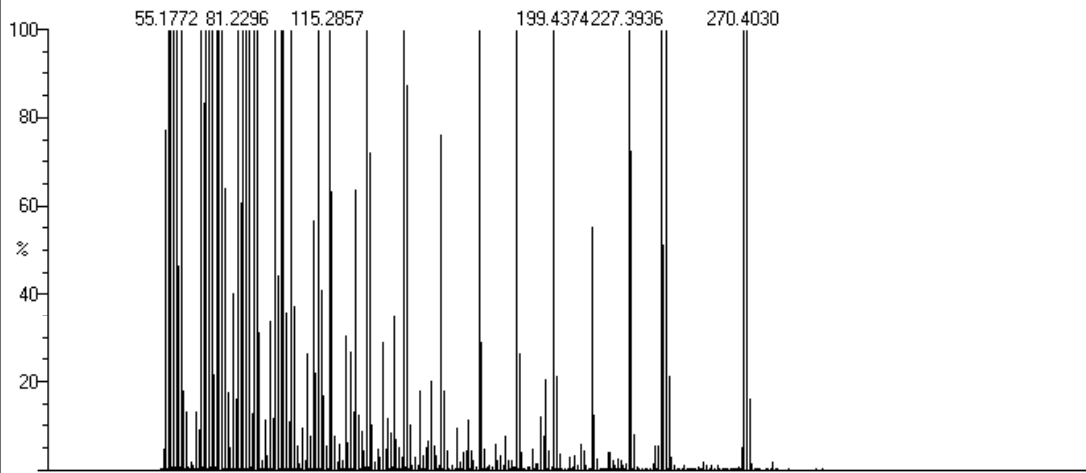


NIST MS 2 of 100 (62220-23- #ions=205
N-[2-[1-Piperazyl]ethyl]-N'-[2-thiophosphatoethyl]-1,3-propanamine

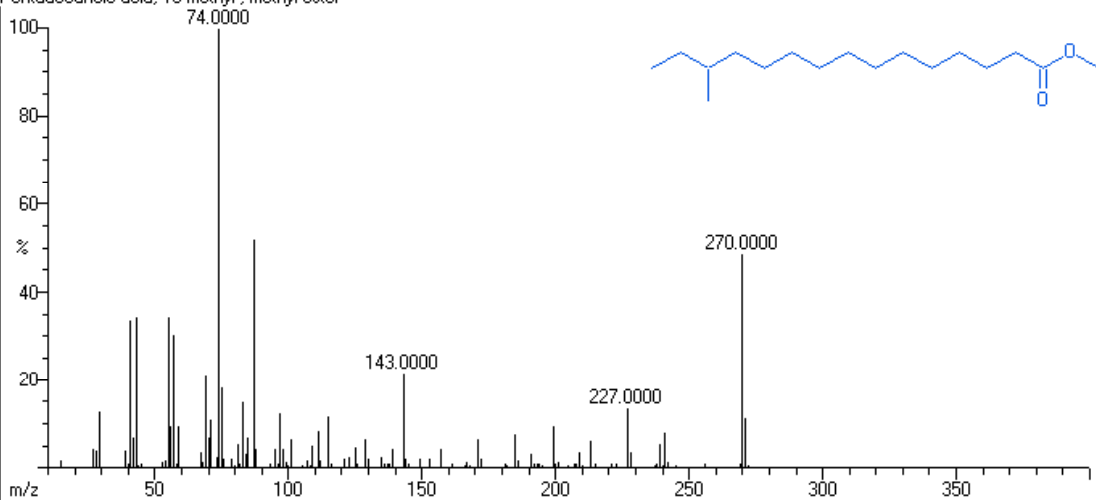


THRI DRAVAGAM

Scan: 561 TIC=64329504 Base=100%FS #ions=1562 RT=15.07

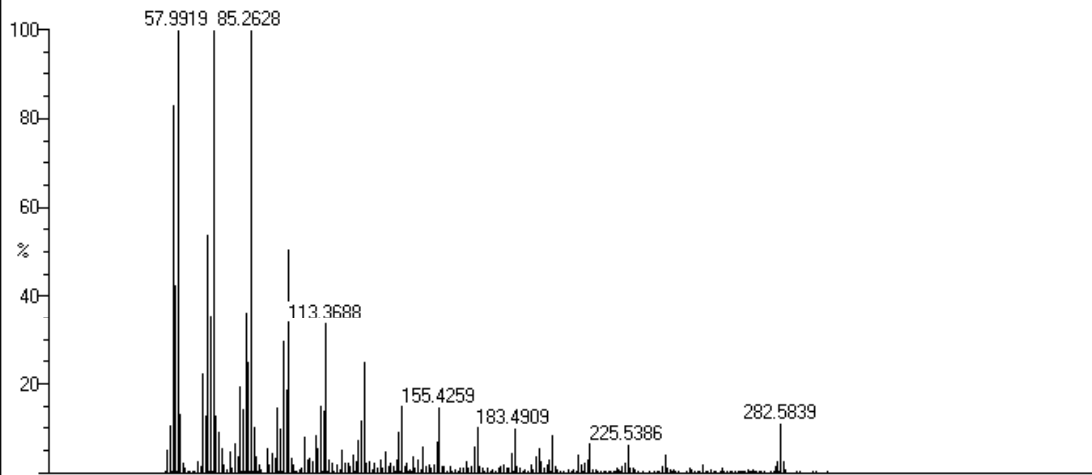


NIST MS 1 of 100 (5487-50-3 #ions=193
Pentadecanoic acid, 13-methyl-, methyl ester



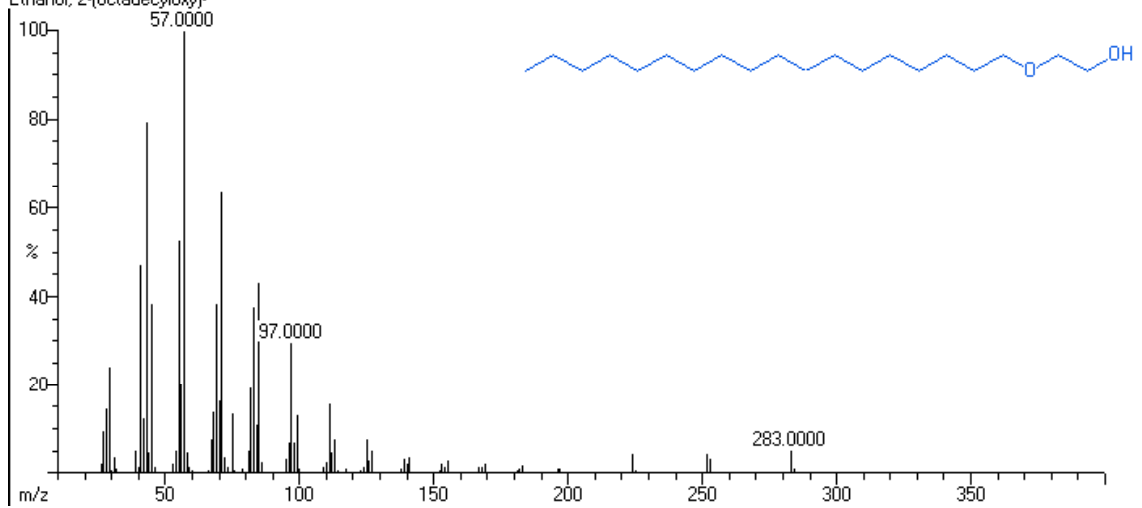
THRI DRAYAGAM

Scan: 589 TIC=18348352 Base=100%FS #ions=1477 RT=15.77



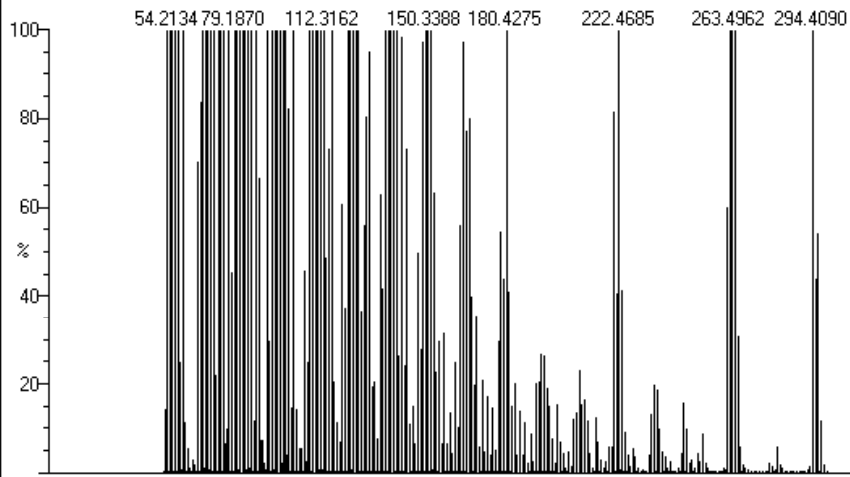
NIST MS 1 of 100 (2136-72-3) #ions=228

Ethanol, 2-(octadecyloxy)-

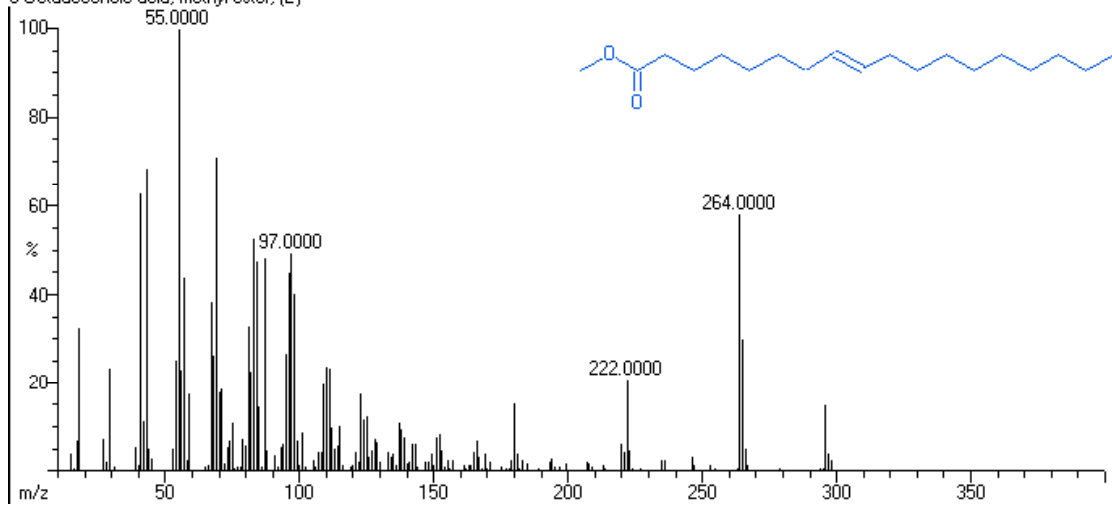


THRI DRAVAGAM

Scan: 630 TIC=102348304 Base=100%FS #ions=1252 RT=16.8



NIST MS 1 of 100 (26528-50- #ions=278
8-Octadecenoic acid, methyl ester, (E)-



GAS CHROMATOGRAPHY RESULTS OF THIRIKADUGU DRAVAGAM

Table-7

S.No	RT	Name of the compound	Molecular formula	Molecular weight(gm/mol)
1.	7.5	9-Methyl-Z-10-Pentadecan -1-ol	C ₁₀ H ₂₀ O	156.27
2.	8.87	Tetradecane,2,6,10-trimethyl	C ₁₇ H ₃₆	240.4677
3.	10.17	2-(2-Azepan-1-yl-2-oxoethyl)-1-hydroxy-1-phenyl-octahydro-pyridol(1,2-a)azepin-4-one	C ₂₄ H ₃₄ N ₂ O ₃	398.53836
4.	11.4	1-Hexadecanol,2-Methyl	C ₁₇ H ₃₆ O	256.4671
5.	12.58	N(2(1-Piperazyl)ethyl)-N-(2-thiophosphatoethyl)1,3-propanamine	C ₁₁ H ₂₇ N ₄ O ₃ PS	326.395842
6.	15.07	Pentadecanoic acid,13-Methyl,methyl ester	CH ₃ (CH ₂) ₁₃ COOH	242.40
7.	15.77	Ethanol,2-(octadecyloxy)	C ₂₀ H ₄₂ O ₂	314.55
8.	16.08	8-Octadecenoic acid,methyl ester,(E)	C ₁₉ H ₃₆ O ₂	296.48794

Through GC-MS Analysis, can find the name, molecular weight and structure of the components of the test drug Thirikadugu dravagam. Images shows the characteristic Gas chromatograph of *Thirikadugu dravagam*.

In this sample there are 8 compounds were identified, they are the following

- 9-Methyl-Z-10-Pentadecan-1-ol
- Tetradecane,2,6,10-trimethyl-
- 2-(2 Azepan-1-yl-2-oxoethyl)-1-hydroxy-1-phenyl-octahydro-pyrido(1,2-a)azepin-4-one
- 1-Hexadecanol,2-Methyl
- N-(2-(1-piperazyl)-N-(2-thiophosphatoethyl)-1,3-propanamine
- Pentadecanoic acid, 13-methyl-methyl ester
- Ethanol,2-(octadecyloxy)
- 8-octadecenoic acid, methyl ester,(E)

**TOXICITY STUDY OF *THIRIKADUGU DRAVAGAM*
ON WISTAR ALBINO RATS**

6.1 ACUTE ORAL TOXICITY STUDY

Table :8 Dose finding experiment and its behavioural Signs of Acute oral toxicity

No	Dose ml/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	5	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	10	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1.Alertness 2.Aggressiveness 3.Pilo erection 4.Grooming 5.Gripping 6.Touch Response 7.Decreased Motor activity 8.Tremors 9.Convulsions 10.Muscle Spasm 11.catatonia 12.Muscle relaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos 17.Diarrhoea 18. Writhing 19.Respiration 20.Mortality

(+) Indicates presence

(-) Indicates absence

Interpretation

The acute toxicity result shows no mortality rate up to dose level of 10ml/kg. The normal behavioural changes were observed in first four hours and no mortality was reported after 14 days observation. Hence the test drug *Thirikadugu dravagam* is safe up to the dose of level of 10ml/Kg in oral administration.

**REPEATE 28 DAYS ORAL TOXICITY STUDY OF THIRIKADUGU
DRAVAGAM ON WISTAR ALBINO RATS**

Table : 9 Body weight of wistar albino rat groups when exposed to *thirikadugu dravagam*

Treatment	0th day	5th day	10th day	15st day	20th day	25th day	28th day
Control	155.25 ±2.54	159.47± 6.25	161.55 ±4.48	164.53± 2.71	166.77 ±6.11	169.21 ±4.22	171.66 ±3.20
Mid dose	171.15 ±6.58	173.83± 6.55	175.33 ± 4.31	176.53± 4.25	178.32 ±3.28	180.16 ±4.37	182.83 ±5.37
High dose	158.83 ±9.54	159.66± 6.09	160.50 ±3.58	163.01± 7.26	165.33 ±7.54	168.53 ±8.31	170.16 ±5.61

Data are expressed as mean ± SEM (n = 10 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett's test

Table: 10 Relative Organ Weight of wistar albino rat groups when exposed to *thirikadugu dravagam*

Dose	Relative Organ Weight of rats					
	Liver	Kidney	Brain	Lungs	Heart	Spleen
Control	2.8±0.1	0.66±0.02	0.38±0.22	0.29±0.01	0.29±0.01	0.15±0.01
Mid dose	2.89±0.1	0.65±0.02	0.42±0.01	0.30±0.02	0.31±0.01	0.17±0.01
High dose	3.01±0.1	0.66±0.03	0.42±0.01	0.31±0.01	0.31±0.01	0.16±0.01

Data are expressed as mean ± SEM (n = 10 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett's test

Table : 11 Sub acute toxicity study - Food intake (gms/day) of Wistar albino rats group exposed *Thirikadugu dravagam*

Treatment	0th day	7th day	14th day	21th day	28th day
Control	37.83±1.8	36.50±1.67	38.83±1.31	39.66±1.46	40.66±1.50
Mid dose	40.16±0.38	42.33±0.60	42.83±0.61	43.16±1.32	44.13±1.37
High dose	40.63±0.58	42.66±1.09	43.01±1.56	43.53±1.31	44.16±1.67

N.S- Not Significant, **($p < 0.01$), *($p < 0.05$), $n = 10$ values are mean \pm S.D (One way ANOVA followed by Dunnett's test)

Table : 12 Sub acute toxicity study - Water intake (ml/day) of Wistar albino rats group exposed to *THIRIKADUGU DRAVAGAM*

Treatment	0th day	7th day	14th day	21th day	28th day
Control	30.0±0.48	31.16±0.22	31.13±0.33	32.2±0.16	35.46±0.34
Mid dose	31.73±0.89	32.52±1.22	32.41±1.11	34.45±1.05	35.64±1.42
High dose	32.66±1.15	35.01±1.64	33.33±1.77	34.16±1.43	36.5±1.52

Data are expressed as mean \pm SEM ($n = 10$ for each group), * $P < 0.05$, ** $P < 0.01$ were considered significant using One way ANOVA followed by Dunnett's test

Table: 13 :Hematological Parameter of Thirikadugu dravagam

Haematological parameter	Control	<i>Thirikadugu dravagam</i>	
		Mid dose	High dose
Total R.B.C. count ($\times 10^6 \text{ mm}^{-3}$).	9.09 \pm 0.15	9.11 \pm 0.85	9.61 \pm 0.16
Total W.B.C. Count ($\times 10^3 \text{ mm}^{-3}$).	12.67 \pm 0.22	12.62 \pm 0.526	12.72 \pm 0.072
Haemoglobin (Hb) (g/dl)	14.61 \pm 0.36	14.68 \pm 0.475	14.81 \pm 0.781
Hematocrit (%).	44.21 \pm 1.01	42.7 \pm 0.852	42.75 \pm 1.072
Platelets ($\times 10^3 \text{ mm}^{-3}$).	830.91 \pm 24.01	832.21 \pm 21.55	833.58 \pm 15.25
Lymphocytes(%).	72.7 \pm 1.32	75.28 \pm 2.63	77.8 \pm 5.58
Neutrophils (%).	20.6 \pm 0.65	19.6 \pm 1.357	19.9 \pm 0.69

Data are expressed as mean \pm SEM (n = 6 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett's test

Chart I : Sub acute toxicity - The mean value of Total RBC, WBC and HB of control and treated groups Of wistar albino rats exposed to *Thirikadugu dravagam*

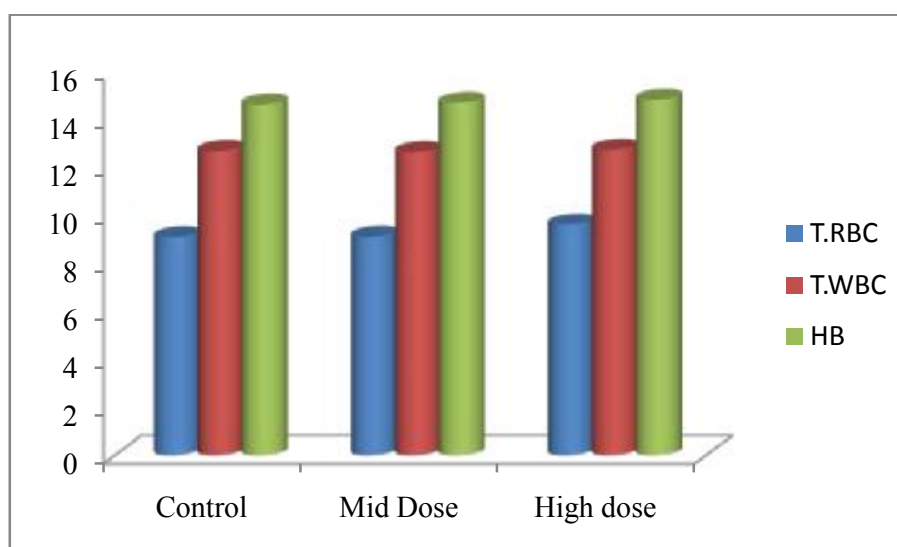


Chart 2 - Sub acute toxicity - The mean value of Total Neutrophil, leucocyte and hematocrit of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*.

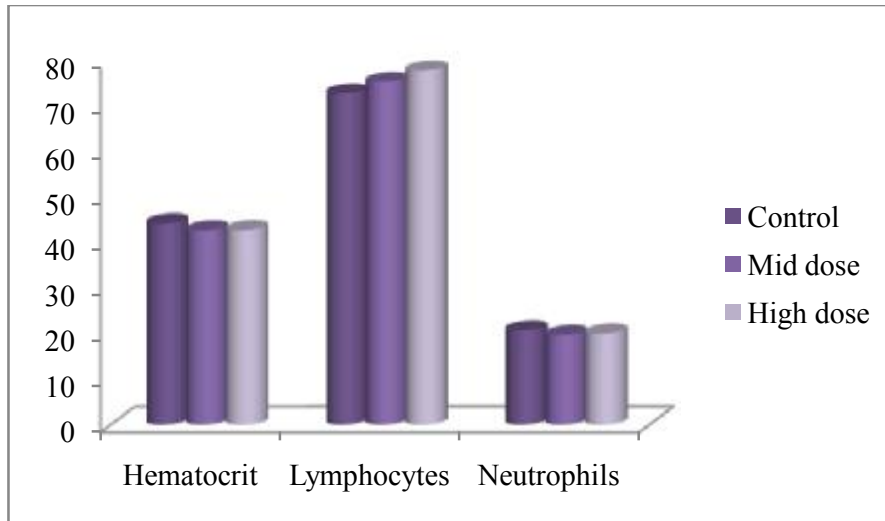


Chart -3 Sub acute toxicity - The mean value of Platelets of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*.

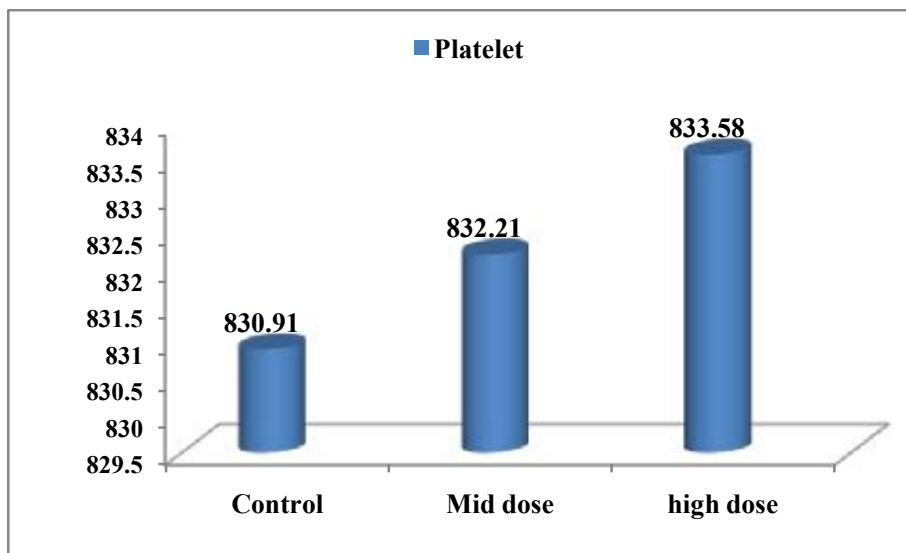


Table: 14 Biochemical Parameters of Thirikadugu dravagam

Biochemical parameter	Control	Trail drug	
		Mid dose	High dose
Creatinine (mg/dl)	0.52±0.079	0.54±0.07	0.57±0.29
Urea (mg/dl)	14.30 ± 0.47	14.33±0.49	14.74±1.078
Blood Glucose(R)	109±0.41	110±0.371	118±0.261
Triglycerides (mg/dl)	50.20±1.13	52.23±1.08	49.17±1.86
Total Cholesterol (mg/dl)	46.60±1.21	45.460±1.08	45.836±1.05
Total protein (mg/dl)	4.40±0.267	4.12±0.371	4.220±0.41
Albumin (g/dl)	3.20±0.41	3.30±0.351	3.29±0.261
AST (IU/L)	121.41±2.68	119.8±4.67	120.76±4.086
ALT (IU/L)	68.40±1.57	69.012±2.32	69.99±3.521
ALP (IU/L)	112.6±4.67	115.01±1.021	116.41±2.108
T. Bilirubin (mg/dl)	0.91±0.32	0.83±1.012	0.88±0.182

Values are mean ±S.E.M. (n=10) ***p<0.001, ** p<0.01, * p<0.05 compared with the control animals.

Chart -4 Sub acute toxicity - The mean value of Urea, creatinine of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*

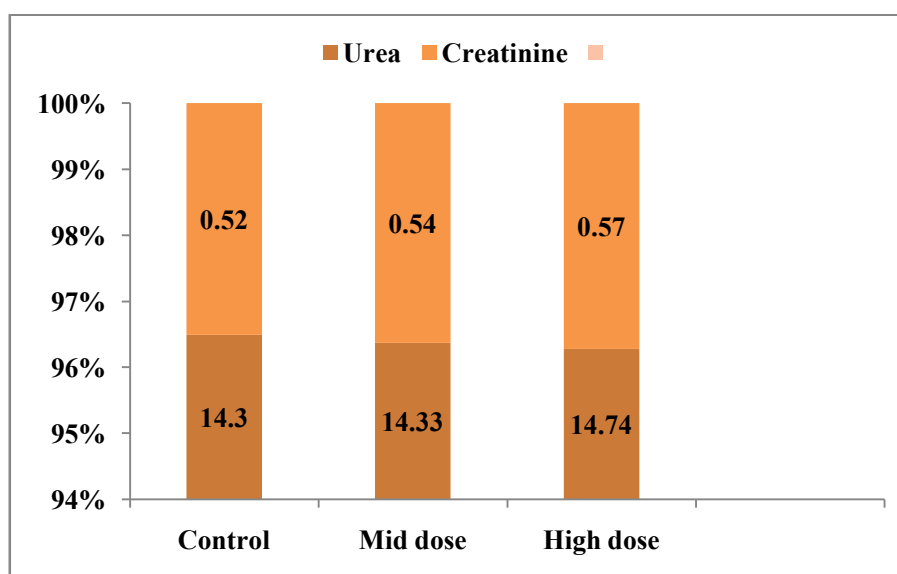


Chart-5 Sub acute toxicity - The mean value of T.Cholestrol, Triglycerides,B lood Glucose of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*

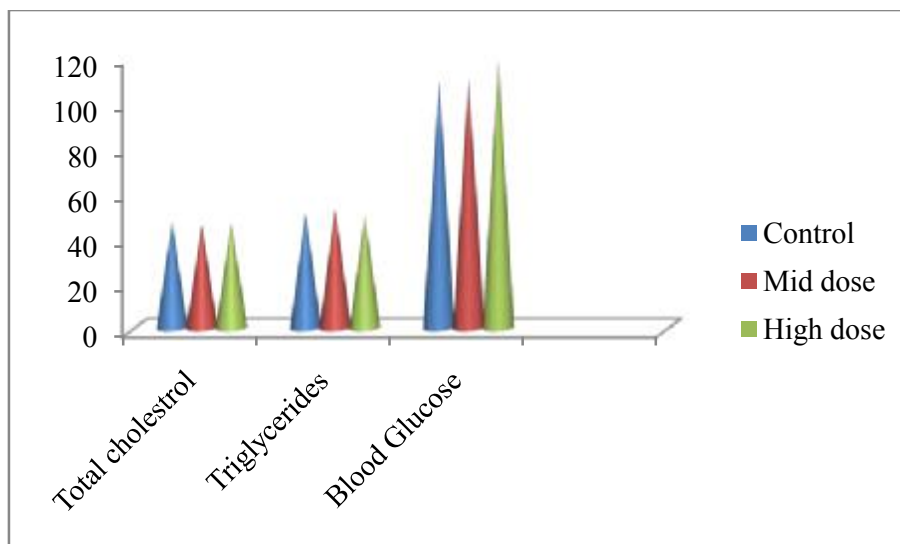
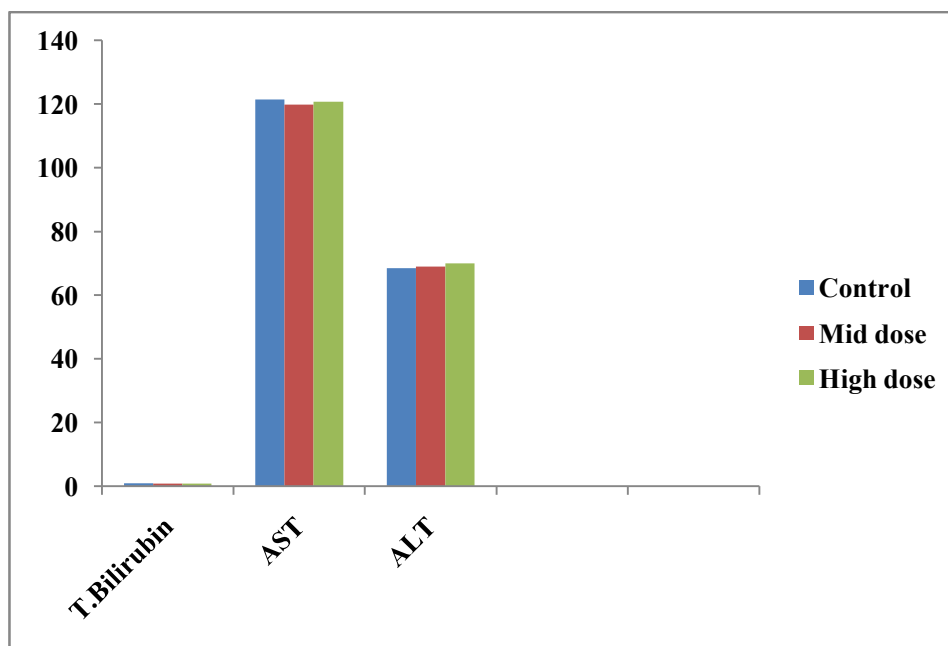


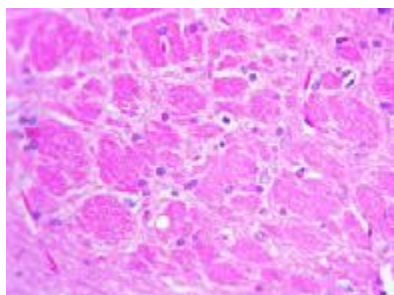
Chart-6 Sub acute toxicity - The mean value of T.Bilirubin, AST,ALT of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*



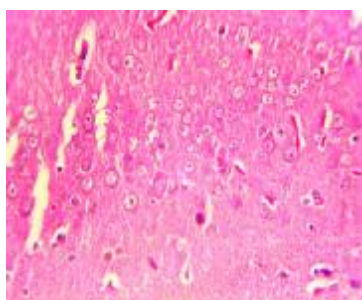
**HISTOPATHOLOGY OF *THIRIKADUGU DRAVAGAM* – REPEATED
DOSE 28 DAYS ORAL TOXICITY STUDY**

BRAIN

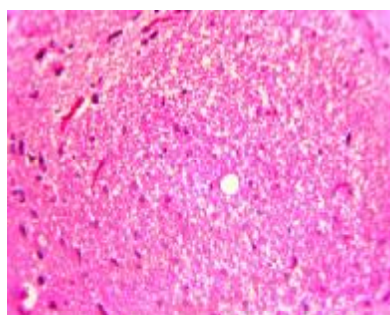
1Control



Mid dose

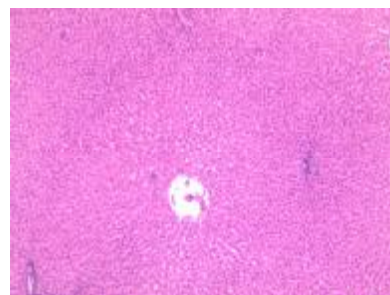


High dose

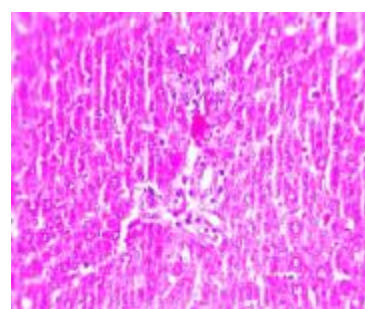


LIVER

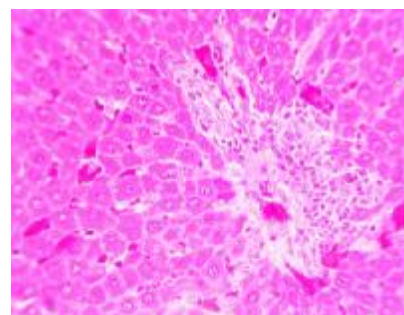
Control



Mid dose

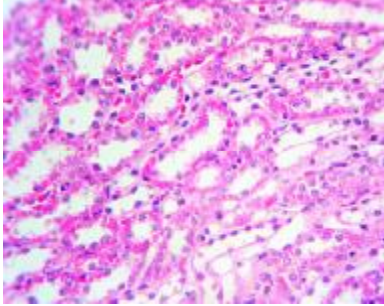


High dose

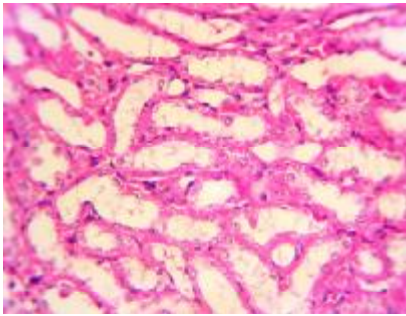


KIDNEY

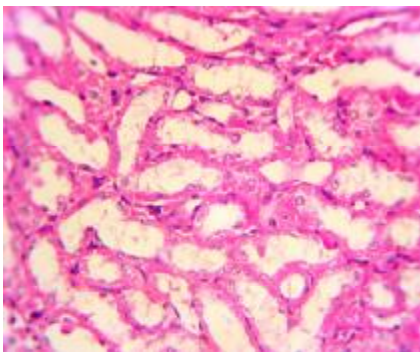
Control



Mid dose

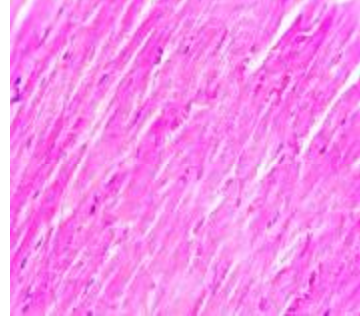


High dose

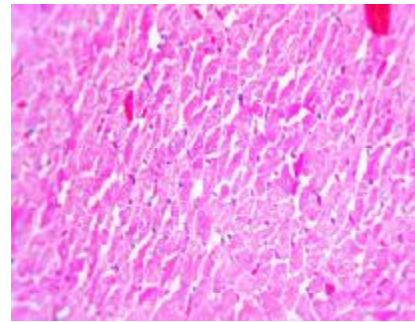


HEART

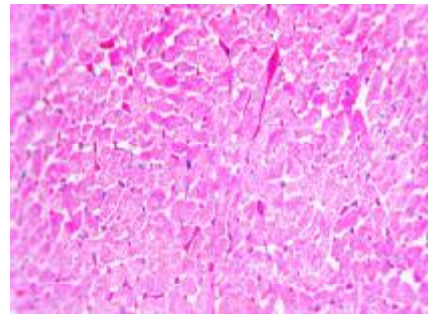
Control



Mid dose

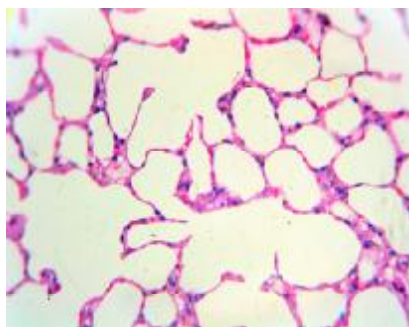


High dose

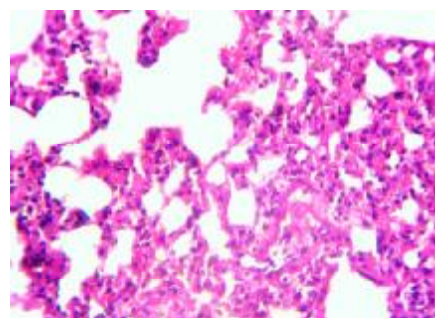


LUNGS

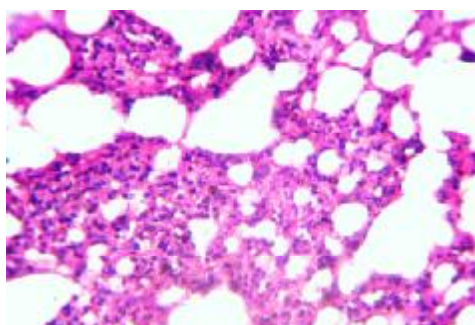
Control



Mid dose



High dose



RESULT

The above slide shows the histopathology studies of sub-acute toxicity studies. There is no toxicological abnormality seen in vital organ after administration of the test drug *Thirikadugu dravagam*. Thus the safety of the drug is revealed so that it can be administered for longtime without any side effects

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days for sub acute toxicity study. There was no significant change in the body weight for the control and treatment group throughout the dosing period of 28 days.

The results of haematological investigations conducted on day 29th day revealed no significant changes in the haematological values when compared with those of respective controls. This gave clear justification that bone marrow and spleen were not influenced by *Thirikadugu Dravagam*.

Results of Biochemical investigations conducted on days 29 and recorded in revealed the no significant changes in the values of different parameters studied when compared with those of respective controls; Urea, SGOT,SGPT, Bilirubin were within the limits.

The other cardio vascular risk markers were also within normal ensured that Thirikadugu Dravagamdid not influence the Cardio vascular system.

The vital organs such as liver, heart, kidneys, lungs and brain were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Cross pathological investigation was carried out and histopathology of vital organ reveled normal histological appearance when compared with the control.

Organ weights of treated animals with respective control animals on day 29 was found to be comparable with respective control group. Gross pathological examination of animals did not reveal any abnormalities. Histopathology examination did not reveal any abnormal macroscopic changes.

Table ; 15 6.3 Sub-chronic toxicity study - Body weight of wistar albino rats when exposed to Thirikadugu dravagam

DAYS	GROUPS			
	Control	Low dose	Mid dose	High dose
1	163.6±33.683	150.1 ± 21.105	158.6± 13.57	168.5± 28.75
15	172.8 ± 28.870	161.5 ± 21.706	175.7 ± 29.01	182.8 ± 32.48
30	185.8 ± 28.310	186.7 ± 14.88	186.8 ± 32.11	190 ± 28.94
45	204.5± 27.73	205.5± 29.76	206.7± 19.75	207± 22.75
60	227.6±33.683	233.6±23.683	237.6±33.683	224.6±23.783
75	242.8 ± 26.85	247.8 ± 28.870	242.8 ± 28.870	243.8 ±26.870
90	265± 27.320	267± 27.320	268± 27.320	266± 36.320*

NS Non Significant, **($p > 0.01$),*($p > 0.05$), n=6 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Table 16 : Sub-Chronic Toxicity Study – Hematological parameters of Thirikadugu Dravagam

Parameters	control	TD (lowdose)	TD (Mid dose)	TD (High dose)
RBC cells/cu.mm	7.5± 0.38	7.23±0.40	7.26±0.22	7.21±0.37
WBC cells/cu.mm	9586±417	11016±483	11016±549	11350±500
Platelet cells/ul	3.4±0.27	3.68±0.11	3.41±0.34	3.26±0.22
PCV %	38.85±2.70	39.5±2.33	39.5±2.33	40.1±1.6
HB (g/dl)	12.95±0.90	13.16± 0.78	13.2± 1.02	13.2±0.35
MCV	93.5±6.89	95±2	91±7.37	93.66± 3.22
MCH	32.83±2.31	32.66±3.72	34.5±5.85	33.5±1.37
MCHC	34.83±2.48	32.16±2.92	34.16±2.48	33.83±2.71

**Table 17 Biochemical parameter Sub-Chronic Toxicity Study
of Thirikadugu Dravagam**

Parameters	control	TD low dose	TD Mid dose	TD High dose
Glucose mg/dl	94.66±14.34	114.16±6.27	120.5±6.59	107±14.05
Urea mg/dl	29.5±8.89	26.66±3.88	30.33±12.90	34.83±4.99
Creatinine mg/dl	0.58±0.13	0.51±0.14	0.58±0.20	0.51±0.11
T.Bilirubin mg/dl	0.58±0.07	0.65±0.10	0.81±0.07	0.71±0.17
SGOT (U/dl)	22.33±7.31	24±8.22	22±5.44	26.16±5.60
SGPT (U/dl)	25.66±4.96	24±3.84	28±5.65	27.83±5.98
ALP (U/dl)	63.±8.07	77.33±17	75.16±11	60.5±9.93
T.Cholestrol mg/dl	119.16±7.11	114±5.17	105.83±11.12	112.6±4.80
HDL mg/dl	37±4.14	41.16±2.78	42.33±3.38	39.16±3.48
LDL mg/dl	55.66±6.12	44.66±5.50	36.5±12.72	47.66±1.36
VLDL mg/dl	28.56±5.76	28.76±4.31	28.63±3.44	24.5±0.95

Data are expressed as mean ± SEM (n = 6 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett's test

Chart 8-Sub Chronic toxicity study- The mean value of T.RBC, Hb &platelet of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*

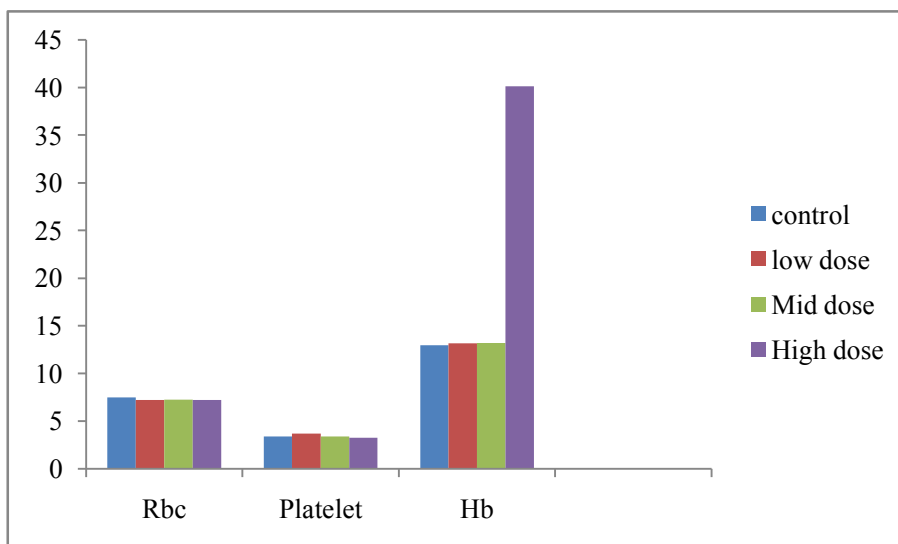


Chart-9 Sub Chronic toxicity study- The mean value of MCV, MCH, MCHC & PCV of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*

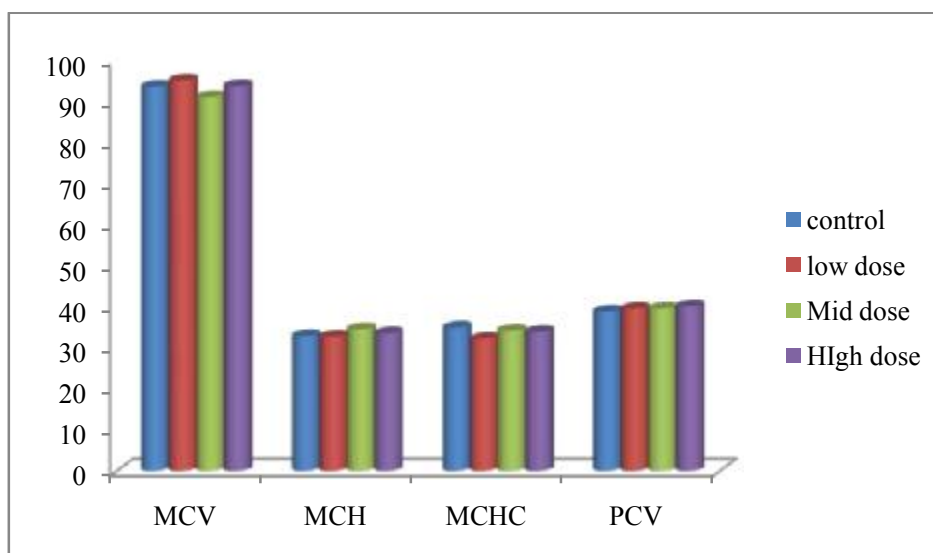


Chart 10 Sub Chronic toxicity study- The mean value of WBC of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*

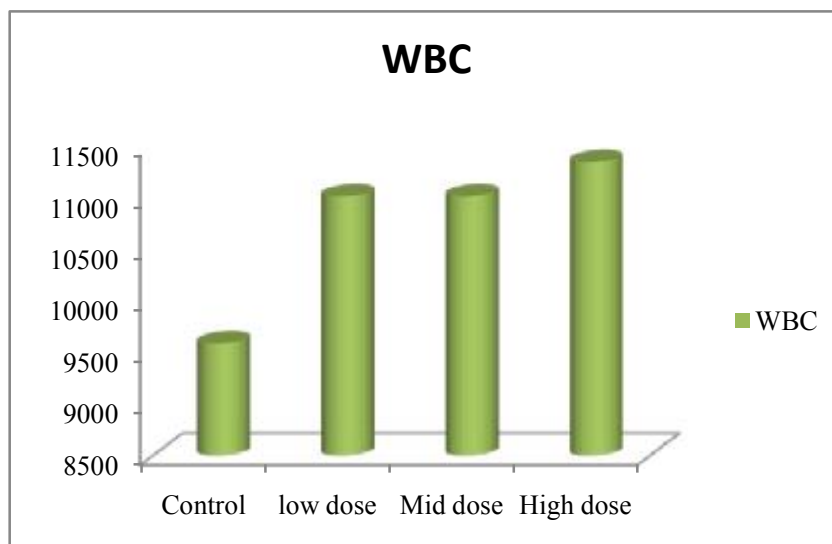


Chart 11- Sub Chronic toxicity study- The mean value of urea and creatinine of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*

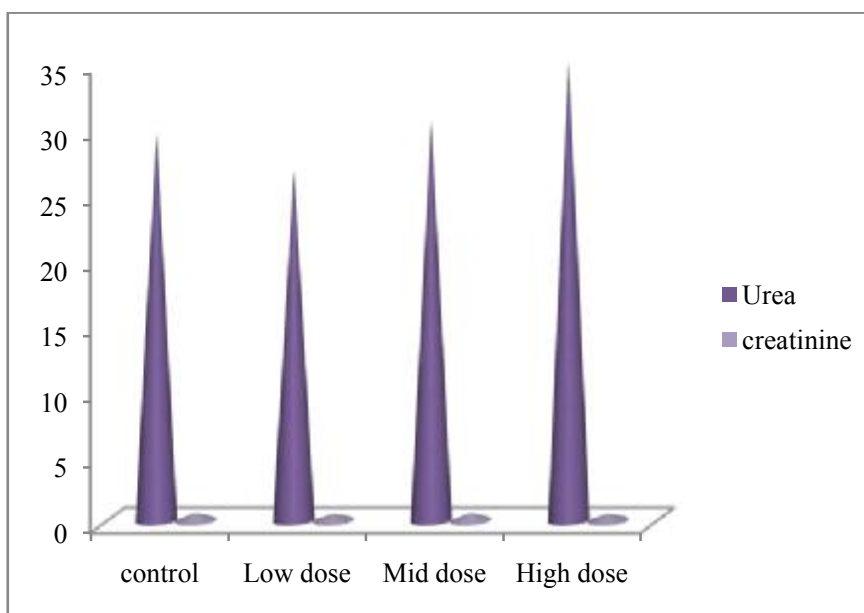


Chart 12- Sub Chronic toxicity study- The mean value of lipid profile of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*

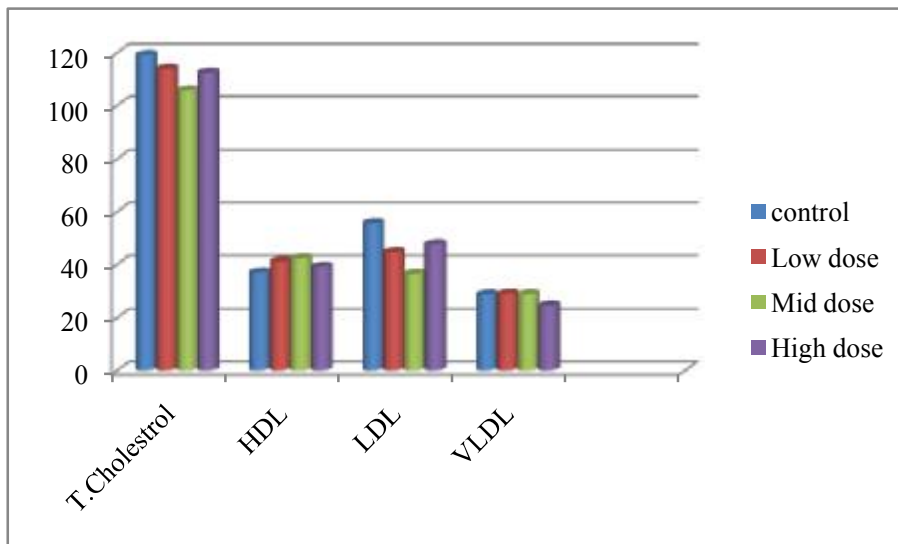


Chart 13- Sub Chronic toxicity study- The mean value of liver function test of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*

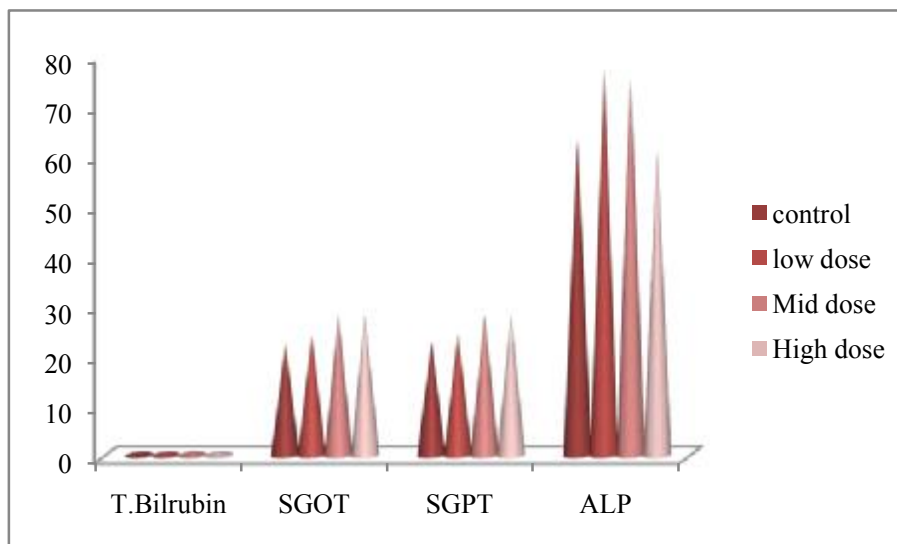
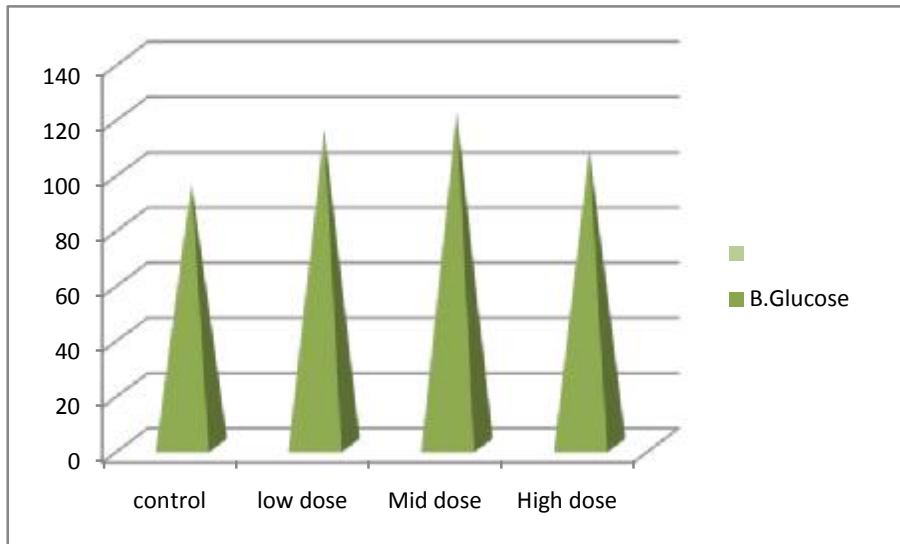


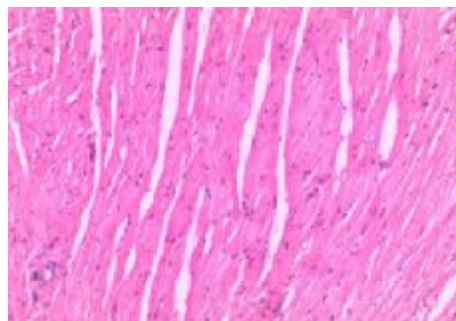
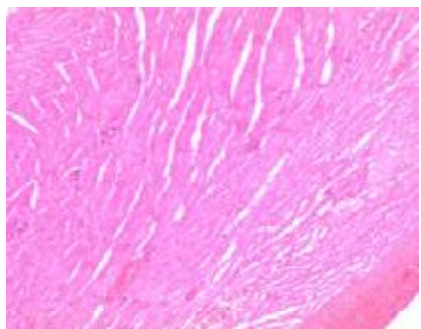
Chart 14-Sub Chronic toxicity study- The mean value of Blood glucose of control and treated groups of wistar albino rats exposed to *Thirikadugu dravagam*



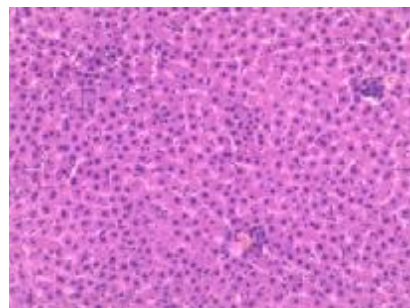
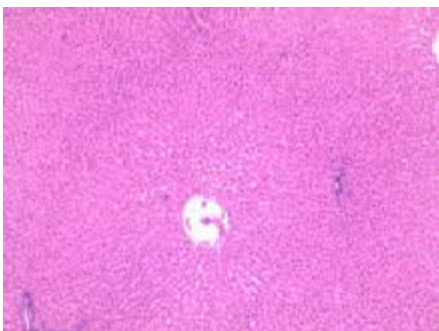
6.3 -Histopathology Reports for Sub chronic toxicity studies

Histopathology reports of Sub-chronic toxicity study of *Thirikadugu dravagam* when exposed to Control group of wistar albino rat.

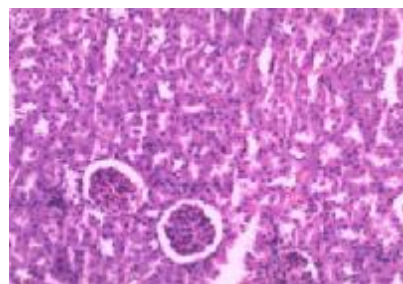
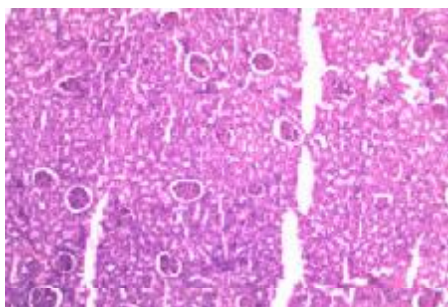
HEART



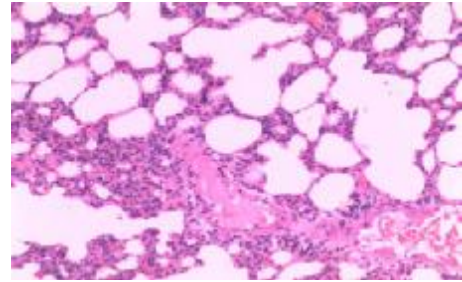
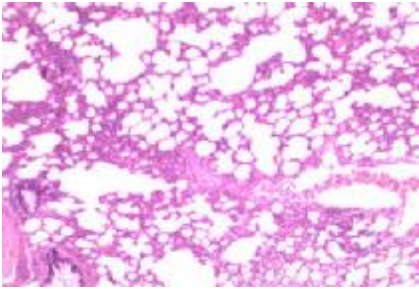
LIVER



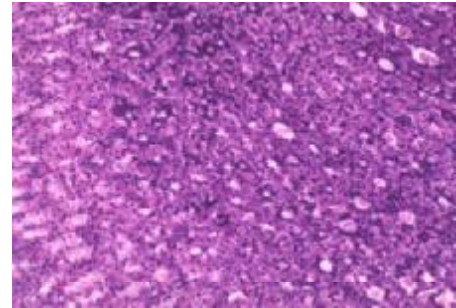
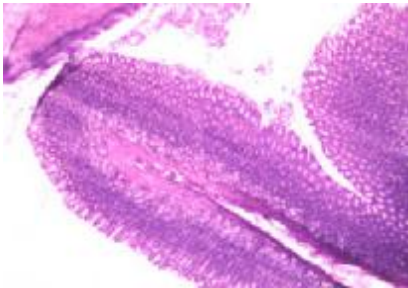
KIDNEY



LUNGS

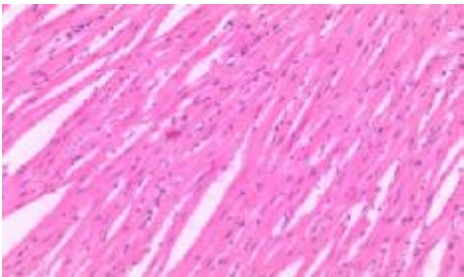
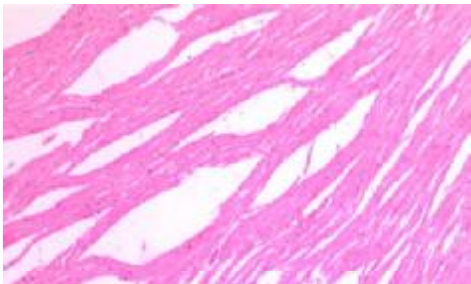


STOMACH

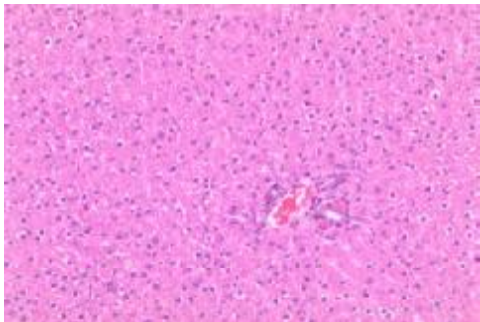
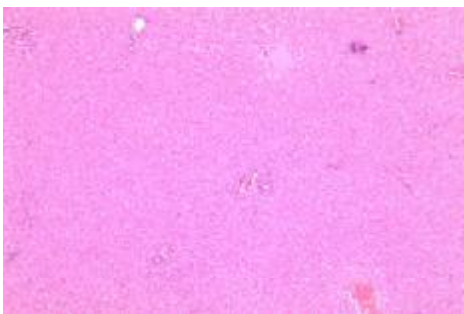


Histopathology reports of Sub-chronic toxicity study of *Thirikadugu dravagam* when exposed to High dose group of wistar albino rat.

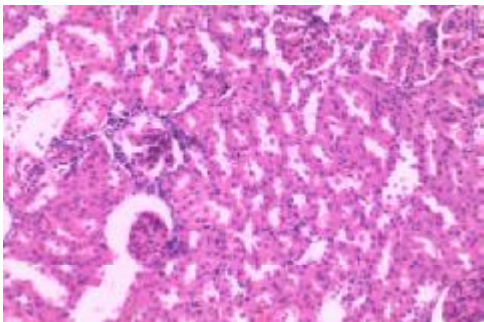
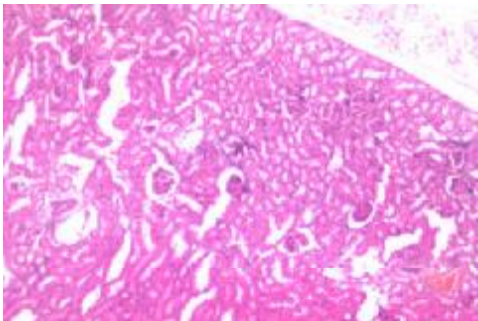
HEART



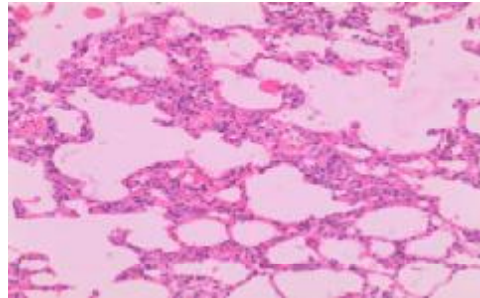
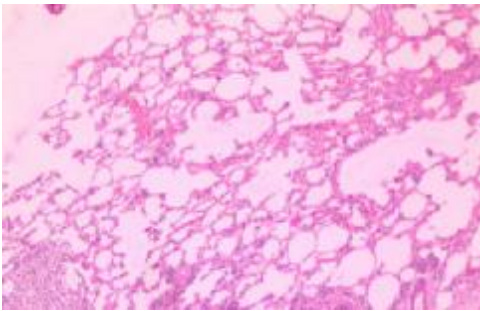
LIVER



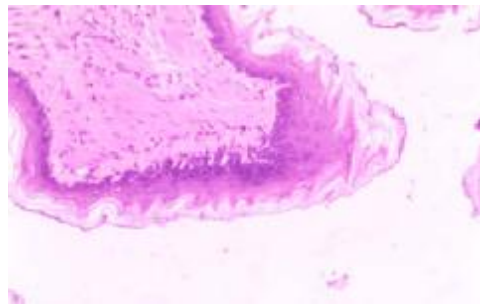
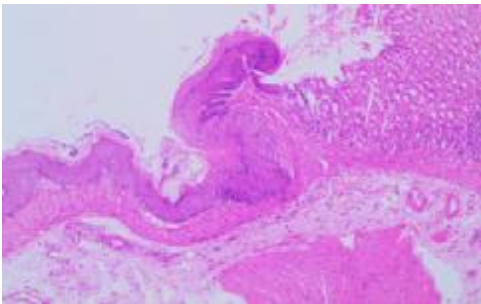
KIDNEY



LUNGS



STOMACH



Histopathology report

Kidney

- Junction between cortex and medulla appears distant
- Derangement in Interstitial connective tissue was observed in sample belongs to 4HM
- No signs of cellular necrosis
- Proximal and distal convoluted tubule appears normal

Heart

- Myocardial fiber mass appears denser with no signs of degeneration or fibrosis.
- Appearance of cardiomyocyte was normal with dark nuclear region. The nuclei of muscle fibers appear oval arrangement.

Liver

- Appearance of portal vein was normal
- Appearance of hepatic cord was normal and radial in nature, no signs of cellular degeneration

Lung

- Lung parenchyma appears normal with regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis
- Perivascular region appears normal, Alveolar septa and wall appeared widen and normal
- No signs of lymphocyte cuffing
- No signs of airway secretion and bronchial secretion
- Bronchial blood vessels and connective tissue appears normal with no sings of pulmonary edema

Stomach

- Light microscopic observation of both the sample reveals normal histology of rat gastric wall composed of mucosa, muscularismucosa, submucosa, muscularispropiria and adventitia.
- No signs of ulceration were observed

Interpretation of Sub-chronic toxicity of *Thirikadugu dravagam*

Sub-chronic oral toxicity repeated dose of *Thirikadugu dravagam* on rats were conducted. All animals from the treated dose survived throughout the dosing period of 90 days. Various parameters were studied and the interpretation of the study result is discussed below.

Body weight

The result of the body weight of rats exposed to control and the *Thirikadugu dravagam* of different dose groups exhibited overall weight gain throughout the dosing period of 90 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

Haematological investigation interpretation:

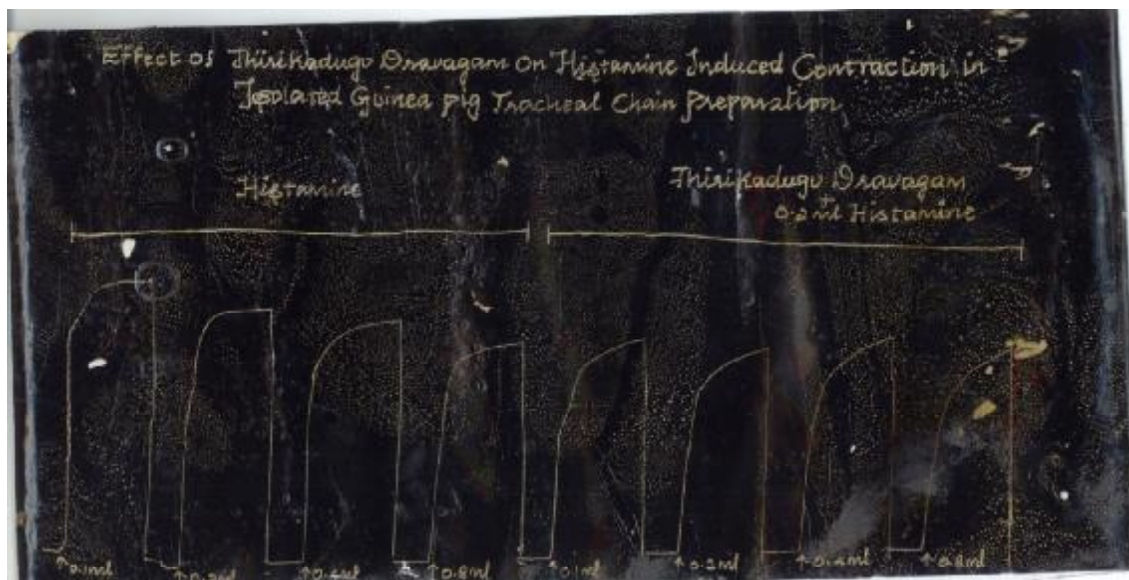
The haematological investigation results of the rats conducted on 91st day after the repeated dose of the drug revealed the values of different parameters. The increase and decrease in the values obtained were all within the normal biological and laboratory limits.

Biochemical investigation interpretation:

The biochemical investigations were conducted on 90th day and the result is produced. The results revealed there are no significant changes in the values of different parameters with that of the control. values were within the normal biological and laboratory limits.

7.1 PHARMACOLOGICAL RESULTS OF THIRIKADUGU DRAVAGAM

Bronchodilator activity using isolated guinea pig tracheal chain preparation



Result

The contractile of tracheal chain in vitro has often been utilized for the study of contractile response of agonists as well as antagonist. spasmogen such as histamine produces dose dependent contraction of guinea pig tracheal chain preparation. In the present study, the trial drug *Thirikadugu dravagam* significantly inhibited the histamine- induced contractions of isolated guinea pig tracheal chain preparation, indicating Bronchodilator activity

7.2 Anti-histaminic activity Of Thirikadugu dravagam using Guinea pig

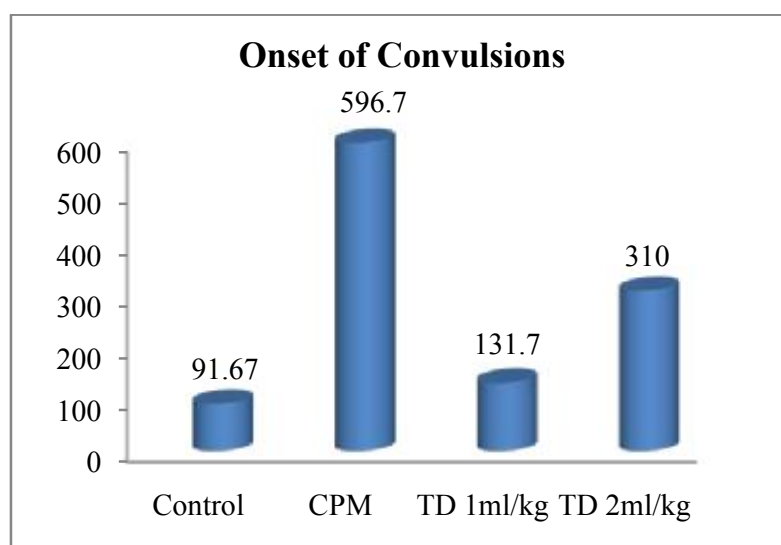
Table :18

Serial No	Group	Onset of Convulsion in sec.
1	Control	91.67± 2.028
2	Standard (Chlopheniramine maleate)	596.7± 46.31***
3	Thirikadugu Dravagam (1ml/kg)	131.7± 6.00 *
4	Thirikadugu Dravagam (2ml/kg)	310.0± 20.82 **

Values are mean ±S.E.M. (n=6) ***p<0.001, ** p<0.01, * p<0.05 compared with the control animals.

Result

The result of present study suggested that guinea pig exposed to histamine aerosol showed Signs of progressive Dyspnea leading to convulsions. The two doses of *Thirikadugu dravagam* prolonged the latent period of convulsion as compared to control following the exposure to histamine aerosol. Thus our findings suggest that *Thirikadugu dravagam* possess significant antihistaminic activity.



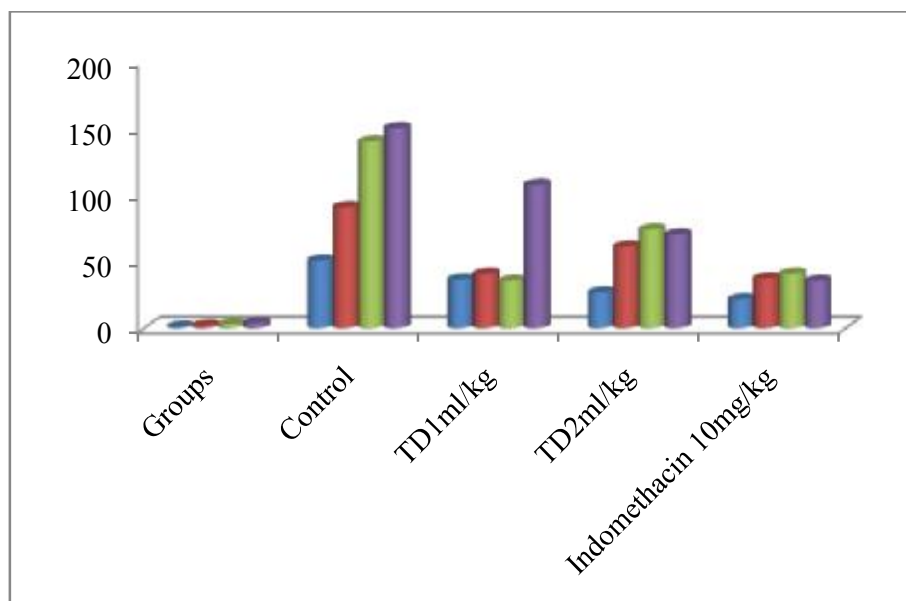
7.3 Anti-inflammatory activity:

Results:

Treatment	Percentage inflammation after carageenan injection at hr			
	1	2	3	4
Control	51.08±3.12	91.25±7.78	140.15±8.27	150.16±2.87
T.Dravagam 1ml/kg	36.50±5.58 **	78.50±3.54 *	100.18±2.29 **	107.45±5.68 **
T.Dravagam 2ml/kg	26.62±2.26 ***	61.12±3.90 ***	74.16±6.16 ***	70.34±2.12 ***
Indomethacin 10mg/kg	21.45±7.87 ***	37.43±3.23 ***	40.79±2.94 ***	35.50±3.14 ***

Values are mean ±S.E.M. (n=6) ***p<0.001, ** p<0.01, * p<0.05 compared with the control animals.

Thirikadugu dravagam exhibited significant anti-inflammatory activity when compare to the control group.



Discussion

9. DISCUSSION

The drug **Thirikadugu dravagam** was selected from the siddha literature “yaakobu vaithiya chinthamani” 700 to evaluate the safety and Pharmacological profile in animal models.

The ingredients of the test drug was identified and authenticated by Botanist, The standard operative procedure were followed.

Review of various information Relevant to the study Both Modern and Siddha Aspect supported the study.

According to AYUSH protocol Physical characterization, like PH, Specific Gravity were evaluated. PH of Thirikadugu dravagam is 4.65, Eventhough it is acidic, Siddhars indicated this for internal use with equal amount of water, to Neutralise the acid.

Specific Gravity is commonly used in pharmaceutical laboratory to determine the concentration of the solution. Specific gravity of Thirikadugu dravagam is 0.9992.

Elemental analysis is also essential to ensure the Safety and efficacy of drug. Test drug shows heavy metals below the detected limit.

GC-MS: Have been used for identification of large number of components present in natural and biological systems. Hence the drug is consider as safe drug.

chemical analysis: chemical analysis of the drug Thirikadugu dravagam reveals that the presence Chloride. Calcium, alkaoloids.

All these analytical studies shows, The drug is safe and shows Sufficient Molecules of therapeutic benefits for the safety and efficacy of the Drug Thirikadugu Dravagam.

Toxicological studies:

Before going to clinical study, It is essential to evaluate the toxicity stiudes. TheSE studies are important to Globalise the siddha system of medicine. Based on these Acute, Sub-acute, and Sub-chronic studies were carried out using wistar albino rats.

This study reveals that, no significant toxic effect of the drug Thirikadugu dravagam upto the higher dose level of 10ml/kg in acute oral toxicity, sub- acute and sub chronic toxicity studies. Therefore the drug *Thirikadugu dravagam* is safe on animal experimentation, and there is no toxicity of administration. Hence it can be classified under the category of drug with non-toxic.

Pharmacological studies:

Pharmacological study was carried out in animal models Tthe experimental data showed that Thirikadugu dravagam has bronchodilator, Anti-histaminic Anti-inflammatory activity and the results are as follows,

Bronchodilator activity:

The contractile of tracheal chain in vitro has often been utilized for the study of contractile response of agonists as well as antagonist.spasmogen such as histamine produces dose dependent contraction of guinea pig tracheal chain preparation. In the present study, the trial drug *Thirikadugu dravagam* significantly inhibited the histamine- induced contractions of isolated guinea pig tracheal chain preparation, indicating Bronchodilator activity

Anti-histaminic activity:

The result of present study suggested that guinea pig exposed to histamine aerosol showed sings of progressive Dysponea leading to convulsions. The two doses of *Thirikadugu dravagam* prolonged the latent period of convulsion as compared to control following the exposure to histamine aerosol. Thus our findings suggest that *Thirikadugu dravagam* possess significant antihistaminic activity.

Anti- inflammatory activity:

Thirikadugu dravagam exhibited significant anti-inflammatory activity when compare to the control

Summary

10 . Summary

- The test drug *Thirikadugu dravagam* was selected from the siddha literature “*Yaaakobu vaithiyachinthamani*” for its Bronchodilator, Antihistaminic and Anti-inflammatory activities.
- The test drug was prepared by the given procedure. All the ingredients were identified and authenticated by the experts.
- Review of literature in various categories was carried out. Siddha aspect, botanical aspect disclosed about the drug .
- The drug was subjected to analysis such as physicochemical, photochemical, chemical analysis, ICPOES, GC-MS which provided the key ingredients present in the drug thus it accounts the efficacy of the drug
- Toxicological study was made according to OECD guidelines comprising acute, sub-acute and sub chronic toxicity study. It screens the safety of the drug.
- Pharmacological study was done. It revealed of *Thirikadugu dravagam* in animal model, Bronchodilator, Anti-histaminic activity using Guinea pig and Anti-inflammatory activities in wistar albino rats.
- Results and discussion gives the proper justifications to prove the potency of the drug.
- Conclusion gives a compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.
- Thus the herbal formulation *Thirikadugu dravagam* is validated for its Safety and efficacy for treating Bronchial asthma and it would be a great drug of choice.

Conclusion

11. CONCLUSION

From the literature evidence, physicochemical analysis, Chemical analysis, Toxicological evaluation and Pharmacological studies, the drug *Thirikadugu Drvagam* has Bronchodilator, Anti-histaminic and anti-inflammatory activity. It is concluded that the drug *Thirikadugu Dravagam* can be used in the management of Bronchial asthma and the related Respiratory disorders.

Annexure



K.K. COLLEGE OF PHARMACY

(Approved by AICTE, PCI & Government of Tamilnadu and
Affiliated to The Tamilnadu Dr. MGR Medical University)

1/161, Sankaralinganar Road, • Gerugambakkam, • Chennai - 600128
Phone : (044) 32546162, Tele/Fax : 23821272

Ref: 4525/KKCP/2015

Date: 10.08.2015

APPROVAL CERTIFICATE

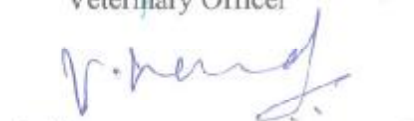
This is to certify that the project title "*Safety and pharmacological profile of THIRIKADUGU DRAVAGAM*" has been approved by IAEC and the details are furnished under

Project Code	Name of the species	Breakup sexwise	Weight	Number proposed	Number approved
KKCP/2015/029	Wistar Albino rat	25 Male + 31 female	150-200gms	60	56 male - 25 female- 31
	Guinea pig	2 male	350-400gms	6	2
Albino rat - fifty six only ; Guinea pig - two only					

Chairman IAEC


(Prof. A. Meena)


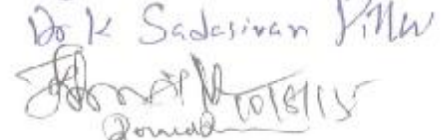

Veterinary Officer


(V. VAIDHYALINGAM)

CPCSEA Nominee


(Dr. C. Kathirvelan)

Members


Dr. K. Sadasivan Pillai

20/08/2015

CERTIFICATE

This is certify that the project title.....SAFETY PROFILE OF
....."THIRIKADUGU DRAVAGAM" (12.756.12 Fazole
Wistar Albino rats)
has been approved by the IAEC. (NO: NIS/IAEC-I/2016/04)

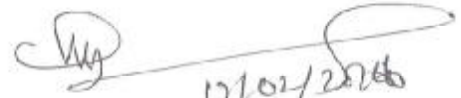
Name of Chairman/Member Secretary IAEC:
nominee:

Dr. B.R.SENTHILKUMAR

Signature with date

Name of CPCSEA

K. NACHIMUTHU


12/02/2016

Chairman/Member Secretary of IAEC:


24, Feb 2016

CPCSEA nominee:

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



NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

CERTIFICATE OF BOTANICAL AUTHENTICITY

Certified that the following plant drugs used in the Siddha formulation "Thirikadugu Dravagam" (Internal) taken up for Post Graduation Dissertation studies by Dr.M.Elakkiya, M.D.(S), II year, Department of Gurupadam, 2015, are identified and authenticated through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology, Micromorphology and Taxonomical methods as

Zingiber officinale Rose. (Zingiberaceae), Rhizome.

Piper nigrum Linn. (Piperaceae), Fruit

Piper longum Linn. (Piperaceae), Root

Elettaria cardamomum Maton (Zingiberaceae), Fruit

Carum copticum Benth. & Hook. f. (Apiaceae), Fruit

Cuminum cyminum Linn. (Apiaceae), Fruit

Certificate No: NISMB1842015

Date: 26-08-2015

Authorized Signatory

DR. D. ARAVIND, M.D.(S),M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha
Chennai - 600 047, INDIA



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

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

This certificate is awarded to

Dr./Mr./Ms. **M. ELAKKIYA**

for participating as **Resource Person** / Delegate in the Fourteenth 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 5th to 9th May 2014.


Dr. N. **KABILAN** M.D. (Siddha)
Reader, Dept. of Siddha


Dr. **JHANSTICHARIES**, M.D.
Registrar


Prof. Dr. D. **SHANTHARAM**, M.D., D.Diab.,
Vice-Chancellor

PROJECT COMPLETION CERTIFICATE

This is to certify that the project entitled **Safety and Pharmacological Profile of Thirikadugu Dravagam** has been approved by the IAEC committee of our institution. Dr. M. Elakkiya has completed the project work under the guidance of Dr. R. Prakash, Professor, Dept., of Pharmacology, K.K. College of Pharmacy, Chennai.



(Dr. R. Prakash)

Professor,

Project Guide



(Dr. A. Meena)

Principal





SOPHISTICATED ANALYTICAL INSTRUMENT FACILITY
INDIAN INSTITUTE OF TECHNOLOGY, MADRAS
Chennai - 600 036. INDIA

CERTIFICATE

This is to certify that Herbal Drug **Thirikadugu Dravagam** formulated by **Dr.M.Elakiya**, III year M.D(S), Department of GUNAPADAM, National Institute of Siddha, Chennai-47. Was analysed (qualitative/quantitative) by, GC-MS and ICPOES methods at SAIF, IITM, Chennai-36, during March 2016.

[DR.R.MURUGESAN]



Dr. R. Murugesan
Senior Scientific Officer
SAIF, IIT, Madras, Chennai-36.

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