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Pre-clinical and clinicl study on Amukkara kizhangu Chooranam for Hypolipidemic Activity in the management of Athimetham (Hyperlipidemia)

&

Pre-clinical and clinical study on Sarva Noi Linga Chenduram for lithontriptic Activity in the management of kalladaippu. (Renal calculi)

(DISSERTATION SUBJECT)

For the partial fulfillment of the

requirement to the Degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH II - GUNAPADAM

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Bonafide certificate

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TRIAL DRUG I: AMUKKARA KIZHANGU CHOORANAM

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INTRODUCTION

Siddha System is one of the ancient systems of medicine in the world . It is mainly based on three vital humours named Vali,Azhal and Iyyam. The inimitable of this system is efficacy of a single drug with various adjuvant evidenced for assortment of diseases. Siddha system not only deals with diseases , treatment , prevention , cure and also lifestyle.

Athimetham (Hyperlipidemia) is one of the life style modification diseases in our country. Hyperlipidemia which is increased levels of lipids. Hyperlipidemia includes both hypercholestremia and hypertriglyceridemia. Although it does not show any symptoms it lay concrete for many diseases that cardiovascular diseases and stroke are very common due to atherosclerosis. Hyperlipidemia is one of the major risk factor for coronary heart disease (CHD) a major leading cause of mortality in developed countries, will soon become the pre-eminent health problem worldwide.

Recent epidemiological studies reveals that there is an increase in lipid levels globally. There is wide variation in the prevalence, awareness, and treatment of hyperlipidemia between populations. According to WHO MONICA PROJECT the prevalence of hypercholesterolaemia varied across populations from 3% to 53% in men, and from 4% to 40% in women. Awareness of hypercholesterolaemia varied from 1% to 33% in men, and from 0% to 31% in women. In most populations, over 50% of men and women on lipid-lowering drugs.[1]

The World Health Organization (WHO) reports that high cholesterol contributes to 56% of cases of coronary heart disease worldwide and causes more than 4 million deaths each year. In most parts of the world, the number of female deaths attributed to high cholesterol is slightly higher than the number of male deaths.[2]

India is a developing country has been showing an increase in the incidence of hyperlipidemia, for the past few decades .In young adult Indian population the prevalence of dyslipidemia was observed to be higher in males than in females. Among participants who had a total Cholesterol (TC) concentration 200mg/dl, 38.7% were males and 23.3% were females. High density lipoprotein cholesterol (HDL-C) was abnormally low in

64.2% males and 33.8% in females. The increase of prevalence of hypercholesterolemia and hypertriglyceridemia was more prominent in 31-40 age group than in 30 age group[3] .

Another epidemiological study in South India conducted by Sri Ramachandra University, Chennai , mentioned that the prevalence of abnormal serum lipid levels was more prominent in the age group of 40-59 years in both the sexes. High levels of triglycerides were identified in 41.5% and very high levels in 1.2%, LDL- cholesterol levels were high in 32.9% and very high in 7.45%, and a higher total cholesterol levels were found in 25.35%. HDL- cholesterol levels were found to be low in 34.35%.[4]

It is the right time for the measures to be taken for this disease. Many Siddha medicines have been indicated for increased levels of lipids. Several indigenous plants have been claimed to possess hypolipidemic and hypocholesteremic properties that may be beneficial to reduce the risk of cardiovascular diseases. In Siddha text Amukkara Kizhangu Chooranam is indicated for Athimetham.[5]

Athimetham is a one of the kabam related disorder. Amukkara Kizhangu Chooranam has kaippu suvai. It's vibagam is kaarppu . It is mentioned in the siddha text that **கார்ப்பு சுவை உடற் பசையையும் ,கொழுப்பையும்,வயிற்றில் கபத்தினால் உண்டாகும் துர்நீரையும் வரட்டும்** .Many studies have been conducted in this herb. Flavanoids of this herb showed hypolipidemic activity in alloxan induced diabetic rats.[6]

Another study showed that dietary herbal supplementation with *Withania somnifera* exhibited a significant reduction in levels of egg yolk total lipids, egg yolk cholesterol and egg yolk triglycerides of birds. [7]

Amukkara Kizhangu Chooranam has not been evaluated for hypolipidemic activity in diet induced hypercholestremic rat and clinical trial so far. This study is different from previous studies regarding adjuvant , dosage forms and methods.

Hence the researcher has selected "Amukkara Kizhangu Chooranam"to evaluate its Hypolipidemic activity and therapeutic effect in the management of Athimetham.

Aim:

To evaluate the safety and efficacy of Amukkara Kizhangu Chooranam (Withania somnifera) for Hypolipidemic activity in the management of Athimetham (Hyperlipidemia).

OBJECTIVE:

Primary objective:

To evaluate the Hypolipidemic activity of Amukkara Kizhangu Chooranam (Withania somnifera) in preclinical studies.

Secondary objective:

Biochemical analysis.

HPTLC.

To evaluate the efficacy of Amukkara Kizhangu Chooranam (Withania somnifera) in clinical trial for Hypolipidemic activity in the management of Athimetham (Hyperlipidemia).

MATERIALS AND METHODS

STANDARD OPERATIVE PROCEDURE

COLLECTION AND AUTHENTICATION OF RAW DRUG:

Amukkara Kizhangu was procured from Raw drug store in Chennai and authenticated by competent authority in the department of Gunapadam , National Institute of Siddha, Chennai.

PURIFICATION OF AMUKKARA[5]:

Amukkara Kizhangu was boiled with milk and then dried in the shadow.

METHOD OF MEDICINE PREPARATION:

Purified Amukkara Kizhangu was pulverised by an electric grinder in to a fine powder and then it was sieved by using a fine silk cloth (vasthra kaayam).The fine Powder was purified by pittavial method. Then it was dried and ultrafiltered by a cotton cloth and made in to fine powder again. The powder was stored in a clean dry airtight glass bottle.

LABELLING:

Name of the preparation	:	Amukkara Kizhangu Chooranam
Quantity of the drug	:	Amukkara Kizhangu Chooranam [28g]
Dose	:	2 gm bd
Adjuvant or Vehicle	:	Honey
Indication	:	Athimetham
Date of manufacturing	:	The drug was prepared in 3 batches 13/3/12, 15/6/12,16/9/12
Expiry	:	3 months.

Withania somnifera plant

Withania somnifera raw drug

AMUKKARA KIZHANGU CHOORANAM

REVIEW OF LITERATURE

SIDDHA ASPECT

அமுக்குராக்கிழங்கு -AMUKKURA-KIZHANGU.[5]

Withania somnifera(Linn)

வேறுபெயர்கள் :

அமுக்கிரி
அமுக்குரவி
அமுக்குரவு
அமுக்கினங்கிழங்கு
அசுவகந்தி
அசுவம்
இருளிச்செவி
கிடிச்செவி
வராககர்ணி

பயன்படும் உறுப்பு : இலை,விதை,வேர்(கிழங்கு)

சுவை : கைப்பு

வீரியம் : வெப்பம்

பிரிவு : கார்ப்பு.

செய்கை:

இலை : வெப்பகற்றி.

காய் : சிறுநீர்ப்பெருக்கி

கிழங்கு : உடற்றேற்றி
ஆண்மைபெருக்கி
வீக்கமுருக்கி
உரமாக்கி
சிறுநீர்ப்பெருக்கி
உறக்கமுண்டாக்கி
உடல்வெப்பகற்றி

குணம்:

இக்கிழங்கு கயம் வளிக்குட்டங்கள் கரப்பான், சுரம், வீக்கம் இவைகளை போக்கும். பசித்தீயையுண்டாக்கும்.

பொதுகுணம்:

கொஞ்சந் துவர்ப்பாங் கொடியகயம் சூலையரி
மிஞ்சுகரப் பான்பாண்டு வெப்புதப்பு- விஞ்சி
முசுவுறு தோடமும்போ மோகம்அன லுண்டாம்
அசுவகந் திக்கென் ற்றி

.....
.....கோல
நகுட வெருண்டுதிர நாட்டுவையேன் மேலை
நகுட வெருண்டுறுவாழ் நாள்

அமுக்கினாங்கிழங்கு,பொடி நெய் முதலியன செய்து பயன்படுத்தினால்,உறுதி,அழகு நீண்ட ஆயுள் முதலியவைகள் உண்டாகும்.

மேல் பூச்சு:

அமுக்கினாங்கிழங்குப் பச்சையாய்க் கொண்டுவந்து பசுவின் நீர் விட்டரைத்து கொதிக்கவைத்து, (கழலை)கிரந்தி, (கழுத்துக் கழலை)கண்டமாலை,வீக்கம்,இடுப்புவலி இவைகளுக்குப் பற்றிட, இவைகள் விலகும்.

இதைச் சுக்குடன் சேர்த்து வெந்நீர்விட்டரைத்து, வீக்கங்களுக்குப் போடக் கரையும்.

கிழங்கு அல்லது இலையை அரைத்து மேகக்கட்டி, நோயுடன்கூடிய வீக்கம், புண் இவைகட்குப் பூசலாம்

குடிநீர்:

கால் முதல் அரை பலம் எடையுள்ள இலையை விதிப்படி குடிநீர் செய்து கொடுக்கச் சுரந்தணியும், அல்லது ஊறல் நீர் செய்துங் கொடுக்கலாம்

அமுக்குராக் பொடி:

- கிழங்கைப் பாலில் வேகவைத்து அலம்பி ,உலர்த்தி,பொடி செய்து ஒரு வேளைக்கு 2 முதல் 4 கிராம் வரை தேனிற் கலந்து கொடுக்க,வளி ஐயம் இவற்றால் பிறந்த நோய்கள், வீக்கம்,பசியின்மை, உடல் பருமன் இவைகள் போம்.

- நெய்யுடன் கலந்து கொடுக்க,ஓய்ச்சல் பெருமூச்சு போம். உடற்கு வன்மைதரும்,விந்துவைப் பெருக்கும்.

- அமுக்கிராக் கிழங்குப் பொடி 1 பங்கும் கற்கண்டு 3 பங்கும் சேர்த்து வேளைக்கு 4 கிராம் காலை மாலை உட்கொண்டு, அரை அல்லது ஓர் ஆழாக்குப் பசுவின் பால் சாப்பிட்டுவர, நரம்புத் தளர்ச்சி ஆகிய இவைகள் நீங்கும் ,உடல் வன்மை பெரும், அழகு தரும்.

BOTANICAL ASPECT

Botanical Classification[8]

- Kingdom : Plantae.
- Division : Angiospermae.
- Class : Dicotiledoneae.
- Order : Tubiflorae.
- Family : Solanaceae.
- Genus : withania
- Species : somnifera .

Common Name[9]:

English	-	Winter cherry
Latin	-	Withania somnifera
Sanskrit	-	Ashwagandha
Hindi	-	Asgandh
Tamil	-	Asuragandhi, Amukkira
Kannada	-	Keramaddinagaddi
Telgu	-	Vajigandha, Pennerugadda
Malayalam	-	Amukkuram, Trittavu.
Marathi	-	Askandha
Marathi	-	Asgundh, Kanchuki, Askandha
Bengali	-	Ashvagandh
Punjabi	-	Asgand
Urdu	-	Asgandanagaori

Ashwagandha is a small, branched, perennial woody shrub that grows usually about 2 feet in height and is naturally found in diverse areas ranging from Africa, the Mediterranean and East into India. Because of its wide range, there is considerable morphological and chemo typical variations in terms of local species[10].

Flower:

Ashwagandha has sessile, axillary, greenish or lurid yellow flowers. They are hermaphrodite (has both male and female organs).

Fruit:

The fruit is Orange-red berry, smooth, oblong, rounded or somewhat produced at base. The fruit is harvested in the late fall and the bright yellow seeds are dried for planting in the following spring.

Roots :

It has a more or less tuberous root

Seed:

The seeds are yellow and scurfy.

Plant Constituents of Withania [11]

Contains:

- Alkaloids
- Anaferine
- Isopelietierine
- Saponins
- Sitoindoside VII
- Sitoindoside VIII
- Steroidal Lactones
- Withaferins
- Withanolides
- Sitoindoside IX
- Sitoindoside X
- Iron

Action:

- Adaptogen [normalizes physical functioning depending on what the individual needs, e.g. it will lower high blood pressure, but raise low blood pressure]
- Anti-inflammatory [an agent to ease inflammation]
- Anti-tumor (in high doses)
- Nervine [an agent that has a calming or soothing effect on the nerves, any agent that acts on the nervous system to restore the nerves to their natural state]
- Sedative [a soothing agent that reduces nervousness, distress or irritation]
- Tonic [an agent that tones, strengthens and invigorates organs or the entire organism giving a feeling of well-being].

JOURNAL REVIEW OF WITHANIA SOMNIFERA

The major biochemical constituents of Withania somnifera root are steroidal alkaloids and steroidal lactonesin a class of constituents called withanolides.[1]

A series of animal studies show Withania somnifera to have profound effects on the hematopoietic system, acting as an chemoprotective agent[2]..[3]

The immunomodulatory activities[11] of i.e. extracts from Withania somnifera (L.) Dunal (Solanaceae), namely WST and WS2, were studied in mice for immune inflammation, active paw anaphylaxis and delayed type hypersensitivity (DTH). Immunomodulatory effect was assessed in IgE-mediated anaphylaxis as reduction of ovalbumin-induced paw edema, in animals treated with WS2 at doses of 150 and 300 mg/kg, and the results were compared with the standard drug disodium chromoglycate. In the DTH model, the modulatory effect was assessed as potentiation or suppression of the reaction, revealing an increase or decrease in mean foot pad thickness, respectively. Potentiation of the DTH reaction was observed in animals treated with cyclophosphamide at a dose of 20 mg/kg, WST at a dose of 1000 mg/kg and WS2 at a dose of 300 mg/kg. On the other hand, cyclophosphamide-induced potentiation of DTH reaction was suppressed in animals treated with WST and WS2. A significant increase in white blood cell counts and platelet counts was observed in animals treated with WST. A protective effect in cyclophosphamide-induced myelosuppression was observed in animals treated with WST and WS2, revealing a significant increase in white blood cell counts and platelet counts. Cyclophosphamide-induced immunosuppression was counteracted by treatment with WS2, revealing significant increase in hemagglutinating antibody responses and hemolytic antibody responses towards sheep red blood cells.

In a mouse study, administration of a powdered root extract from Withania somnifera was found to enhance total whiteblood cell count. In addition, this extract inhibited delayed-type hypersensitivity reactions and enhanced phagocytic activity of macrophages when compared to a control group.[4]

Nitric oxide has been determined to have a significant effect on macrophage cytotoxicity against microorganisms and tumor cells. Withania somnifera increased NO production in mouse macrophages in a concentration-dependent manner. This effect was attributed to increased production of inducible nitric oxide synthase, an enzyme generated in response to inflammatory mediators and known to inhibit the growth of many pathogens.[5]

Withania somnifera exhibited stimulatory effects,both *in vitro* and *in vivo*, on the generation of cytotoxic T lymphocytes, and demonstrated the potential to reduce tumor growth.[6]

The chemopreventive effect was demonstrated in a study of Withania somnifera root extract on induced skin cancer in Swiss albino mice given Withania somnifera before and during exposure to the skin cancer causing agent 7,12-dimethylbenz[a]anthracene. A significant decrease in incidence and average number of skin lesions was demonstrated compared to the control group. Additionally, levels of reduced glutathione, superoxide dismutase, catalase,and glutathione peroxidase in the exposed tissue returned to near normal values following administration of the extract. The chemopreventive activity is thought to be due in part to the antioxidant free radical scavenging activity of the extract.[7]

An *in vitro* study showed withanolides from Withania somnifera inhibited growth in human breast, central nervous system, lung, and colon cancer cell lines comparable to doxorubicin. Withaferin A more effectively inhibited growth of breast and colon cancer cell lines than did doxoorubicin. These results suggest Withania somnifera extracts may prevent or inhibit tumor growth in cancer patients, and suggest a potential for development of new chemotherapeutic agents. Withania somnifera extracts may prevent or inhibit tumor growth in cancer patients, and suggest a potential for development of new chemotherapeutic agents.[8.]

In an animal study assessing the anxiolytic and antidepressive actions of Withania somnifera compared to commonly prescribed pharmaceuticals, an extract of the root was administered orally to rats once daily for five days. The results were compared to a group administered the benzodiazepine lorazepam for anxiolytic activity, and the tricyclic antidepressant imipramine for antidepressant investigation. Both the Withania somnifera

group and the lorazepam group demonstrated reduced brain levels of a marker of clinical anxiety. Withania somnifera also exhibited an antidepressant effect comparable to that induced by imipramine in the forced swim-induced "behavioral despair" and"learned helplessness" tests[9].

Flavonoids were determined in the extracts of *W. somnifera* root (WSREt) and leaf (WSLEt). The amounts of total flavonoids found in WSREt and WSLEt were 530 and 520 mg/100 g dry weight (DW), respectively. Hypoglycaemic and hypolipidaemic effects of WSREt and WSLEt were also investigated in alloxan-induced diabetic rats. WSREt and WSLEt and the standard drug glibenclamide were orally administered daily to diabetic rats for eight weeks. After the treatment period, urine sugar, blood glucose, haemoglobin (Hb), glycosylated haemoglobin (HbA1C), liver glycogen, serum and tissues lipids, serum and tissues proteins, liver glucose-6-phosphatase (G6P) and serum enzymes like aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP) levels were determined. The levels of urine sugar, blood glucose, HbA1C, G6P, AST, ALT, ACP, ALP, serum lipids except high density lipoprotein-bound cholesterol (HDL-c) and tissues like liver, kidney and heart lipids were significantly ($p < 0.05$) increased, however Hb, total protein, albumin, albumin:globulin (A:G) ratio, tissues protein and glycogen were significantly ($p < 0.05$) decreased in alloxan-induced diabetic rats. Treatment of the diabetic rats with WSREt, WSLEt and glibenclamide restored the changes of the above parameters to their normal level after eight weeks of treatment, indicating that WSREt and WSLEt possess hypoglycaemic and hypolipidaemic activities in alloxan-induced diabetes mellitus (DM) rats[10].

PHYSICAL PROPERTIES

The physical properties for the drug Amukkara Kizhangu Chooranam was carried out in Sri Ramachandra University, Chennai.

pH at 10% of aqueous solution:

Five grams of the sample was weighed accurately and placed in clear 100 ml beaker. Then 50 ml of distilled water was added to it and dissolved well. After 30 minutes it was then applied in to pH meter at standard buffer solution of 4.0,7.0,9.2(trial drug 1 table 2)

Ash Values:

The Ash values are a measure of the inorganic constituents present in the raw drug. A high ash content explains its unsuitable nature to be used as a drug (trial drug table 2)

Total Ash:

A little of extract was taken in a silica crucible previously ignited, cooled and weighed. It was incinerated by gradually increasing the heat not exceeding dull red heat (450°C) until free from carbon, cooled and weighed. The percentage of ash was calculated with reference to air- dried drug. The procedure was repeated to get the constant weight. (trial drug 1table 2)

Water soluble ash:

The total ash was boiled with 25 ml water and filtered through ash less filter paper (Whatmann4.1). It was followed by washing with hot water .The filter paper was dried and ignited in the silica crucible, cooled and the water insoluble ash was weighed. The water-soluble ash can be calculated by subtracting the water insoluble ash from the total ash. (trial drug1table2)

Acid insoluble ash:

The total ash obtained was boiled for 5 minutes with 25 ml of (10% w/v) dilute hydrochloric acid and filtering through ash less filter paper (Whatmann 4.1). The filter paper was ignited in the silica crucible, cooled and insoluble ash was weighed. (trial drug 1 table 2)

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

HPTLC Fingerprint - RH1

HPTLC for the drug Amukkara Kizhangu Chooranam was carried out in Sri Ramachandra University, Chennai.

Sample Preparation

100 mg of extract was weighed and dissolved in 70% methanol to get a concentration of 10mg/ml concentration this is then used for injection.

Chromatographic Conditions

SampleName	:	AKC powder
Sample-ID	:	110
Stationary Phase	:	Silica gel 60 F 254
Mobile Phase	:	chloroform: methanol (9:1)
Scanning Wavelength	:	404 nm
Applied volume	:	10µl
Development mode	:	Ascending mode

Significance of HPTLC fingerprinting in Standardisation

Standardisation of traditional medicine has become mandatory in the present national and international scientific scenario, as they have to stand competing with stringent regulatory methods and also clinically. HPTLC is one of the versatile chromatographic methods presently available for the rapid analysis of herbal drugs due to several reasons. Firstly the time required for the demonstration of the most of the characteristic constituents of a drug is very quick and short. Secondly, in addition to qualitative detection, HPTLC also provides semi-quantitative information on the major active constituents of a drug, thus enabling an assessment of drug quality. Thirdly the fingerprint obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. Hence in order to check the identity, purity and standardise the quantity of active principles in the herbal extracts a HPTLC has been obtained.

The distribution of phyto-constituents in a plant depends on various factors such as soil, time of collection period of storage, etc. So, it is necessary to standardize the extract being used for pharmacological studies. HPTLC serves as a convenient tool for finding out the distribution pattern of phyto constituents which is unique to each plant. The HPTLC finger-printing profile establishes the identity and purity of the raw drug being used. It helps in the authentification of the plant material.

Chromatographic Conditions

The finger printing has been done using the following chromatographic conditions. Chromatography was performed on a10x10 cm pre activated HPTLC silica gel 60F 254 plate. Samples were applied to the plate as 6mm wide band with an automatic TLC applicator Linomat 5 with N2 flow (CAMAG, Switzerland), 8mm from the bottom. Densitometric scanning was performed on CAMAG scanner III.The plates were pre-washed by methanol and activated at 60^0 C for 5 minutes prior to chromatography. The slit dimension was kept at 5 minutes x 0.45 minutes and 20 minutes scanning speed was employed. The mobile phase was chosen after running each plant in different mobile phases of varying polarity (Toluene, Toluene: Ethyl acetate and Ethyl acetate: Methanol) and 10 ml of mobile phase was used per chromatography. Linear

ascending development was carried out in 20 cm x 10-em twin glass chamber saturated with the mobile phase.

Chromatographic Analysis

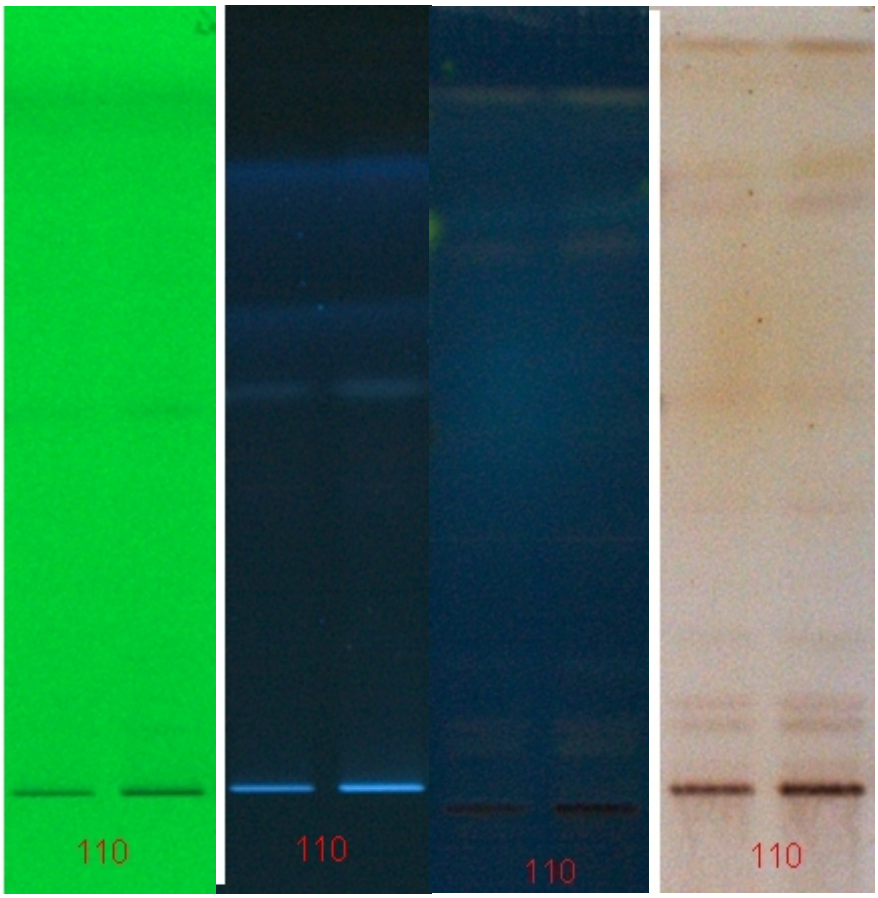
The hydro alcoholic extracts of the plants have been prepared at a concentration of 10 mg/10 ml in alcohol and were spotted using CAMAG Linomat 5 applicator. The method was optimized by selecting appropriate mobile phase for respective plant extracts and developed in a twin trough chamber, 20 x 10 cm at 25°C. The plates were dried by hair dryer. The developed plates were scanned at appropriate wavelength using CAMAG TLC scanner 3 and photo-documented using CAMAG REPROSTAR 3(graph 1-9).

Inferences:

HPTLC fingerprint of RH -1 shows four peaks at Rf values 0.25, 0.31, 0.41 & 0.95. The peak correspond to the Rf value 0.31 has maximum peak area of 7256.5. At this stage it is difficult to confirm the individual components present in the extract, but from our lab experience on phytochemical analysis, we suggest that the major peaks found in the fingerprint may be acidic glycosides / resins. Since, in the present chromatographic conditions, the above mentioned components will be eluted easy.

Fingerprint chromatogram of RH -1 at 404nm

Amukkara kizhangu Chooranam



BIOCHEMICAL ANALYSIS OF AMUKKARA KIZHANGU CHOORANAM

The biochemical analysis of the Amukkara Kizhangu Chooranam was carried out in the Biochemistry lab, NIS.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Appearance of sample	Light yellow in colour	
2.	Solubility: a. A little(500mg) of the sample was shaken well with distilled water. b. A little(500mg) of the sample was shaken well with con. HCl/Con. H_2So_4	Sparingly soluble	Absence of Silicate
3.	Action of Heat: A small amount(500mg) of the sample was taken in a dry test tube and heated gartly at first and then strong.	No white fumes evolved	Absence of Carbonate
4.	Flame Test: A small amount(500mg) 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 appeared.	Absence of Copper
5.	Ash Test: A filter paper was soaked into a mixture of sample and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No yellow colour flame appeared.	Absence of sodium

Preparation of Extract:

5gm of Amukkura Kizhangu Choornam[Withania sonifera.] was weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water.

S.No	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 b. 2ml of the above prepared extracts was added with 2ml of dil-HCl was added until the effervescence ceases off. Then 2ml of dil.Barium chloride solution was added.	No cloudy appearance.	Absence of Sulphate
2.	Test For Chloride: 2ml of the above prepared extract was added with dil. HCl till the effervescence ceases. Then 2ml of dil.silver nitrate solution was added.	No cloudy appearance.	Absence of Chloride
3.	Test For Phosphate: 2ml of the extract was treated with 2ml of dil.ammonium molybdate solution and 2ml of con.HNo3.	No Yellow appearance present	Absence of Phosphate
4.	Test For Carbonate: 2ml of the extract was treated with 2ml dil. Magnesium sulphate solution	No Cloudy appearance.	Absence of carbonate
5.	Test For Nitrate: 1gm of the substance was heated with copper turning and concentrated H2So4 and viewed the test tube vertically down.	No Brown gas evolved.	Absence of Nitrate
6.	Test For Sulphide: 1gm of the substance was treated with 2ml of con. HCL	No Rotten Egg Smelling gas.	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 was placed.	No Characteristic changes	Absence of Nitrite
9.	Test For Borate: 2 Pinches(50mg) of the substance was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	No Bluish green colour flame.	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 obtained.	Absence of Lead
2.	Test For Copper: a. One pinch(50mg) of substance was made into paste with con. HClin a watch glass and introduced into the non-luminuous part of the flame.	No Blue colour flame No Blue colour precipitate formed.	Absence of copper
3.	Test For Aluminium: To the 2ml of extract, dil.sodium hydroxide was added in 5 drops to excess.	NoYellow colour appeared.	Absence of aluminium
4.	Test For Iron: a. To the 2ml of extract,2ml of dil.ammonium solution was added. b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 was added	blood red colour appeared.	presence 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 was added.	No White precipitate was formed	Absence of Zinc
6.	Test For Calcium: 2ml of the extract was added with 2ml of 4% dil.ammonium oxalate solution	No Cloudy appearance and white precipitate was obtained	Absence of calcium
7.	Test For Magnesium: To 2ml of extract dil.sodium hydroxide solution was added in drops to excess.	No White precipitate was 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 substance 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 Yellowish precipitate was obtained.	Absence of Potassium
10.	Test For Sodium: 2 pinches(50mg) of the substance was made into paste by using HCl and introduced into the blue flame of Bunsen burner.	yellow colour flame appeared	Presence of sodium
11.	Test For Mercury: 2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.	No yellow precipitate was obtained	Absence of mercury
12.	Test For Arsenic: 2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.	No brownwish red precipitate was 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.	Brick red colour not developed	Absence of reducing sugar
3.	Test For The Alkaloids: a) 2ml of the extract was treated with 2ml of dil.potassium iodide solution. b) 2ml of the extract was treated with 2ml of dil.picric acid. c) 2ml of the extract was treated with 2ml of dil.phosphotungstic acid.	. Yellow colour developed	- Presence of Alkaloid

4.	Test For Tannic Acid: 2ml of extract was treated with 2ml of dil.ferric chloride solution	black precipitate was obtained	Absence of Tannic acid
5.	Test For Unsaturated Compound: To the 2ml of extract 2ml of dil.Potassium permanganate solution was added.	Potassium permanganate was not decolourised	Absence of unsaturated compound
6.	Test For Amino Acid: 2 drops of the extract was placed on a filter paper and dried well. 20ml of Biurette reagent was added.	No Violet colour developed	Absence of amino acids
7.	Test For Type Of Compound: 2ml of the extract was treated with 2 ml of dil.ferric chloride solution.	No green colour developed No red colour developed No violet colour developed No blue colour developed	Absence of oxy quinole pinephrine and pyro catechol Anti pyrine, Aliphatic amino acids and meconic acid are absent Apomorphine salicylate and Resorcinol are absent Morphine, Phenol cresol and hydro uinone are absent

ACUTE AND SUB ACUTE TOXICITY STUDY ON AMUKKARA KIZHANGU CHOORANAM IN RODENTS

Animals:

Mice of either sex weighing 25-30g and rats weighing 210-240g were obtained from the animal house of Vels University. The animals were used with the approval of the Institute animal ethics committee and obtained from Vels University, Chennai. They were fed with a balanced standard pellet diet and maintained under standard laboratory conditions, providing 24-28^{0}C temperature, standard light cycle (12 h light, 12 h dark) and water ad libitum. Animals were kept in cages with raised floors of wide mesh to prevent coprophagy. Animal welfare guidelines were observed during the maintenance period and experimentation. The rats were randomly assigned to control and different treatment groups, six animals per group. (Approval number: XIII/VELS/PCOL/36/2000/CPCSEA/IAEC/08.08.2012). The animals were acclimatized for one week under laboratory conditions.

ACUTE TOXICITY STUDY-OECD 425 GUIDELINES

Acute oral toxicity test for the Amukkara Kizhangu Chooranam was carried out as per OECD Guidelines 425. As with other sequential test designs, care was taken to ensure that animals are available in the appropriate size and age range for the entire study. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food was withheld for further 2 hours in mice. The animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering of the test drug, to observe any death or changes in general behaviour and other physiological activities. Single animals are dosed in sequence usually at 48 h intervals. However, the time interval between dosing is determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose was delayed until one is confident of survival of the previously dosed animal.

Observation of toxicity signs: General behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, change in skin and fur, mortality and the body weight changes were monitored daily. The time of onset, intensity, and duration of these signs, if any, was recorded.

SUB-ACUTE TOXICITY

In a 28-days sub acute toxicity study, twenty four either sex rats were divided into four groups of 6 rats each. Group I that served as normal control was administered with distilled water (p.o.) while groups II, III and IV were administered daily with the Amukkara Kizhangu Chooranam (p.o.) for 28 days at a dose of 100, 200 and 400g/kg respectively. The animals were then observed daily for gross behavioural changes and any other signs of subacute toxicity. The weight of each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per rat was calculated. At the end of the 28 days they were fasted overnight, each animal was anaesthetized with diethylether, following which they were then dissected and blood samples were obtained by cardiac puncture into heparinised tubes. The blood sample collected from each rat was centrifuged with 3000 X g at $4^{o}C$ for 10 min to separate the serum and used for the biochemical assays.

Hematological and blood biochemical analyses:

At the end of the study, all animals were kept fasted for 16-18 h and then anesthetized with anesthetic ether on the 28th day. Blood samples for hematological and blood chemical analysis were taken from retro orbital vein. Heparinized blood samples were taken for determining complete blood count (white blood cell count, differential white blood cell count, platelet count, red blood cell count, hematocrit, and hemoglobin) by semi automated hematology analyzer. The serum from non-heparinized blood was carefully collected for blood chemistry and enzyme analysis (glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, total and direct bilirubins, serum glutamate-oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP)) were automatically determined using autoanalyzer.

Necropsy:

All rats were sacrificed after the blood collection. The positions, shapes, sizes and colors of internal organs were evaluated. The Spleen, Testes, Pancreas, Lungs, Liver, Brain, Heart, Stomach, Intestine, Bone, Ovaries, and Kidney tissues were excised from all rats to visually detect gross lesions, and weighed to determine relative organs' weights and preserved in 10% neutral formalin for histopathological assessment. The tissues were embedded in paraffin, and then sectioned, stained with haematoxylin and eosin and were examined microscopically.

Statistical analysis

Values were represented as mean ± SEM. Data were analysed using one-way analysis of variance (ANOVA) and group means were compared using the Tukey-Kramer Multiple Comparisms Test using GraphPad Instat-V3 software. P values < 0.05 were considered significant((trial drug 1 table 4-13)

RESULTS AND DISCUSSION

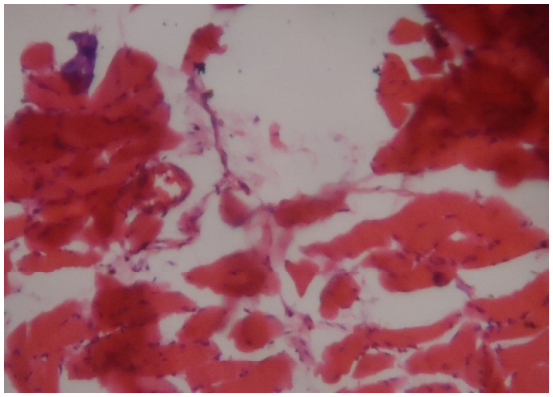
The acute toxicity study of the Amukkara Kizhangu Chooranam indicated no changes in the behavior and in the sensory nervous system responses in the animals. Also no adverse gastrointestinal effects were observed in the mice used in the experiment. All the mice that received upto 2.0g/kg dose of the Amukkara Kizhangu Chooranam survived beyond the 24 hours of observation. Hence the dose was fixed as 100, 200 and 400mg/kg for further sub acute toxicity study. During the subacute toxicity tests, the results obtained on the average daily water, food intake and weekly weight gain are observed. The eating and drinking habit and behavior of all the animals used were normal in both vehicle-treated and Amukkara Kizhangu Chooranam treated animals. The results obtained on the biochemical parameters of rats fed with Amukkara Kizhangu Chooranam for 28 days revealed that essential organs such as the liver, kidneys, spleen and testes were not adversely affected during the subacute administration. Acute and subacute oral administration of Amukkara Kizhangu Chooranam did not cause any significant changes in gross behavioural effects in rodents.

The feed conversion efficiency followed the same pattern, thus indicating a normal metabolism of the animals. Macroscopically, the liver, spleen, lungs, testis and the kidneys showed no discolouration and the textures were consistent when compared with the control group. Histopathological examination revealed that the spleens, livers, lungs, testes and the kidneys of rats administered with Amukkara Kizhangu Chooranam showed no differences relative to those of the control group at the two dose levels, though there was focal proximal tubular epithelial necrosis in the kidney at 400mg/kg.

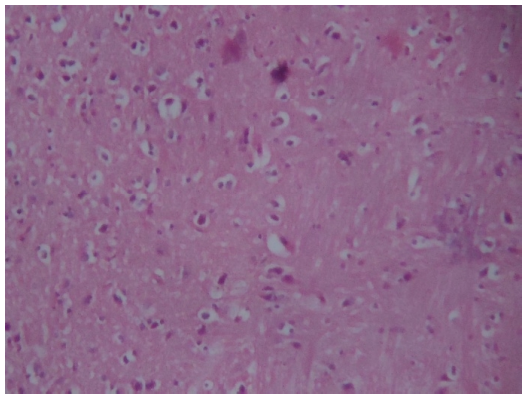
These results indicate that Amukkara Kizhangu Chooranam at 400mg/kg body weight is not toxic to the liver, spleen and testes of rat but has a minor effect on the lungs and kidney. It is well established that changes in the lipid profile and total protein of serum could be indicative of perturbations in the liver or kidney following toxic injury. In conclusion, the present results show that Amukkara kizhangu Chooranam possesses very low toxicity as indicated in our rat model. No deaths or signs of toxicity were observed in the rats that received the Amukkara Kizhangu Chooranam up to an oral acute dose of 2g/kg thus establishing its safety in use.

HISTO-PATHOLOGICAL SLIDES – TOXICITY STUDIES

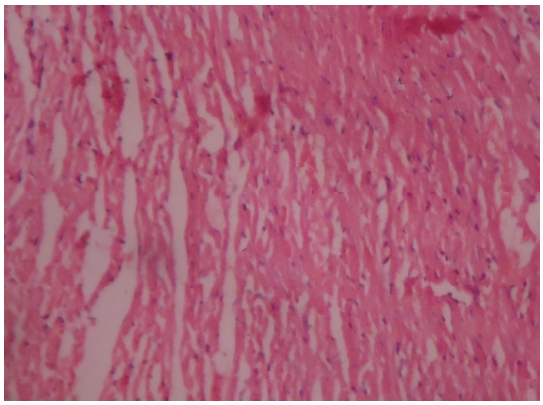
FOR TRIAL DRUG 1



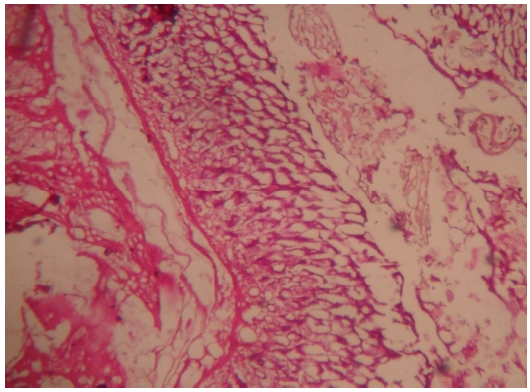
Bone 400 mg



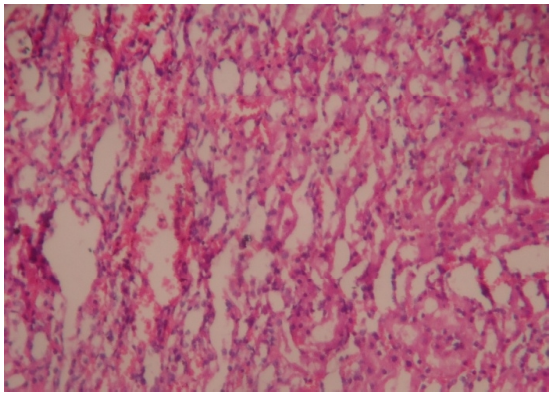
Brain 400 mg



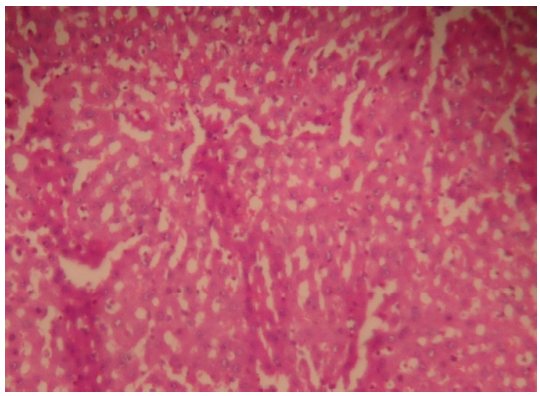
Heart 400mg



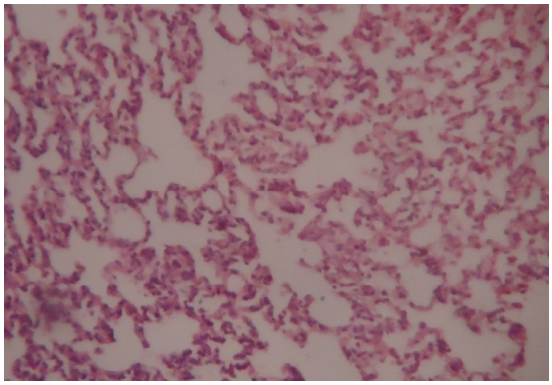
Intestines400mg



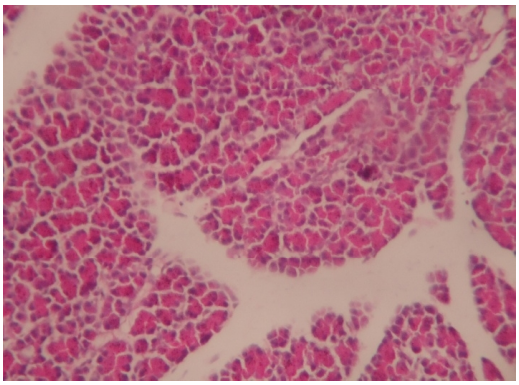
Kidney 400mg



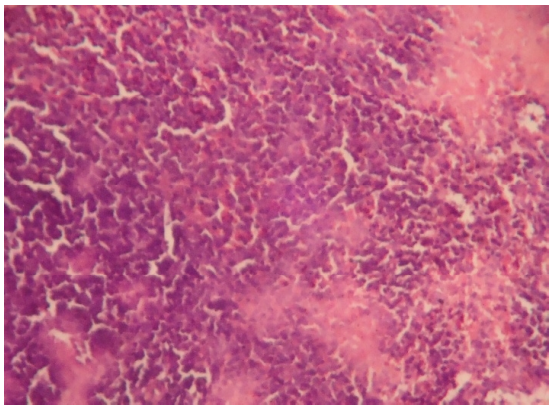
Liver400mg



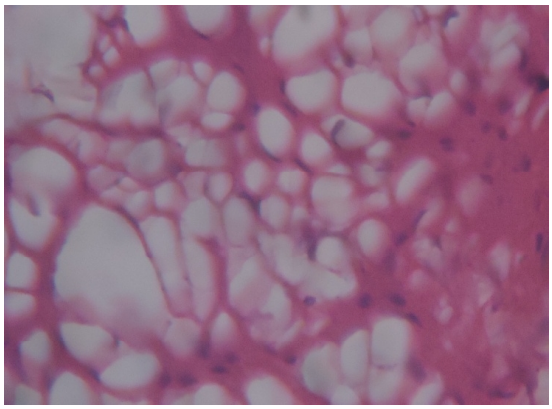
Lungs400mg



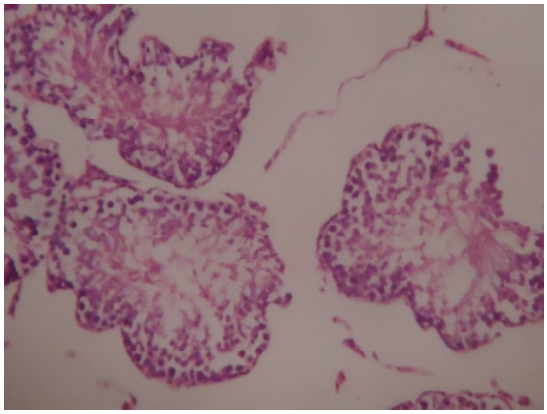
Pancreas400mg



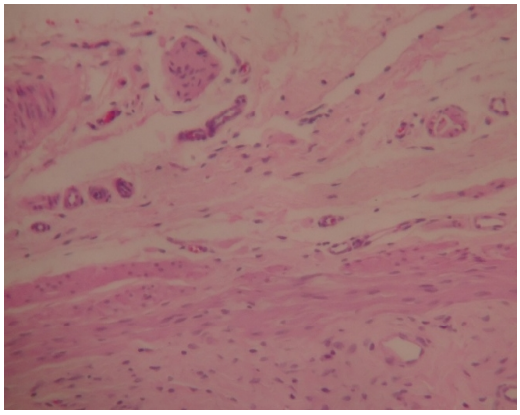
Spleen 400mg



stomach400mg



Testis 400mg



Ovaries 400mg

HYPOLIPIDEMIC ACTIVITY OF AMUKKARA KIZHANGU CHOORANAM(AKC) IN CHOLESTEROL RICH DIET INDUCED HYPERLIPIDEMIC RATS

AIM

To evaluate the hypolipidemic activity of Amukkara Kizhangu Chooranam in cholesterol rich diet induced hyperlipidemic rats

MATERIALS AND METHODS

Chemicals: Cholesterol, Sodium cholate and coconut oil were all purchased from SD-fine chemicals, India, Lovastatin was procured form Ranbaxy labs. Ltd., Gurgaon, India. All other reagents used were of analytical grade. The various chemicals employed for different procedures were of analytical grade supplied by BDH Glaxo laboratories, E.Merck and Sigma Diagnostic (india) Pvt.Ltd. Commercially available BUF was purchased for the present work from a local shop. Standard Lovastatin at a dose of 10 mg kg^{-1} was prepared by suspending bulk in aqueous 0.5% Carboxy methylcellulose.

Experimental animals - Adult albino rats 9-12 months old and weighing around 250g were selected (Approval number: XIII/ VELS/PCOL/36/2000/CPCSEA/ IAEC/08.08.2012) and and all the animals were fed with BUF for induction of lipid profile for one week. On eighth day the blood samples were collected and animals showing remarkable elevation of lipid parameter were divided into further five groups, six animals of each and those animals were treated with test drug AKC at the appropriate dose levels once daily in oral route with the help of oral gavage continuously. The total duration of treatment was 21days and the cholesterol rich diet along with normal pellet diet was given to the test animals to maintain the elevated biochemical profile during the drug treatment period. The grouping pattern was as follows.

Group 1: Normal

Group 2: High cholesterol diet control

Group 3: High cholesterol diet treated with AKC 100 mg kg^{-1} b.w., p.o.

Group 4: High cholesterol diet treated with AKC 200 mg kg^{-1} b.w., p.o.

Group 5: Standard Lovastatin 10 mg kg^{-1} body weight (b.wt.), orally (p.o.)

Diet preparation- Normal rat feed supplied by Sai durga feeds, Bangalore was fed to normal control group in measured quantities and it was found that a rat consumed an average weight of 14g feed daily. The normal rat was powdered and mixed with fat so as to fix 21% fat in the diet for control, groups 2-5, and similar high fat diets mixed with AKC 100-200mg/kg. The mixture of feeds were wetted with a little water and made into balls and dried in an oven for feeding it daily. Water was supplied in bottles to each group so that controls and tests were paired fed. The body weight was measured at about every 7 days interval.

After 21days of drug feeding the rats were sacrificed on overnight fasting. Their blood was collected in centrifuge tubes by punching the retro orbital vein and the serum was separated after an hour. It was used for the estimation of lipid parameters and enzyme activities. The liver was also collected and preserved in ice cold beakers for various estimations. Kits provided by sigma diagnostics Pvt. Ltd. Were used for lipid and enzyme estimations according to standard methods. Extractions of tissues were carried out for various estimations.

Blood sample collection and analysis:

On the 8th and 28^{th} day, blood was collected by retro-orbital puncture technique, under mild ether anesthesia after 8 h fasting and allowed to clot for 30 min at room temperature. Blood samples were centrifuged at 3000 rpm for 20 min. Serum was separated and stored at -20°C until biochemical estimations were carried out. Serum samples were analyzed spectrophotometrically for total serum cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) was estimated using diagnostic kits which were procured from Lab-Care Diagnostics Pvt. Ltd., Mumbai, India.

Very Low Density Lipoprotein (VLDL), High Density Lipoprotein ratio (HDL-C ratio), Atherogenic Index (AI) and low density lipoprotein cholesterol (LDL-C) were calculated.

Extraction for cholesterol– Acute weighed (0.5g) tissue was ground with 4g of anhydrous sodium sulphate using mortor and pestle. An extract using chloroform methanol mixture (1:1) was made 1:5 volumes and diluted to 20ml and centrifuged. 2ml of this supernatant was evaporated and redissolved in 1ml acetic acid 0.05ml of this extract was used for the estimation of total cholesterol. Serum VLDL+LDL cholesterol was determined by substracting HDL cholesterol from total cholesterol.

Extraction for AST and ALP – Accurately weighed 0.5g tissue was ground in a mortor with pestle under cold conditions. 2ml of phosphate buffer (P^H 7.4) was added and centrifuged in a refrigerated centrifuge at 2000g. The supernatant was for the assay of enzyme. Serum lipid parameters such as total cholesterol, HDL cholesterol and VLDL+LDL cholesterol and serum enzyme such as aspartate transaminase (AST) and alkaline phosphate (ALP) were estimated by standard methods.

Statistical analysis: Experimental results were Mean±SEM (Standard Error of Mean) of 6 animals. The results were statistically analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests to determine level of significance. Data were considered statistically significant only when value of $p<0.05$.

RESULTS AND DISCUSSION

From the acute toxicity study, it was confirmed that the Amukkara Kizhangu Chooranam is non toxic upto 2000mg/kg on oral administration in mice. Hence, the one tenth of this maximum tolerable dose and its lower dose was considered for further pharmacological study. After 21days of AKC treatment at the different dose levels of cholesterol rich diet induced hyperlipidemic rats showed significant increase in body weight after fourteen days ($P<0.01$) when compared to normal control. The result on the lipid profile was observed that the diets containing BUF increased very significantly the lipid profile i.e., total cholesterol. The administration of AKC at 100 and 200mg/kg dose levels along with high fat diets significantly ameliorated the deleterious effects of these animal fats and in addition the 200mg/kg dose significantly increased HDL cholesterol which has a protective action against CHD.

The total cholesterol in the AKC 100 and 200mg/kg treated group showed 142.00±4.37 and 138.81±3.48mg/dl respectively, whereas the hyperlidemic control showed 179.46±5.13mg/dl. Similarly, the triglyceride level was 159.33±4.48 and 147.10±3.52mg/dl and in control it was 285.17±4.10mg/dl. The LDL level was significantly reduced in AKC 100 and 200mg/kg treated animals towards 41.64±3.00 and 70.15±3.64 from 87.34±4.15mg/dl. In this it was noticeable that the lower dose group showing overall maximum beneficial effect in animal models. The high dose group showing moderate activity and the exact reason is not clear. The VLDL was effectively reduced to normal range on AKC treatment compared to control the effect is statistically significant and comparable to that of standard drug treatment.

The artheogenic Index i.e the ratio of total cholesterol/HDL cholesterol in the fat fed groups increased as 4.65±0.22 but after AKC treatment it was significantly altered to 3.11±0.04 and 3.48±0.04 respectively. The AI in standard drug Lovastatin treated animals it was 2.86±0.06which was almost equivalent to normal. It was noted that the damage is not completely prevented by any of the above doses of AKC but their use may lessen the atherogenic effects of the animal fats in diets. It appears the BUF is more harmful in their hyperlipidemic and related effects. Most of the altered parameters are ameliorated significantly and the lipid profile was better controlled that the enzyme levels on incorporation of any of the two doses in the high fat diets.

The SGOT and SGPT levels were altered in drug AKC treated animals to 159.87±5.75, 198.40±5.44 and 64.11±2.31, 101.02±2.64 from 234.48±5.50 and 130.40±4.56 respectively. The total protein, Urea and Glucose levels were also altered towards normal on AKC treatment. In treated groups a significantly reduced level of HMG CoA reductase, the rate limiting enzyme in cholesterol synthesis may be responsible for the fall in cholesterol level. Modern lipid lowering agents i.e., statins (Atrovastatin, Simvastatin, Rosuvastatin etc.) are expensive. The most important adverse effects of statins are liver and muscle toxicity. Other risk factors are hepatic dysfunction, renal insufficiency, hypothyroidism, advanced age and serious infections. The liver, Heart and Kidney weight was signicantly increased in hyperlipidemic rats which was normalized in the AKC treated animals.

The hyperlipidemic and particularly the hypercholesterolemic effects of the animal fats may be due to the higher percentage of saturated fatty acids. i.e. 54-68% in them. A richer content of cholesterol on BUF may account for a greater hypercholesterolemic effect. Hyperlipidemia is one of the major risk factor for cardiovascular disease like atherosclerosis. Atherosclerosis is a generalized and inflammatory vascular disease frequently associated with renal disease and dysfunction. Diverse renal vascular diseases, including atherosclerotic renal vascular disease, account for more than one third of all cases of end stage renal disease. An enhancement in the activities may be due to various reasons. viz; as a result of stimulation of different metabolic pathways leading to the synthesis of cholesterol from dietary fats and also from the interconversion of aminoacids and breakdown of phospholipids and related compounds under a stress of high fat diets.

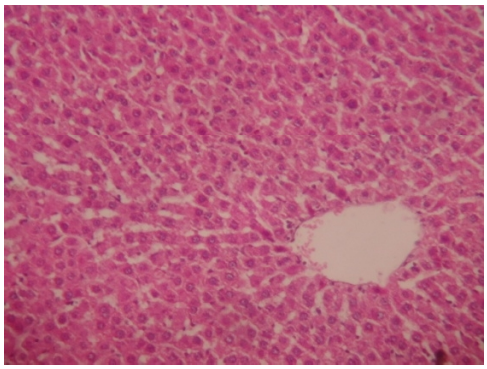
All these results emphasise the need for incorporation of the AKC in our daily diet as a measure to protect our body from atherosclerosis and related diseases. Abundant evidence supports the link between hyperlipidemia and atherosclerosis. Clinical trials showed that lowering lipids reduces the morbidity and mortality associated with cardiovascular complications. It is well known that HDL-Cholesterol levels have a protective role in Coronary artery disease. Similarly increased level of serum LDL-cholesterol results in increased risk for the development of atherosclerosis.

The increased level of HDL- cholesterol and decreased cholesterol level along with its LDL fraction which is evident from the results could be due to an increased cholesterol excretion and decreased cholesterol absorption through gastro intestinal tract. Thus the decreasing cholesterol levels in the body under the influence of AKC could have enhanced the enzymatic by a positive feedback mechanism. The rats fed with high cholesterol diet exhibited significant increase in TC, LDL-C and VLDL and significant decrease in HDL-C, HDL-C ratio as compared to the normal animals.

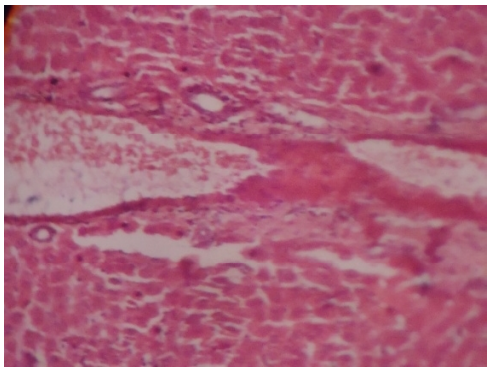
CONCLUSION

From the toxicity study, it was established that the Amukkara Kizhangu Chooranam is non toxic upto 2000mg/kg. The test drug Amukkara Kizhangu Chooranam for 21 days treatment significantly lowered the total cholesterol, triglycerides and other biochemical parameters elevated on cholesterol rich diet. Histopathological reports substantiate the beneficial effect of test drug on the reduction in the fat deposition in the liver. Based on the above results, it can be concluded that the Amukkara Kizhangu Chooranam is an effective drug in the treatment of hyperlipidemia at the dose level of 100mg/kg. The overall beneficial effect of Amukkara Kizhangu Chooranam was observed in low dose treatment only in animal models.

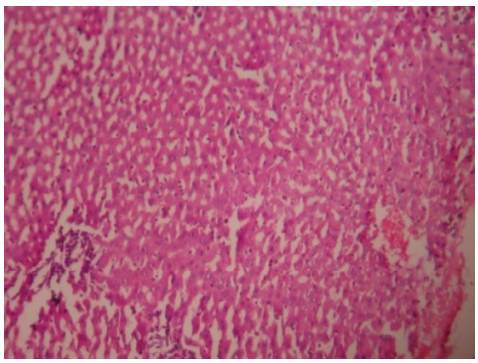
HISTO-PATHOLOGICAL SLIDES – PHARMACOLOGICAL STUDIES FOR TRIAL DRUG 1



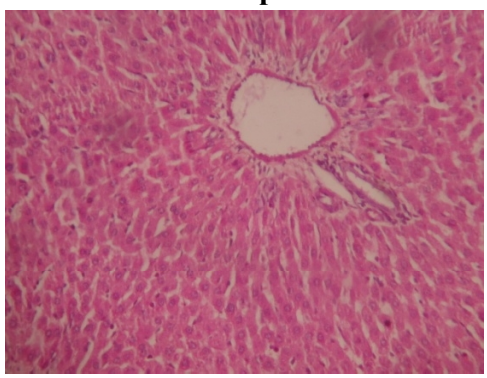
Normal control



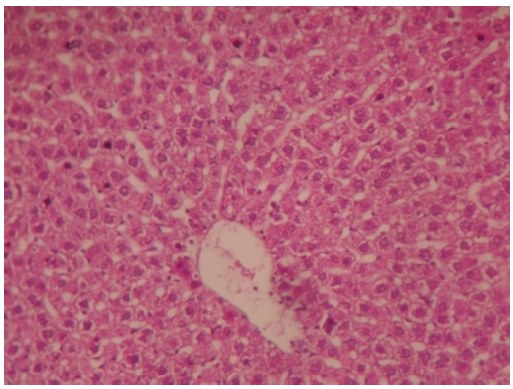
Hyperlipidemic control –cells with fat Deposition.



AKC 100mg/kg moderate accumution of fats



AKC 200 mg/kg-intact cells with normal Cellular architecture.



Lovastatin treated

HYPERLIPIDEMIA[12]

Hyperlipidemia is a heterogeneous group of disorders characterized by an excess of lipids in the bloodstream. These lipids include cholesterol, cholesterol esters, phospholipids, and triglycerides

SYNONYMS

- Hypercholesterolemia
- Hypertriglyceridemia
- Hyperlipoproteinemia
- Dyslipidemia
- High serum cholesterol

EPIDEMIOLOGY:

The World Health Organization (WHO) reports that high cholesterol contributes to 56% of cases of Coronary Heart Disease worldwide and causes more than 4 million deaths each year.

AGE

- Total and LDL-C rise about 20% in men aged 20 to 50 years
- Total and LDL-C rise steadily about 30% in women aged 20 to 60 years
- Younger women have lower levels than men
- Homozygous familial hypercholesterolemia manifests itself from birth

GENDER

Incidence is higher among men than women.

SOCIOECONOMIC STATUS

- Awareness of dietary factors that affect plasma lipid levels increases with higher educational levels
- Low-cost food items are often higher in saturated fats and lower in nutritional value

ETIOLOGY:

Common causes

- Familial combined hypercholesterolemia is the most common primary lipid disorder, characterized by moderate elevation of plasma triglycerides and cholesterol and reduced plasma HDL-C
- Familial Hypertriglyceridemia

Dietary causes include:

- Fat intake per total calories greater than 40%
- Saturated fat intake per total calories greater than 10%
- Cholesterol intake greater than 300 mg per day
- Habitual excessive alcohol use

Lifestyle contributing factors include:

- Habitual excessive alcohol use
- Obesity
- Lack of exercise

Drugs associated with Hyperlipidemia include:

- Anabolic steroids
- Retinoids
- Birth control pills and estrogens
- Corticosteroids
- Thiazide diuretics
- Protease inhibitors
- Beta-blockers

SYMPTOMS:

Usually asymptomatic

Primary type I:

Type I Hyperlipidemia is quite uncommon according to Harrison's Principles of Internal Medicine. It is also called familial Hyperchylomicronemia and Buerger-Gruetz syndrome. This disorder causes high chylomicrons, the proteins that carry fat from the intestine to the liver. It can cause abdominal pain, pancreatitis, fat deposits in the skin and eyes and a large liver and spleen. Treatment involves eating a healthy diet.

Primary type II:

Type II Hyperlipidemia is divided into type IIa and type IIb. Type IIa is also known as familial hypercholesterolemia and type IIb is also known as familial combined Hyperlipidemia. Type lIa results in high LDL, or "bad" cholesterol, levels. Type IIa also raises levels of LDL, as well as a similar lipoprotein, VLDL, which results in elevated fat levels in the blood. These conditions cause fat deposits under the skin and around the eyes, and are treated medically and with dietary control.

Primary type III:

Type III Hyperlipidemia is an uncommon disorder also known as familial Dysbetalipoproteinemia, remnant removal disease or broad-beta disease. It results in high levels of LDL and carries a very significant risk of heart disease. It is treated with medicine and diet.

Primary type IV:

Type IV is also known as familial Hyperlipidemia. Cholesterol levels tend to be normal and fat is elevated in the blood as VLDL levels are elevated. It is also treated with medicines and proper diet.

Primary type V:

Type V is another rare type that is characterized by elevated chylomicrons and VLDL. It is also known as endogenous Hypertriglyceridemia.

Accquired:

According to "Greenspan's Basic & Clinical Endocrinology" by Dr. David Gardner, acquired Hyperlipidemia is high fat and cholesterol in the blood due to other conditions or medications. Diabetes, low thyroid hormone levels, kidney disease and some other metabolic disorders cause Hyperlipidemia. Some drugs can also cause Hyperlipidemia, including alcohol, diuretics, estrogens and beta blockers.

Complications:

Arteriosclerotic heart disease:

A serious complication associated with hyperlipidemia is a condition called arteriosclerotic heart disease, coronary heart disease or hardening of the arteries. Plaque formation narrows the arteries and prevents blood and oxygen from reaching the heart. As the disease progresses the blood vessels may become so constricted that blood and oxygen are unable to reach the heart, resulting in breathing problems, chest pain or heart attack.

Heart attack:

People who have Hyperlipidemia are at risk for an early heart attack .A heart attack can occur when blood clots prevent blood flow through the coronary arteries to the heart. When the heart does not receive an adequate amount of blood and oxygen, the heart muscle may become damaged or die. Hyperlipidemia may experience a heart attack when cholesterol plaques accumulate in the coronary arteries and block blood and oxygen from reaching the heart.

Stroke:

A stroke can occur when reduced blood flow to the brain deprives the brain tissue of oxygen and nutrients. When the brain does not receive blood and oxygen for several minutes, the brain cells begin to die. Hyperlipidemia may experience a stroke when fatty plaques loosen from their constricted coronary arteries, lodge in the brain and block blood flow to that part of the body.

CLINICAL STUDY

Clinical trial on Amukkara Kizhangu Chooranam in the management of Athimetham(Hyperlipidemia) for Hypolipidemic activity got approved by institutional ethical committee, NIS on 24/12/2011. Approval no is NIS/IEC/2011/3/13b-24/12/2011.

Based on the protocol approved by IEC,NIS the study was conducted on Athimetham (Hyperlipidemia) patients.The study was conducted in National Institute of Siddha , Ayothidass Pandithar Hospital, Chennai -47.

Study type : pilot study

Sample size : 20 patients

SUBJECT SELECTION

Patients reporting at OPD of Ayothidoss Pandithar hospital with inclusion criteria were subjected to screening test & documented using screening proforma.

INCLUSION CRITERIA:

Age : 20-60 years.

Sex : male and female

Weight : male above 50 kg

Female above 45 kg.

Increased levels of any one of the following:

- Serum total cholesterol (220-400mgs/dl)
- Serum triglycerides.(170-350mgs/dl)
- Low density lipo protein.(150-300mgs/dl)
- Very low density lipo protein.(50-100mgs/dl)

Family history of hyperlipidemia.

Patient who was already diagnosed as hyperlipidemia.

Patients who are willing to provide blood for investigations before and after treatment.

Patients who are willing to attend OPD once in 7 days.

EXCLUSION CRITERIA:

- Chronic renal failure
- Alcoholism
- Liver disorder
- Pregnancy and lactation
- Drugs
- Any other serious illness

WITHDRAWL CRITERIA:

- development of any adverse reaction
- occurrence of any other serious illness
- Non-co-operation of the patient

Trial drug	:	Ammukara Kizhangu Chooranam
Dose	:	2g twice daily.
Vehicle	:	honey
Duration	:	30 days.

Conduct of the study:

Athimetham patients who satisfied the inclusion and exclusion criteria were admitted to the clinical trial. Patients informed consent was obtained. Routine haematological,urine investigations along with lipid profile were assessed before and after treatment. Trial drug was issued to them once in 7 days .Each time they were assessed clinically .Haematological investigations were taken before and after treatment. . Patients was informed to report about adverse effects if any.

Among 20 patients 45% patients were male 55%were female (trial drug 1 table 20 ,trial drug 1 chart 1)

Among 20 patients 40% patients were in the age group of 30-45 years. (trial drug 1 table 20, bar diagram 8)

Among 20 patients 60 %patients were in the age group of 45-60 years**.** (trial drug 1 table 20)

Among 20 patients, 16 patients showed increase in serum total Cholesterol ,11 patients showed increase in LDL, 10 patients sowed increase in VLDL and 18 patients showed increase in TGL.

After the treatment with Amukkara Kizhangu Chooranam for 30 days, among 20 patients 80% showed decrease in serum total cholesterol, 70% showed decrease in TGL, 45% showed decrease inLDL,40%showed decrease in VLDL. No adverse effects found during the conduct of study **. (**trial drug table 21)

Amukkara Kizhangu Chooranam reduced serum total Cholesterol ,TGL ,LDL and VLDL and it is statistically significant.

DISCUSSION

The principle aim of this study was to assess the pre-clinical safety and efficacy and to evaluate the therapeutic efficacy of the drug Amukkara Kizhangu Chooranam in the management of Athimetham (Hyperlipidemia).

The literary evidence from the text Gunapadam mooligai vaguppu and modern science reviews strongly supports the Hypolipidemic activity of the drug.

Biochemical analysis:

The biochemical analysis of the drug reveals the presence of alkaloids ,iron, glycosides.

Toxicological studies:

In acute toxicity study the results indicate that no deaths or signs of toxicity were observed in the rats that received Amukkara Kizhangu Chooranam up to an oral acute dose of 2g/kg thus establishing its safety in use.

During the sub acute toxicity studies Amukkara Kizhangu Chooranam at 400mg/kg body weight is not toxic to the liver, spleen and testes of rat but has a minor effect on the lungs and kidney.

Pharmacological studies:

The test drug Amukkara Kizhangu Chooranam for 21 days treatment significantly lowered the total cholesterol, triglycerides and other biochemical parameters elevated on cholesterol rich diet. Amukkara Kizhangu Chooranam is an effective drug in the treatment of hyperlipidemia at the dose level of 100mg/kg.

In treated groups a significantly reduced level of HMG CoA reductase the rate limiting enzyme in cholesterol synthesis may be responsible for the fall in cholesterol level.

Clinical observation:

In case of clinical trial , the treatment with Amukkara Kizhangu Chooranam for 30 days,80% showed decrease in serum total cholesterol , 70% showed decrease in TGL,45% showed decrease in LDL,40% showed decrease in VLDL.

The drug has significantly reduced the serum cholesterol and triglyceride level. So the drug may contribute to prevent the risk of cardiac disease.

Bio-statistics:

Statistically, the paired 't' test shows statistical significance for the drug Amukkara Kizhangu Chooranam in the management of Athimetham.

'p' value for S.T.cholesterol is 0.001 .TGL is 0.002 **(**TRIAL DRUG 1 table 22).

Previous studies :

Previous studies showed that the herb possess immuno modulator , haemopoetic ,anti tumor ,anti depressant activity .[30]

Another study proved that flavanoids of withania somnifera WSREt and WSLEt showed hypoglycemic and hypolipedemic activities in alloxan induced diabetic rats. [4]

Siddha Aspect:

அமுக்குராக்கிழங்கின்

சுவை : கைப்பு

வீரியம் : வெப்பம்

பிரிவு : கார்ப்பு.

கார்ப்பு சுவை உடற் பசையையும் , கொழுப்பையும்,வயிற்றில் கபத்தினால் உண்டாகும் துர்நீரையும் வரட்டும் .[13,14]

Athimetham is one of the kabam related disorder .Since Amukkara Kizhangu Chooranam has kaarppu suvai it equalizes the increased kabam humour it and will reduce the lipids as mentioned in siddha text. [13, 14]

The present study is unlike from previous studies on the subject of suvai basis , inducing hyperlipidemia ,dosage form, vehicle and includes clinical trial.

Further the precise mechanism and the active constituents of Amukkara Kizhangu Chooranam which is responsible for its Hypolipidemic activity and related pharmacological responses are still to be determined and further chronic toxicological studies are also to be established.

Abundant evidence supports the link between Hyperlipidemia and atherosclerosis.

Increased level of serum LDL-cholesterol and TGL results in increased risk for the development of atherosclerosis. As per the above studies Amukkara Kizhangu Chooranam may prevent the mortality and morbidity due to atherosclerosis in case of cardio vascular diseases and stroke.

So Amukkara Kizhangu Chooranam will be a better choice of drug in the management of Athimetham(Hyperlipidemia).

SUMMARY

- The literary evidence strongly supports the hypolipidemic activity of Amukkara Kizhangu Chooranam.
- The drug Amukkara Kizhangu chooranam has been selected for this study to evaluate its hypolipidemic activity in the management of Athimetham(Hyperlipidemia).
- Biochemical analysis of the drug Amukkara Kizhangu Chooranam reveals the presence of iron,glycosides, alkaloids & Amino acids.
- In acute toxicity study all the mice that received upto 2.0g/kg dose of the Amukkara Kizhangu Chooranam survived beyond the 24 hours of observation. No deaths or signs of toxicity were observed in the rats that received the Amukkara kizhangu Chooranam up to an oral acute dose of 2g/kg thus establishing its safety in use.
- During the sub acute toxicity studies Amukkara kizhangu Chooranam at 400mg/kg body weight is not toxic to the liver, spleen and testes of rat but has a minor effect on the lungs and kidney. The present results show that Amukkara kizhangu Chooranam possesses very low toxicity as indicated in our rat model.
- The test drug Amukkara Kizhangu Chooranam treatment significantly lowered the total cholesterol, triglycerides and other biochemical parameters elevated on cholesterol rich diet
- A number of 20 patients were included in clinical trial satisfying the inclusion criteria .

  The drug Amukkara Kizhangu Chooranam was given to the patients once in 7 days for 30 days.
- Among 20 patients 80% showed decrease in serum total cholesterol, 70% showed decrease in TGL, 45% showed decrease inLDL, 40% showed decrease in VLDL after the treatment with Amukkara Kizhangu Chooranam for 30 days.

- No adverse effect was developed during the treatment period.
- Statistical analysis- paired ‘t’ test, showed “P” value for Serum total Cholesterol is 0.001 ,LDL is 0.14 ,VLDL is 0.037 and TGL IS 0.002. So the drug Amukkara Kizhangu Chooranam considered to be statistically significant in the management of Athimetham .
- The drug Amukkara Kizhangu Chooranam ensures
- No significant toxicity
- Hypolipidemic Activity.
- No side effects
- No undoing effects
- Encouraging clinical results.
- From the clinical studies and statistical analysis it is proved that the drug Amukkara Kizhangu Chooranam is statistically significant for hypolipidemic activity in the
  management of Athimetham (Hyperlipidemia) in prospective days.

CONCLUSION

- The literature and research journal review of the plant supports that it has Hypolipidemic activity.
- The safety studies (acute toxicity and repeated oral toxicity) studies conducted revealed that the trial drug Amukkara Kizhangu Chooranam is safe. Hence it can be reasonably assumed that the drug is safe for human use.
- The pharmacological study conducted in animal model showed significant **Hypolipidemic activity.**
- Clinical study revealed the therapeutic efficacy of the trial drug by showing, reduction in serum total cholesterol ,TGL significantly. There were no adverse reactions reported during the clinical trial.
- Hence, the drug **Amukkara Kizhangu Chooranam** can be used in the management of **Athimetham (Hyperlipidemia)**

INTRODUCTION

Siddha System is one of the traditional and pioneer systems of medicine. Siddha medicines are broadly classified in to internal medicines and external medicines each of 32 types. They are prepared from herbs, metals, minerals and biological resources. A total number of 4,448 diseases is mentioned in Siddha text as well as with line of treatment.

Kalladaippu is one of the diseases mentioned in siddha text. Kalladaippu can be correlated with renal calculus which is the presence of stones in the kidneys, ureters and bladder. Mankind has been affected by the urinary stones since centuries. Renal calculus is one of the most common urological disorder.

Archeological findings give profound evidence that humans have been suffered from kidney and bladder stones for centuries. Bladder stones were more prevalent during older ages, but kidney stones became more prevalent during the past 100 years[15] The first evidence of urinary stone was found in Egyptian mummy E1amrah eygpt at 4800B.C.[16.]

The high incidence and recurrence rate contribute to making the urolithiasis a serious social problem. Nowadays, urolithiasis must be considered a 'disease in evolution' for several reasons, such as epidemiological changes, evolution of the methods used for diagnosis, and the treatment and prophylaxis of the population considered 'at risk' of stone disease.[17]

The overall probability that an individual will form stones varies in different parts of the world. The risk of developing urolithiasis in adults appears to be higher in the western hemisphere (5–9%) in Europe, 12% in Canada, (13–15%) in USA than in the eastern hemisphere (1–5%), although the highest risks have been reported in some Asian countries such as Saudi Arabia (20.1%).[18]

It is estimated that at least 10% of the population in the industrialized part of the world is afflicted by urinary tract stone disease. Kidney stones are common in industrialized nations with an annual incidence of 0.5% to 1.9%.

Urinary stone constitute one of the commonest diseases in our country and pain due to kidney stones is known as worse than that of labour pain. In India, approximately 5 -7 million patients suffer from stone disease and at least 1/1000 of Indian population needs hospitalization due to kidney stone disease.[16]

In India upper and lower urinary tract stones occur frequently but the incidence shows wide regional variation. The incidence of renal calculi is comparatively low in the southern part of country compared to other parts. [19]

It has been well documented that the incidence of urinary stones is higher in countries with warm or hot climates, probably due to low urinary output and scant fluid intake.[20] 12% of people have stone in their life time. Highest incidence of urinary stone in the age group of 30-45 years and declines after the age of 50. 12% of men and 5 % of women suffer from urinary stone by the age of 70. 50% have their recurrence in 5-10 years. 7-10 of every1000 hospital admission is a renal stone .[16]

First-degree relatives of stone-formers have a 2-16 times higher risk of developing renal stones when compared with the general population. In a stone-former, the probability of having a relative with stones may be as high as 35-65% as compared with 5-20% probability in a non-stone-former.[18]

Treatment options and conservative measures, as well as 'surgical' interventions have also been known for a long time. In the recent few days new modern techniques are available to treat renal calculi which are not cost effective to low and middle socio-economic group. Even though our current preventive measures are definitively good the incidence and recurrence has not yet reduced markedly.

Several Siddha medicines evidenced lithontriptic activity in the management of renal calculi. Sarva Noi Linga Chenduram is one of the herbo-mineral formulation which is mentioned in siddha text for Kalladaippu[32].

The ingredients of Sarva Noi Linga Chenduram are Lingam and Venkaram. In siddha text it is mentioned that "அப்பு பூத உறுப்புகளில் உண்டாகும் நோய்களை நீக்கும்" for Lingam and also Venkaram possess lithontriptic,diuretic activity. The efficacy of this drug for Kalladaippu has not been evaluated so far.

Hence the researcher has selected Sarva Noi Linga Chenduram to evaluate its lithontriptic activity and therapeutic effect in the management of Kalladaippu.

Aim:

To evaluate the safety and efficacy of "Sarva Noi Linga Chenduram" for Lithontriptic activity in the management of Kalladaippu [Renal calculi]

OBJECTIVE:

Primary objective:

To evaluate the lithontriptic activity of "Sarva Noi Linga Chenduram" for Lithontriptic activity in preclinical studies.

Secondary objective:

Bio –chemical analysis.

Atomic Absorption spectrometer Study.

To evaluate the therapeutic efficacy of "Sarva Noi Linga Chenduram" in clinical trial for Lithontriptic activity in the management of Kalladaippu[Renal Calculi].

MATERIALS AND METHODS

STANDARD OPERATIVE PROCEDURE:

COLLECTION AND AUTHENTICATION OF RAW DRUG:

The raw drugs were procured from raw drug store in Chennai and authenticated by competent authority of Department of Gunapadam ,National Institute of Siddha, Chennai.

PREPARATION OF THE MEDICINE:

INGREDIENTS:

Purified Lingam (Cinnabar) - 35g

Purified Venkaram (Borax) - 140g

PURIFICATION METHODS:

Purification of Lingam: [21]

It was kept on a mud vessel and heated in low fire. The juices of citrus lemon, Acalypa indica, cow's milk are mixed in equal proportions. The mixed liquid was poured drop by drop on lingam while heating.

Purification of Venkaram: [22]

Venkaram ground by the citrus lemon juice and then dried it

METHOD OF MEDICINE PREPARATION:

Lingam was grinded into tiny particles. Venkaram was placed in a mud vessel and heated in a low fire. When venkaram started melting purified lingam was sprinkled little by little. It had to be mixed well. Before melting of venkaram all quantity of lingam was sprinkled. After that the medicine was taken away from the heat. By the time, it got completely condensed. Then it was well ground in the kalvam and stored in an air tight container.

LABELLING:

Name of the preparation	:	Sarva Noi Linga Chenduram.
Quantity of the drugs	:	Lingam 35g, Venkaram140g.
Dose	:	130 mg, twice a day.
Adjuvant or Vehicle	:	Mullangi (Raphanus sativus) juice.
Indication	:	Kalladaippu [Renal calculi]
Date of manufacturing	:	17/3/12
Date of expiry	:	75 years from the date of manufacturing

LINGAM BEFORE PURIFICATION LINGAM AFTER PURIFICATION

VENKARAM

BEFORE PURIFICATION AFTER PURIFICATION

SARVA NOI LINGA CHENDURAM

SIDDHA ASPECT

இலிங்கம்[25]

வேறு பெயர்கள்:

ஆண்குறி

இங்குலிகம்

இராசம்

கடைவன்னி

கர்ப்பம்

கலிக்கம்

காஞ்சனம்

காரணம்

சண்டகம்

சமரசம்

சானியம்

செந்தூரம்

மணிராகம்

மிலேச்சம்

வனி

வன்னி

குணம்:

இதற்கு கனத் தன்மையும், நெருப்பிலிடப் புகையுந் தன்மையும் நீரில் கரையாத்தன்மையும் உண்டு, வாசனையும் உருசியும் கிடையா.

பொது குணம்:

" பேதிசுரஞ் சந்தி பெருவிரண நீரொடுத
காதகடி காசங் கரப்பான்புண்-ணோத
வுருவுலிங்க சங்கதமா யூறுகட்டி யும்போங்
குருவிலிங்க சங்கமத்தைக் கொள். "

" ஆதி யிரதவுருக் காதலாற் சாதிலிங்க
மோதி லிரதகுண முற்றுடலிற்-றீதுபுரி
குட்டங் கிரந்தி கொடுஞ்சூலை வாதமுத
லுட்டங்கு நோய்களையோட் டும்."

தோற்றத்தில் பாதரச உருக்காகிய சிவந்த நிறத்தை உடைய சாதிலிங்கமும்,அது சேர்ந்த மற்ற மருந்துகளும், அந்த இரச குணத்தைக் கொண்டு துன்பத்தை உண்டுபண்ணுகின்றபேதி, சுரம், சந்நிபாதம், தீராப்புண்கள், அதிமூத்திரம்,காணாக்கடி, விடம், காசம், கரப்பான், சிரங்கு, சொல்வதற்கும் பார்ப்பதற்கும், வெறுப்புத் தோன்றும் பரவு நுணாக்காய்க் கிரந்தி ,குட்டம்,கொடுமை செய்கின்ற சூலை வாத நோய் முதலியவைகளையும், மற்றும் உடலில் மறைந்து இருக்கும் பிணிகளையும் நீக்கும்.

" நிலத்தி லெழுந்த பிணிநீங்காக் கிரந்தி
சலத்துடனே சூலைவெடிதானகற்றும்-பலத்ததாம்
சாதிலிங்கத் தின்குணத்தைச் சாற்றினேன் சன்னிமுதல்
ஓதுசுரம் போமே ஒளிந்து."

நிலத்தெழுந்த பிணி-பிருதிவி பூத உறுப்புகளில் உண்டாம் நோய்கள்;சலப்பிணி-அப்பு பூத உறுப்புகளில் உண்டாகும் நோய்கள்.

சுத்தி முறைகள்:

- பழச்சாறு,பசும்பால்,மேனிச்சாறு இம்மூன்றையும் சமவெடைகூட்டி, இலிங்கத்திற்குச் சுருக்கிட்டெடுக்க,இது சுத்தியாம்.
- முலைப்பாலிலும் , எலுமிச்சங்கனி இரசத்திலும் முறையே ஒவ்வொரு நாள் ஊற வைக்க வேண்டும்.

அளவு:

உள் மருந்து : 10 உளுந்தெடை (650 மி கி) வரை உள்ளுக்கு கொடுக்கவும்

புகை : 1/2 வராகன்.

(பைஷஜ கல்பம்).

வெங்காரம் [25(a)]

வேறு பெயர்கள் :

பொரிகாரம்

காரம்

உருக்கினம்

உருக்கு மித்திரன்

டங்கணம்

தூமத்தையடக்கி

சுவை:

இனிப்புடன் கூடிய துவர்ப்புச் சுவையை உடையது

வீரியம் : வெப்பம்

“வெங்காரம் வெய்தெனிலும் நோய் தீர்க்கும்” என்ற அடியால் உனரலாம். .

செய்கை :

- குளிர்ச்சி உண்டாக்கி.
- சிறுநீர்பெருக்கி
- ருதுஉண்டாக்கி
- பிரசவகாரி
- கற்கரைச்சி

வெளியாட்சி:

- சமனகாரி
- உடல் தேற்றி
- அழுகலகற்றி
- துவர்ப்பி

பொது குணம் :

"சொறிபுடையெண் குன்மநமை சோரி யாசம்
பறிகிரகணி கல்லானம் பன்னோய்-னெறியைத்
தடங்கணங்க பங்கிருமி சர்ப்பவிடஞ் சந்நி
யிடங்கணங்க லக்கற்போ மெண்."

வெங்காரத்தினால், தவளைச் சொறி, புடை, எண்வகைக் குன்மம், தினவு, இரத்தமூலம், ஒழுக்குக் கிரகணி, அஸ்மரி, பங்குவாதம், பல் நோய், நாளவழியை தடுக்கின்ற மூத்திரகிரிச்சரங்கள் ,கபாதிக்கம், புழு ,பாம்பு முதலியவைகளால் உண்டாகும் நஞ்சு,சந்நிபாதம் முதலிய நோய்கள் நீங்கும்.

சுத்தி முறைகள்:

- பசுவின் சாணப்பாலில் இதனைக் கழுவி உலர்த்த வேண்டும்.
- இதனை நீர்வற்றும்படி பொரித்துக் கொள்ள வேண்டும்.
- இதனைச் சட்டியில் இட்டுப் பொரித்துக் காடியிலாவது பழச்சாற்றிலாவது அரைத்து உலர்த்தி எடுக்க வேண்டும்
- பழச்சாற்றிலாவது அரிசிக் கழுநீரிலாவது அரைத்து உலர்த்திக் கொள்ள வேண்டும்

உபயோகங்கள்:

பொரித்த வெங்காரத்தை 5 (650 மி கிராம்) முதல் 10 குன்றியெடை (1.3 கிராம்) வரை இளநீரில் போட்டுக் கொடுக்க நீர்க்கட்டு குணமாகும்.

MINEROLOGICAL ASPECT

LINGAM- CINNABAR [23, 24]

Cinnabar:

Cinnabar is a primary ore of mercury, a pigment and as a minerals specimen which was mined by the Roman Empire for its mercury content and it has been the main ore of mercury throughout the centuries.

Chemical formula : HgS, Mercury Sulfide.

Class : Sulfides and Sulfosalts

Occurrence

Generally cinnabar occurs as a vein-filling mineral associated with recent volcanic activity and alkaline hot springs. Cinnabar is deposited by epithermal ascending aqueous solutions (those near surface and not too hot) far removed from their igneous source.

PHYSICAL CHARACTERISTICS:

- Color is a bright scarlet or cinnamon red to a brick red.
- Luster is adamantine to submetallic in darker specimens.
- Transparency crystals are translucent to transparent.
- Crystal System is trigonal.
- Crystal Habits: individual, well formed, large crystals are scarce; crusts and crystal complexes are more common; may be massive, or in capillary needles. Crystals that are found tend to be the six sided trigonal scalahedrons that appear to have opposing three sided pyramids. It also forms modified rhombohedrons, prismatic and twinned crystals as discribed above.
- Cleavage is perfect in three directions, forming prisms.
- Fracture is uneven to splintery.

- Hardness is 2 - 2.5.
- Specific Gravity is approximately 8.1+ (very heavy for a non-metallic mineral)
- Streak is red
- Associated Minerals are realgar, pyrite, dolomite, quartz, stibnite and mercury.
- Other Characteristics: silghtly sectile and crystals can be striated.
- Notable Occurances include Almaden, Spain, Idria, Serbia, Hunan Prov., China and California, Oregon, Texas, and Arkansas, USA.
- Best Field Indicators are crystal habit, density, cleavage, softness and color.

Other forms of cinnabar

- Hepatic cinnabar is an impure variety from the mines of Idrija in the Carniola region of Slovenia, in which the cinnabar is mixed with bituminous and earthy matter.
- Metacinnabarite is a black-colored form of HgS, which crystallizes in the cubic form.
- Synthetic cinnabar is produced by treatment of Hg(II)
- salts with hydrogen sulfide to precipitate black, synthetic metacinnabarite, which is then heated in water. This conversion is promoted by the presence of sodium sulfide.
- Hypercinnabar, crystallise in the hexagonal form.

VENKARAM – BORAX [26,27.]

Borax, also known as sodium borate, sodium tetraborate, or disodium tetraborate, is an important boron compound, a mineral, and a salt of boric acid.

- **Chemistry** : Na2B4O7 -10H2O, Hydrated sodium borate.
- **Class** : Carbonates
- **Subclass** : Borates

Occurrence:

Borax is a complex borate mineral that is found in playa lakes and other evaporite deposits

PHYSICAL CHARACTERISTICS:

- Color is white to clear.
- Luster is vitreous.
- Transparency crystals are transparent to translucent.
- Crystal System is monoclinic.
- Crystal Habits include the blocky to prismatic crystals with a nearly square cross section. Also massive and as crusts.
- Cleavage is perfect in one direction.
- Fracture is conchoidal.
- Hardness is 2 - 2.5
- Specific Gravity is approximately 1.7 (very light)
- Streak is white.
- Associated Minerals are calcite, halite, hanksite, colemanite, ulexite and other borates.
- Other Characteristics: a sweet alkaline taste, alters to chalky white tincalconite with dehydration.
- Notable Occurrences include Trona, Boron, Death Valley and other California localities; Andes Mountains; Turkey and Tibet.
- Best Field Indicators are crystal habit, color, associations, locality, density and hardness.

Structure:

The basic structure of borax contains chains of interlocking BO2(OH) triangles and BO3(OH) tetrahedrons bonded to chains of sodium and water octahedrons. Most old mineral specimens of borax are chalky white due to a chemical reaction from dehydration. They have actually altered (at least on their surface) to the mineral tincalconite, Na2 B4O7-5H2O, with the loss of water. This kind of alteration from one mineral to another leaves the original shape of the crystal. Minerologists refer to this as a pseudomorph, or "fake shape", because the tincalconite has the crystal shape of the predecessing borax.

The term borax is often used for a number of closely related minerals or chemical compounds that differ in their crystal water content.

- Anhydrous borax ($Na_2B_4O_7$)
- Borax pentahydrate ($Na_2B_4O_7{\cdot}5H_2O$)
- Borax decahydrate ($Na_2B_4O_7{\cdot}10H_2O$)

Borax is generally described as $Na_2B_4O_7{\cdot}10H_2O$. However, it is better formulated as $Na_2[B_4O_5(OH)_4]{\cdot}8H_2O$, since borax contains the $[B_4O_5(OH)_4]^{2-}$ ion. In this structure, there are two four-coordinate boron atoms (two BO_4 tetrahedra) and two three-coordinate boron atoms (two BO_3 triangles).

JOURNAL REVIEW OF SARVA NOI LINGA CHENDURUM

Sarva Noi Linga Chenduram mentioned for lithontriptic activity is composed of sulphide of mercury and sodium tetra borate. On the topic of that the following journals are reviewed.

To determine the effect of Linga bhupathi tablets (Siddha formulation of Impcops) on Indian earthworms. Methods: Linga bhupathi (100mg/ tablet) were investigated for activity in Indian earthworms (Pheretima postuma) against piperazine citrate (15mg/ml) and albendazole (20mg/ml) as standard reference and normal saline as control. The time to achieve paralysis of the worms was determined. Results: The two concentration of Linga bhupathi tablet exhibited significant anthelminthic activity ($p<0.001$) when compared with the piperazine citrate, albendazole and normal saline. Conclusion: Linga bhupathi tablet has paralytic effect on Indian earthworms.[1]

Linga kattu has the efficacy to control the fungal growth in lower concentrations[2]

The mechanism of action of mercurial diuretics has been analyzed by examining 32 different organic mercurials and determining their *in vitro* acid lability, rate of excretion, and structural characteristics, and correlating this with their activity as diuretics. All active diuretics are acid labile. No acid stable compounds are diuretics. However, within the acid labile group, distribution, as reflected by rate of excretion, appears to be a second determinant of diuretic potency. The factors which influence distribution and excretion are discussed. The urinary excretory products of mercurials have been identified. In most instances these are mainly the cysteine complex of the administered mercurial. With extremely acid-labile compounds, the urinary excretory product may be identified as mercuric cysteine, thus demonstrating the *in vivo* rupture of the carbon-mercury bond. It is concluded that the diuretic response is attributable to the *in vivo* intrarenal release of mercuric ions and that, with the compounds commonly employed, this occurs with only a minute fraction of the agent that is administered.Some components of mercurial diuretics are given below [3].

A study conducted on female mongrel dogs about structure diuretic activity relationships of organic compounds of mercury. The results showed that the following components possess diuretic activity.[4]

- 3-hydroxy mercuri-2 methoxy -1 propycarbamyl –o-phenoxy acetate.
- 3-acetomercuri-2-methoxy succinyl propyl urea.
- 3-chloromercuri-2-methoxy propyl urea.
- Aceto mercuric-2-methoxy 1-hydroxy propane.
- 3-bromo mercuric propane.
- Mercuric chloride.

PHYSICAL PROPERTIES

The physical properties of Sarva Noi Linga Chenduram was carried out in Sri Ramachandra University Chennai.

pH at 10% of aqueous solution:

Five grams of the sample weighed accurately and placed in clear 100 ml beaker. Then 50 ml of distilled water is added to it and dissolved well. Wait for 30 minutes and then apply in to pH meter at standard buffer solution of 4.0, 7.0, 9.2**. (**trial drug 2 table 3)

Ash Values:

The Ash values are a measure of the inorganic constituents present in the raw drug. A high ash content explains its unsuitable nature to be used as a drug **(**trial drug 2 table 3)

Total Ash:

A little of extract was taken in a silica crucible previously ignited, cooled and weighed. It was incinerated by gradually increasing the heat not exceeding dull red heat (450°C) until free from carbon, cooled and weighed. The percentage of ash was calculated with reference to air- dried drug. The procedure was repeated to get the constant weight. **(**trial drug 2 table 3)

Water soluble ash:

The total ash was boiled with 25 ml water and filtered through ash less filter paper (Whatmann 4.1). It was followed by washing with hot water .The filter paper was dried and ignited in the silica crucible, cooled and the water insoluble ash was weighed.The water-soluble ash can be calculated by subtracting the water insoluble ash from the total ash. **(**trial drug 2 table 3)

Acid insoluble ash:

The total ash obtained was boiled for 5 minutes with 25 ml of (10% w/v) dilute hydrochloric acid and filtering through ash less filter paper (Whatmann 4.1). The filter paper was ignited in the silica crucible, cooled and insoluble ash was weighed. **(**trial drug 2 table 3)

BIO -CHEMICAL ANALYSIS OF SARVA NOI LINGA CHENDURAM

The biochemical analysis of the Sarva Noi Linga Chenduram was carried out in the Biochemistry lab, NIS, Chennai.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Appearance of sample	Light yellow in colour	
2.	Solubility: a. A little(500mg) of the sample was shaken well with distilled water. b. A little(500mg) of the sample was shaken well with con. HCl/Con. H_2So_4	Sparingly soluble	Absence of Silicate
3.	Action of Heat: A small amount(500mg) of the sample was taken in a dry test tube and heated gartly at first and then strong.	No white fumes evolved	Absence of Carbonate
4.	Flame Test: A small amount(500mg) 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 appeared.	Absence of Copper
5.	Ash Test: A filter paper was soaked into a mixture of sample and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	No Yellow colour flame appeared.	Absence of sodium

Preparation of Extract:

5gm of Sarva Noi Linga Chendurum was weighed accurately and placed in a 250ml clean beaker and added with 50ml of distilled water. Then it was boiled well for about 10 minutes. Then it was cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water.

S.No	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 b. 2ml of the above prepared extracts was added with 2ml of dil-HCl was added until the effervescence ceases off. Then 2ml of dil.Barium chloride solution was added.	Cloudy appearance present	Presence of Sulphate
2.	Test For Chloride: 2ml of the above prepared extract was added with dil. HCl till the effervescence ceases. Then 2ml of dil.silver nitrate solution was added.	No cloudy appearance.	Absence of Chloride
3.	Test For Phosphate: 2ml of the extract was treated with 2ml of dil.ammonium molybdate solution and 2ml of con.HNo3.	No Yellow appearance present	Absence of Phosphate

4 .	Test For Carbonate: 2ml of the extract was treated with 2ml dil. Magnesium sulphate solution	No Cloudy appearance.	Absence of carbonate
5.	Test For Nitrate: 1gm of the substance was heated with copper turning and concentrated H2So4 and viewed the test tube vertically down.	No Brown gas evolved.	Absence of Nitrate
6.	Test For Sulphide: 1gm of the substance was treated with 2ml of con. HCL	No Rotten Egg Smelling gas.	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 was placed.	No Characteristic changes	Absence of Nitrite
9.	Test For Borate: 2 Pinches(50mg) of the substance was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	Bluish green colour flame.	presence 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 obtained.	Absence of Lead
2.	Test For Copper: a. One pinch(50mg) of substance was made into paste with con. HClin a watch glass and introduced into the non-luminuous part of the flame.	No Blue colour flame No Blue colour precipitate formed.	Absence of copper
3.	Test For Aluminium: To the 2ml of extract, dil.sodium hydroxide was added in 5 drops to excess.	NoYellow colour appeared.	Absence of aluminium
4.	Test For Iron: a. To the 2ml of extract,2ml of dil.ammonium solution was added. b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 was added	No blood 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 was added.	No White precipitate was formed	Absence of Zinc
6.	Test For Calcium: 2ml of the extract was added with 2ml of 4% dil.ammonium oxalate solution	No Cloudy appearance and white precipitate was obtained	Absence of calcium

7.	Test For Magnesium: To 2ml of extract dil.sodium hydroxide solution was added in drops to excess.	No white precipitate was 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 substance 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 Yellowish precipitate was obtained.	Absence of Potassium
10.	Test For Sodium: 2 pinches(50mg) of the substance was made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame appeared	Absence of sodium
11.	Test For Mercury: 2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.	No yellow precipitate was obtained	Absence of mercury
12.	Test For Arsenic: 2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.	No brownwish red precipitate was 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.	Brick red colour not developed	Absence of reducing sugar
3.	Test For The Alkaloids: a) 2ml of the extract was treated with 2ml of dil.potassium iodide solution. b) 2ml of the extract was treated with 2ml of dil.picric acid. c) 2ml of the extract was treated with 2ml of dil.phosphotungstic acid.	. No Yellow colour developed	- Absence of Alkaloid
s4.	Test For Tannic Acid: 2ml of extract was treated with 2ml of dil.ferric chloride solution	black precipitate was obtained	Absence of Tannic acid
5.	Test For Unsaturated Compound: To the 2ml of extract 2ml of dil.Potassium permanganate solution was added.	Potassium permanganate was not decolourised	Absence of unsaturated compound

6.	Test For Amino Acid: 2 drops of the extract was placed on a filter paper and dried well. 20ml of Biurette reagent was added.	No violet colour developed	Absence of amino acids
7.	Test For Type Of Compound: 2ml of the extract was treated with 2 ml of dil.ferric chloride solution.	No green colour developed No red colour developed No violet colour developed No blue colour developed	Absence of oxy quinole pinephrine and pyro catechol Anti pyrine, Aliphatic amino acids and meconic acid are absent Apomorphine salicylate and Resorcinol are absent Morphine, Phenol cresol and hydro uinone are absent

ATOMIC ABSORBTION SPECTROSCOPY

Atomic Absorbtion Spectroscopy of Sarva Noi Linga Chenduram was carried out in Sri Ramachandra University, Chennai

The elemental analysis of digested samples have been determined by Atomic Absorption Spectrophotometer (AAS model 400 Perkin Elmer). In this method the sample, in the form of a homogeneous liquid, is introduced into a flame where thermal and chemical reactions create "free" atoms capable of absorbing, emitting or fluorescing at characteristic wavelengths.

In Atomic Absorption Spectrophotometer (AAS) the majority of free atoms in the commonly used flames were in the ground state, but that the flames did not also have enough energy to excite these atoms. A light source emitting a narrow spectral line of the characteristic energy is used to excite the free atoms formed in the flame. The decrease in energy (absorption) is then measured.

METHODOLOGY

I. Microwave Digestion For Elemental Analysis

Model Name: Multiwave3000

Digestion Procedure:

200mg of the given sample is placed in a digestion vessel, acid is added and the mixture is heated for several minutes. After the digestion, the samples are diluted to a specific volume. If too much sample is used in wet digestion, the reaction mixture can become violent. The samples are placed in digestion vessels that fit directly into digestion racks. There are several different acids or mixtures of acids used for digestion, the most common of which is concentrated Hydrochloric acid. The samples are heated slowly at a high temperature. After digestion, the samples are diluted to the appropriate volume with deionized H_2O.

II. Elemental Analysis using Atomic Absorption Spectrophotometer

The elemental analysis of digested samples have been determined by Atomic Absorption Spectrophotometer- Flame technique (AAS model 400 Perkin Elmer). Working standard solutions of sulphur, borate, mercury were prepared from stock standard solution of 1000 ppm from MERCK. Using blank solution to zero the instrument performs the Calibration. The standards are then analyzed and their absorbance recorded. A graph of Absorbance Vs Concentration is plotted. Calibration of the instrument was repeated periodically during operation. A blank reading was also taken and necessary correction was made during the calculation of concentration of various elements.

The digested material was made upto 100 ml for analysis in an (AAS) atomic absorption spectrophotometer (Perkin Elmer). The results were calibrated using standard calibration curve.

ACUTE AND SUB ACUTE TOXICITY STUDIES ON SARVA NOI LINGA CHENDURAM IN RODENTS

Animals:

Mice of either sex weighing 25-30g and rats weighing 210-240g were obtained from the animal house of Vels University. The animals were used with the approval of the Institute animal ethics committee and obtained from Vels University, Chennai. They were fed with a balanced standard pellet diet and maintained under standard laboratory conditions, providing 24-28^{0}C temperature, standard light cycle (12 h light, 12 h dark) and water ad libitum. Animals were kept in cages with raised floors of wide mesh to prevent coprophagy. Animal welfare guidelines were observed during the maintenance period and experimentation. The rats were randomly assigned to control and different treatment groups, six animals per group. (Approval number: XIII/VELS/PCOL/36/2000/CPCSEA/IAEC/08.08.2012). The animals were acclimatized for one week under laboratory conditions.

ACUTE TOXICITY STUDY-OECD 425 GUIDELINES

Acute oral toxicity test for the Sarva Noi Linga Chenduram was carried out as per OECD Guidelines 425. As with other sequential test designs, care was taken to ensure that animals are available in the appropriate size and age range for the entire study. The test substance is administered in a single dose by gavage using a stomach tube or a suitable intubation cannula. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food was withheld for a further 2 hours in mice. The animals were observed continuously for the first 4 h and then each hour for the next 24 h and at 6 hourly intervals for the following 48 h after administering of the test drug, to observe any death or changes in general behaviour and other physiological activities. Single animals are dosed in sequence usually at 48 h intervals. However, the time interval between dosing is determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose was delayed until one is confident of survival of the previously dosed animal.

Observation of toxicity signs: General behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, change in skin and fur, mortality and the body weight changes were monitored daily. The time of onset, intensity, and duration of these signs, if any, was recorded.

SUB-ACUTE TOXICITY

In a 28-days sub acute toxicity study, twenty four either sex rats were divided into four groups of 6 rats each. Group I that served as normal control was administered with distilled water (p.o.) while groups II, III and IV were administered daily with the Sarva Noi Linga Chenduram (p.o.) for 28 days at a dose of 50,100,200 mg/kg respectively. The animals were then observed daily for gross behavioural changes and any other signs of subacute toxicity. The weight of each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per rat was calculated. At the end of the 28 days they were fasted overnight, each animal was anaesthetized with diethylether, following which they were then dissected and blood samples were obtained by cardiac puncture into heparinised tubes. The blood sample collected from each rat was centrifuged with 3000 X g at $4^{o}C$ for 10 min to separate the serum and used for the biochemical assays.

Hematological and blood biochemical analyses:

At the end of the study, all animals were kept fasted for 16-18 h and then anesthetized with anesthetic ether on the 28th day. Blood samples for hematological and blood chemical analyses were taken from retro orbital vein. Heparinized blood samples were taken for determining complete blood count (white blood cell count, differential white blood cell count, platelet count, red blood cell count, hematocrit, and hemoglobin) by semiautomated hematology analyzer. The serum from non-heparinized blood was carefully collected for blood chemistry and enzyme analysis (glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, total and direct bilirubins, serum glutamate-oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP)) were automatically determined using autoanalyzer.

Necropsy:

All rats were sacrificed after the blood collection. The positions, shapes, sizes and colors of internal organs were evaluated. The Spleen, Testes, Pancreas, Lungs, Liver, Brain, Heart, Stomach, Intestine, Bone, Ovary, and Kidney tissues were excised from all rats to visually detect gross lesions, and weighed to determine relative organs' weights and preserved in 10% neutral formalin for histopathological assessment. The tissues were embedded in paraffin, and then sectioned, stained with haematoxylin and eosin and were examined microscopically.

Statistical analysis

Values were represented as mean ± SEM. Data were analysed using one-way analysis of variance (ANOVA) and group means were compared using the Tukey-Kramer Multiple Comparisms Test using GraphPad Instat-V3 software. P values < 0.05 were considered significant. **(**trial drug 2 table 5-14)

RESULTS AND DISCUSSION

In acute toxicity study, the animals treated with 1000mg/kg were showed tolerance with negligible toxic signs. Hence the one tenth of the dose was selected as median therapeutic dose for the further study. In sub acute toxicity study, animals were shown significant toxic clinical signs during the dosing period of 28 days. Animals from Sarva Noi Linga Chenduram treated dose groups not survived throughout the dosing period of 28 days and it was found two animals dead after 12days of treatment in high dose. Results of body weight determination of animals of control and different dose groups exhibited reduction in body weight (P>0.05) after one week of the dosing period.

During dosing period, the quantity of food consumed by animals from different dose groups was found to be comparable (P>0.05) and normal with that of control animals. Ophthalmoscopic examination of animals in control and Sarva Noi Linga Chenduram treated group revealed abnormality as liver damage. Urine analysis data of control group and Sarva Noi Linga Chenduram treated group of animals determined revealed abnormalities like increase in urine volume and colour was reddish brown. Gross pathological examination of animals in control as well as the Sarva Noi Linga Chenduram

treated group revealed abnormalities like liver damage at higher dose treated animals and also and microanatomical changes in bone and spleen tissue.

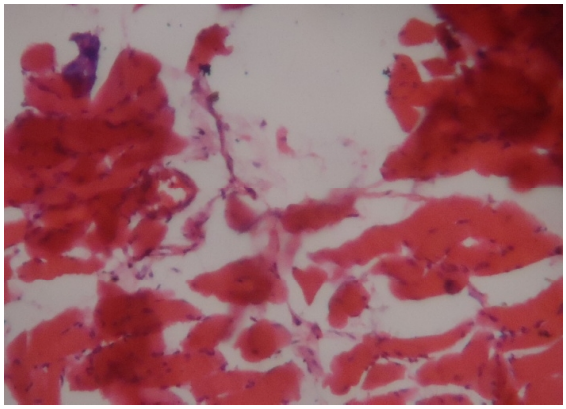
The results of haematological investigations, revealed mild changes ($P>0.05$) when compared with those of respective controls. Results of Biochemical investigations conducted on days 28 revealed the significant changes in the values of different parameters when compared with those of respective controls. Globulin showed increased levels in animals in 50mg/kg dose group ($P<0.01$), Total Protein level is elevated in animals of 100 and 200mg/kg dose group but it is statistically not significant. Uric acid level was elevated in animals of 50 and 200mg/kg group ($P<0.05$). Other all biochemical and Haematological parameters were found to be within normal limit as compared to control group values.

CONCLUSION

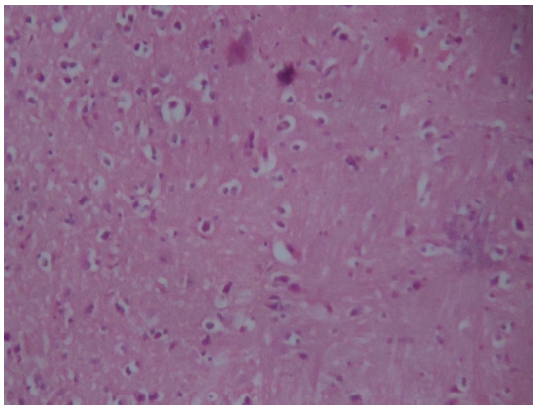
Toxic effect was observed at 200mg/kg of Sarva Noi Linga Chenduram treated via oral route over a period of 28 days. So, it can be concluded that the Sarva Noi Linga Chenduram can be prescribed for therapeutic use in human with the dosage recommendations of upto maximum of 100mg/kg body weight p.o. for long term administrations the 20-30% of dose reduction is very essential to avoid organ damage.

HISTO-PATHOLOGICAL SLIDES – TOXICITY STUDIES

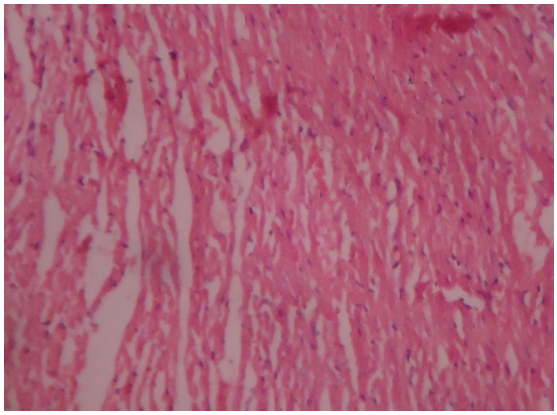
FOR TRIAL DRUG 2



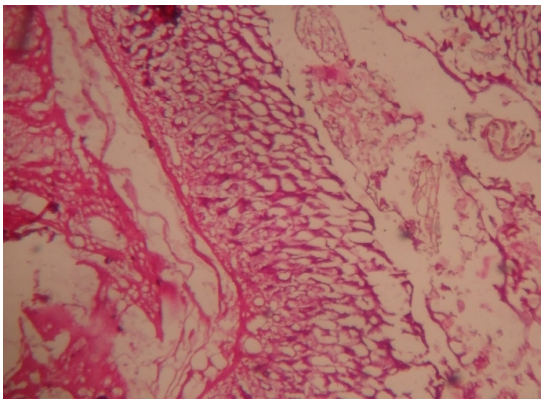
Bone



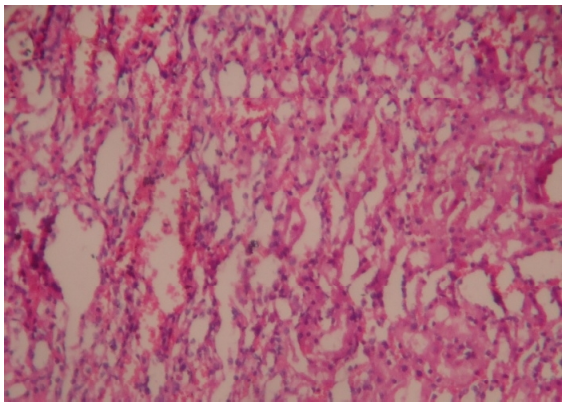
Brain



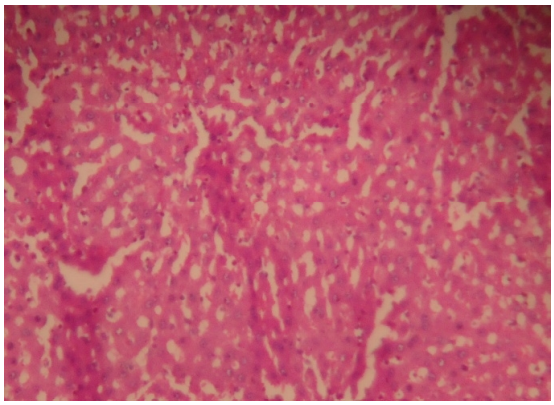
Heart



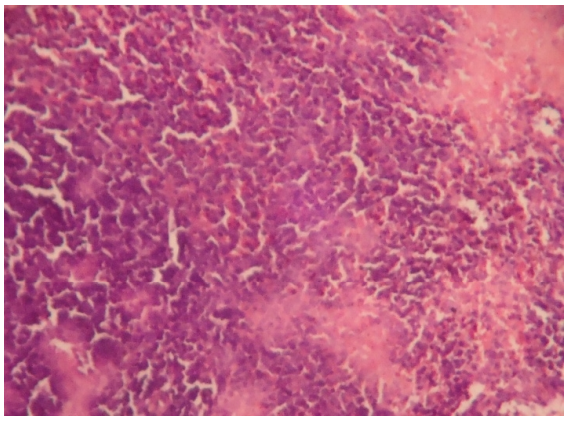
Intestine



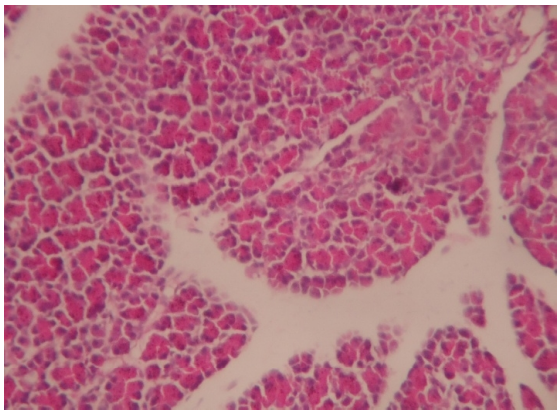
Kidney



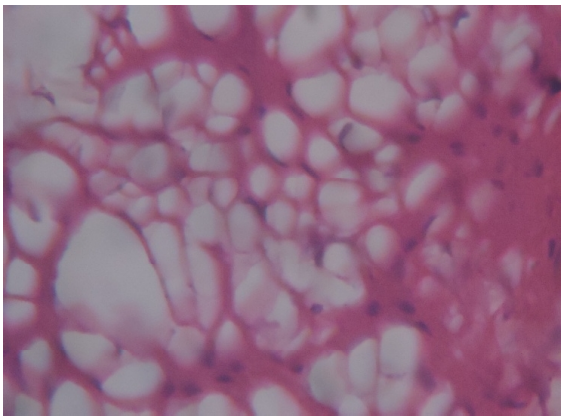
\Liver



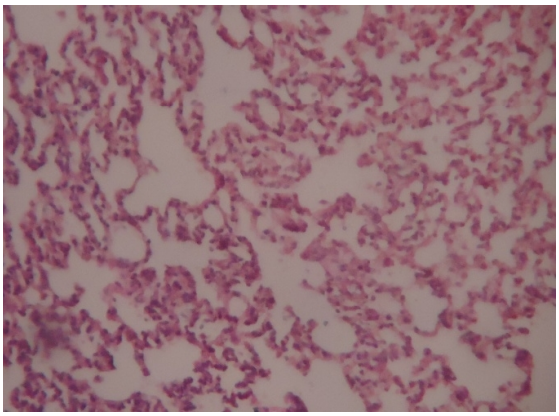
` Lungs



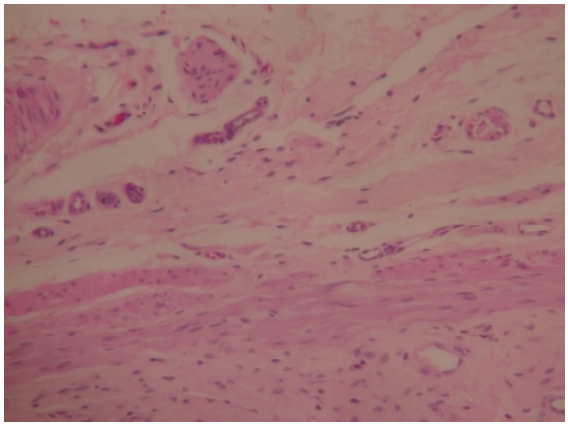
Pancreas



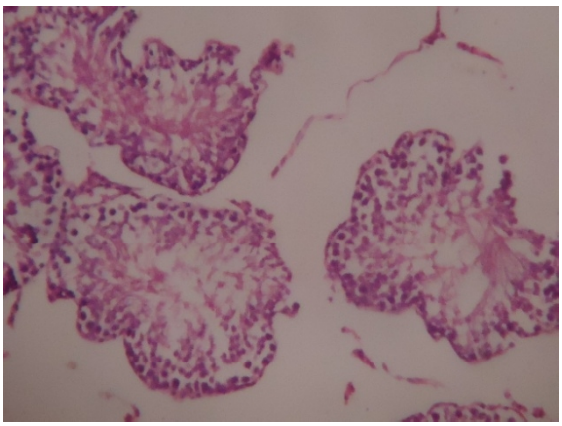
Spleen



stomach



Testis



Ovaries

LITHONTRIPTIC ACTIVITY OF SARVA NOI LINGA CHENDURAM IN ETHYLENE GLYCOL INDUCED LITHIATIC RATS

AIM

To evaluate the Lithontriptic activity of Sarva Noi Linga Chenduram in ethylene glycol induced lithiatic rats.

MATERIALS AND METHODS

Preparation of drug and stock solution

The suspension of siddha drug Sarva Noi Linga Chenduram in 2% (w/v) CMC was prepared for oral administration by gastric intubation method.

Animal selection

For acute toxicity studies, Wistar albino mice of either sex weighing between 28 and 30 g were selected. For the antiurolithiatic study, male Wistar weighing between 180-220 g were used. The animals were acclimatized to standard laboratory conditions (temperature: 25±2°C) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water ad libitum.

(Approval numberXIII/VELS/PCOL/37/2000/CPCSEA/IAEC/08.08.2012).

Acute toxicity studies

The acute oral toxicity study was carried out as per the OECD guidelines 425. One-tenth of the median lethal dose was taken as an effective dose.

Ethylene glycol induced urolithiasis model

Ethylene glycol induced urolithiatic model in rat was be used to assess the effect of Sarva Noi Linga Chenduram. The study is designed to find out the effect of Sarva Noi Linga Chenduram on therapeutic usage against ethylene glycol induced urolithiasis. All rats were housed in metabolic cages for entire duration of the experiment. Animals were divided into five groups containing six animals in each. Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to Groups II-V for induction of renal calculi till 28th day. Group II received Ethylene glycol alone and served as urolithiatic control. Group III received

standard antiurolithiatic drug, cystone (750mg/kg body weight) from 15th day till 28th day. Groups IV received Sarva Noi Linga Chenduram (50mg/kg body weight) from 15th day till 28th day, Group V received Sarva Noi Linga Chenduram (100mg/kg body weight) from 15st day till 28th day.

Group and Treatment

Group 1: Treated with Normal saline

Group 2: Treated with Control (ethylene glycol) + vehicle

Group 3: Treated with Standard (ethylene glycol + Cystone)

Group 4: Treated with Sarva Noi Linga Chenduram (50mg/kg) + ethylene glycol

Group 5: Treated with Sarva Noi Linga Chenduram (100mg/kg) + ethylene glycol

All doses were given once daily by oral route.

Assessment of lithontriptic activity

Collection and analysis of urine:

All animals were kept in individual metabolic cages and urine samples of 24h were collected on 28th day. Animals will be having free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for calcium, phosphate and oxalate content.

Serum Analysis:

After the experimental period, blood was collected from the retro-orbital vein under anesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000x g for 10 min and analyzed for creatinine, uric acid and urea nitrogen.

Kidney homogenate analysis:

The abdomen was cut open to remove both kidneys form each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80°C in a hot air oven. A sample of 100mg of the dried kidney were boiled in 10ml of 1N hydrochloric acid for 30min and homogenized. The

homogenate was centrifuged at 2000x g for 10min and the supernatant was separated. The calcium, phosphate and oxalate content in kidney homogenate were determined.

DIURETIC ACTIVITY:

Standarsization Of Furosemide

Seven groups of six male wistar albino rats were employed four doses of 10,15,20,25-mg/kg b.w of furosemide were administered intraperitonealy to each group of rats separately. The control animals received normal saline alone. The animals were placed in separate cages and the urine output over 24hr period was collected. This procedure was repeated. The most consistent dose (15mg/kg b.w) was adapted for dosing.

Evaluation of diuretic activity

Five groups of six male Wistar albino rats were used. First group received normal saline. Second group received Sarva Noi Linga Chenduram 50mg/kg. The third group received Sarva Noi Linga Chenduram 100mg/kg. The fourth group was administered furosemide 20mg/kg. Immediately after administration of the drug, the rats were placed in metabolic cages, specially designed to separate urine and feacal matter and was observed at room temperature. The animals were denied for food and water during the experiment. The urine volume (ml/day) was measured and then assayed for Na^+ and K^+ and Cl^- concentrations in mMol/l, Cl was measured using routine method.

Statistical analysis:

Results expressed as mean ± S.E.M. Differences among data was determined using one-way ANOVA followed by Dunnet 't' test. **(**trial drug 2 table 14-18 bar diagram 1-4)

RESULTS AND DISCUSSION

The results of acute toxicity study revealed that the Sarva Noi Linga Chenduram is tolerable upto 1000mg/kg and the therapeutic dose was fixed as 50 and 100mg/kg for further pharmacological investigation. Ethylene glycol induced urolithiasis resulted in significant elevation of urine volume, kidney calcium, oxalate, inorganic phosphate, serum blood urea nitrogen, creatinine and uric acid compared to normal control group. Treatment with cystone (750 mg/kg) and Sarva Noi linga Chenduram reduced the bio-

chemical changes induced by ethylene glycol. In order to probe the possible mechanism by which Sarva Noi Linga Chenduram cures renal damage caused by ethylene glycol, investigation on levels of various stone inhibitors like total protein, magnesium and citrate was studied. There was significant rise on total protein, magnesium and citrate after treatment with cystone and Sarva Noi Linga Chendooram.

Administration of ethylene glycol significantly reduced the body weight, urine volume and pH of urine as compared to normal group. Rats treated with cystone and Sarva Noi Linga Chenduram also showed significant decreased in body weight, urine volume and pH of urine as compared to control group. The histopathological study of the kidney sections also supported the above results. In all the stone forming rats there was damage to the last part of the nephron, collecting system and peritubular interstitium as compared to the normal rat kidney architecture. The tubules appeared focally ecstatic and surrounded by inflammatory infiltration.

Flattened epithelium with focal vacuolar degeneration and single cell necrosis bordered the tubules, which focally contained hyaline casts. Inflammatory infiltration was mainly composed of mature lymphocytes infiltrating tubular epithelium. Irregular crystals were present inside the tubules and in the peritubular interstitium, along the nephron and at papillary level. The Sarva Noi Linga Chenduram treated groups showed normal histology of the kidney, and shows normal glomeruli, slight oedema of the tubular cells compared to standard drug treated animals. The kidneys excised from ethylene glycol treated group were larger and heavier than from the control animals. When observed under light microscope, many crystalline deposits in the histological preparations were seen in tubules of all regions of kidney.

In Sarva Noi Linga Chenduram along with EG treated rats, such deposits were small and less abundant. Microscopic examination of kidney sections derived from EG induced urolithiatic rats showed calcification inside the tubules which causes dilation of the proximal tubules. Co-treatment with Sarva Noi Linga Chenduram decreased the calcification in different parts of the renal tubules and also prevented damages to the tubules and calyxes. Organ-body weight ratio is a marker of cell constriction and inflammation. The non-significant effect on the rat kidney-body weight ratio following the administration of various doses of the Sarva Noi Linga Chenduram suggests that the drug did not induce inflammation or constriction of the kidney cells.

Pathologic studies have shown that the renal failure from EG is associated with proximal tubule cell necrosis leading to production of several metabolites (glycol aldehyde, glycolate, glyoxylate and oxalate, in that order) and accumulation of large calcium oxalate monohydrate crystals in tubular lumen.

An Ayurvedic compound preparation (Cystone) was found to contain water soluble substances, which inhibited the initial precipitation of calcium and phosphate ions in the form of a mineral phase bound to the organic matrix and the subsequent growth of the preformed mineral phase. In the present study, concurrent administration of EG with cystone/ Sarva Noi Linga Chenduram causes significant curative effect in EG induced changes. The effect is dose dependent. The effectiveness of Sarva Noi Linga Chenduram is comparable to cystone.

The architectural appearance of the kidneys from the rats in the control group, presented a normal histological appearance with no calcium oxalate depositions with normal glomeruli, tubules surrounded by the Bowmanís capsule, proximal and distal convoluted tubules without any inflammatory changes and normal blood vessels. On the other hand, disrupted renal parenchyma showing loss of structural arrangement of renal tubules, early degenerative changes in glomeruli and focal calcification in glomerulo-tubular structures and congested blood vessels were observed in the renal tissue of urolithiatic rats.

The renal tissue of EG along with Sarva Noi Linga Chenduram shows only few stray areas of calcification in glomeruli and normal tubular structures with no congestion in blood vessels. The renal tissue of standard drug treatment still shows moderate calcification in many tubules and few glomeruli. It has been reported that the kidneys are the principle target organ for ethylene glycol toxicity and administration of ethylene glycol for 3 weeks resulted in insignificant urinary oxalate excretion and deposition of crystals in kidney, hence in our study ethylene glycol was chosen to induce lithiasis. Following the induction of lithiasis the urinary volume and composition were found to be altered.

In our study also the urinary output was markedly decreased in lithiatic control rats on day 28, however in Sarva Noi Linga Chenduram and standard treated rats the urinary volumes were increased when compared to that lithiatic Group. This suggested that Sarva Noi Linga Chenduram might have moderate diuretic effect. Following ethylene

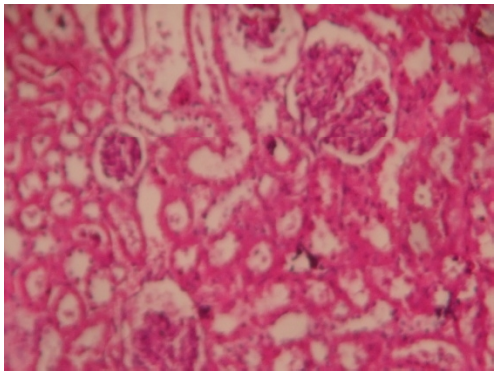
glycol administration the excretion of calcium, oxalate, phosphate and protein were found to be increased in lithiatic group while in standard, test groups these levels were significantly decreased ($P<0.01$).

On contrary to this the magnesium level was decreased in lithiatic group while in standard and Sarva Noi Linga Chenduram treated groups those levels were increased significantly ($P<0.01$). The serum creatinine levels of Sarva Noi Linga Chenduram treated rats restored to normal limits and the creatinine clearance was also found to be improved. The findings of the histopathological studies suggested that no microcrystalline deposition and kidney damage in the Sarva Noi Linga Chenduram treated groups. All these observations enabled us to confirm the inhibitory potential of Sarva Noi Linga Chenduram on ethylene glycol induced lithiasis.

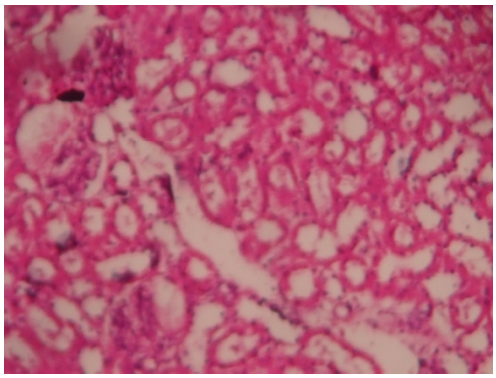
CONCLUSION

The presented data indicate that administration of the Sarva Noi Linga Chenduram to rats with ethylene glycol induced lithiasis reduced the formation of urinary stones, regarding lithontriptic activity of the Sarva Noi Linga Chenduram. The mechanism underlying this effect is still unknown, but is apparently related to diuresis and lowering of urinary concentrations of stone forming constituents. These effects could conclude the antiurolithiatic property of Sarva Noi Linga Chenduram.

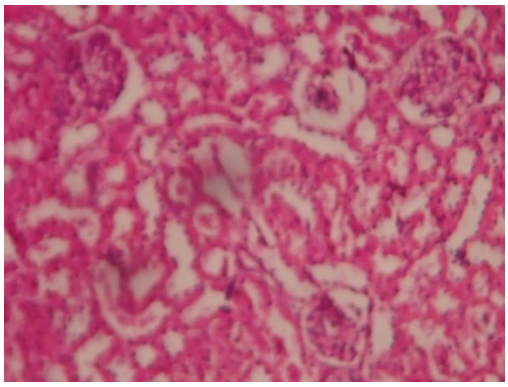
HISTO-PATHOLOGICAL SLIDES – PHARMACOLOGICAL STUDIES FOR TRIAL DRUG 2



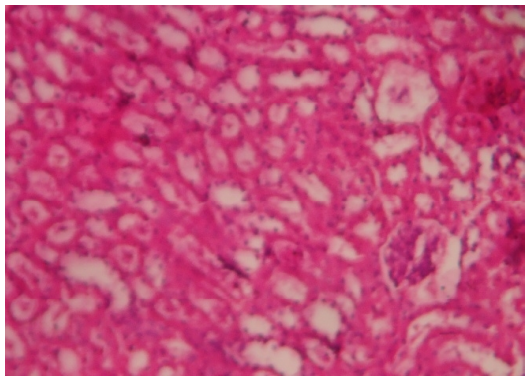
Normal



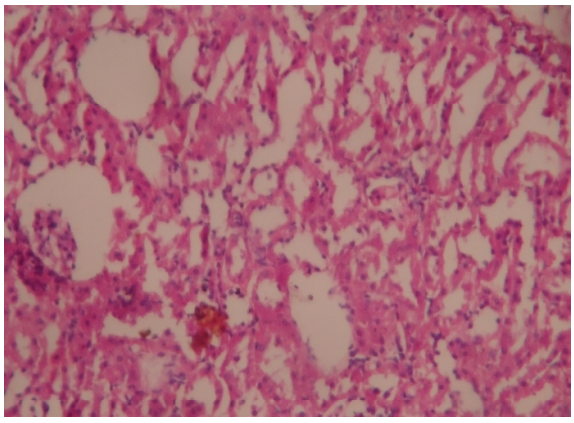
Control



SNLC 50 mg



SNLC 100 Mg



Standard cystone.

SIDDHA ASPECT

கல்லடைப்பு நோய்[28]

வேறு பெயர்:

அச்சமரி

இயல்பு:

சிறுநீர் கழிக்குங்கால் அ து இறங்கிக் கொண்டிருக்கும்போதே திடீரென நீரடைத்தல்,குறிமுனை நோதல்,நீர்ப்புழை எரிதல், இடுப்பின் பின்புறுத்தும், முதுகுத் தண்டின் பக்கத்தும் நோதல்,சிறுநீரில் மணலையொத்த சிறு கற்கள் கலந்திருத்தல் ஆகிய தன்மைகளையுடையதாம்.

நோய் வரும் வழி :

சுனை நீர் பன்னாட்கள் தேங்கிய நீர் இவைகளைப் பருகுவதாலும், மாப்பண்டம், வளிக்குற்றத்தை மிகுதிப்படுத்தும் உணவு முதலியவற்றை உண்பதாலும் விந்து கட்டுப்படுவதாலும் இந்நோய் பிறக்கும் எனக் கூறுவர்.

"கலங்கினதோர் தண்ணீர்தன் குடித்த பேர்க்குக்
கல்லெலும்பு மயிர்மண்தான் கலந்தன் னத்தில்
அலங்கியதோ ரன்னங்க ளருந்த லாலும்
அருகலொடு பழம்பண்ட மருந்த லாலும்
மலங்கினதோர் மாப்பண்ட மருந்த லாலும்
மந்தத்தில் வாயுவாம் பதார்த்தந் தன்னைத்
துலங்கினதோ ருசிதன்னிற் சுவைத்த லாலும்
சுருக்காய்க்கல் லடைப்புவந்து தோன்றுந் தானே"

வகைகள்:

வளி கல்லடைப்பு:

வளிக்குற்றத்தால் பிறந்த கல்லடைப்பு நோயில் உந்திக்குக்கீழ்,சுருக்கு, சுருக்கெனக் குத்தலை உண்டாக்கி நீர் இறங்குங்கால், நின்று நின்று இறங்கும். ஆண் குறி தாங்க முடியாத வலியுடன்,வீங்கிக்காணும். அவ்வலியினால் உட்கார முடியாமல் துன்பமுண்டாதலுமன்றி, அவ்வலியைத் தாங்க முடியாமல் அழுதல் பெருமூச்சுவிடல் வயிறுப்பல் முதலியவற்றைப் பிறப்பிக்கும் . நீரில் நீர்ப்புழையின் மெல்லிய சவ்வும் வெளியாகும்.

அழல் கல்லடைப்பு:

நீர்ப்புழையில் இரும்பைக்காய்ச்சிச் சுட்டதுப் போல் எரிச்சலை உண்டாக்கும்.அன்றியும் , உடல் முற்றும் அனலாக இருத்தலும், சிறுநீருடன் குருதி மிகவும் வெளிப்படுதலும் நீர்ப்புழை முழுவதும் குடைவது போலும், குத்துவதுபோலும், கூச்செறிதல் போலும் ,வலித்தலுமான குறிகுணங்களைக் காட்டி நீரிழியும் போது செந்நிறமான சிறுகற்கள் வெளியாகும்.

ஐயக் கல்லடைப்பு:

கொப்பூழிலே வில்போல் நிமிர்ந்து குத்தலை உண்டாக்கி, இடுப்பு, தொடை இவைகளை குடையும் .ஆண் குறி விறுவிறுத்துக் கடுக்கும். அங்கு வியர்வையுண்டாம்.வெண்மை நிறமுடைய சிறுகற்கள் சிறுநீருடன் வெளியாகும்.

முக்குற்ற கல்லடைப்பு :

நீர்த்தாரையில் அடிப்பக்கத்தில் தாங்க முடியாத வலியும், நீர் விட்டு விட்டு வருதலும் , அதில் நாளொன்றுக்குக் கைந்நிறையளவு சிறுமணல் போன்ற கற்கள் வெளியாதலும்,அதனுடன் வெண்ணீர்(சுக்கிலம்) கசிவதும் ஆகியகுறி குணங்களைக் காட்டும்.

MODERN ASPECT

RENAL CALCULI[29]

Renal calculi are formed when the concentration of certain minerals in the urine like phosphorous, calcium, magnesium, uric acid, oxalate or xanthine become too high resulting in a build-up of crystals (which are normally flushed out of the system during urination) in the urinary tract.

Synonyms:

Renal stone, Kidney stone, Renal calculi, Calculus, Urolithiasis.

AETIOLOGY

- Hyperexcretion of relatively insoluble urinary constituents.
- Physiological changes in urine .
- Altered urinary crystalloids and colloids.
- Decreased urinary output of citrate.
- Vitamin A deficiency.
- Urinary infection.
- Urinary stasis.
- Hyperparathyroidism.
- Prolonged immobilization.
- Deficient intestinal colonization of Oxalobacter formigenes .

PREVALENCE AND INCIDENCE:

It is estimated that at least 10% of the population in the industrialized part of the world is afflicted by urinary tract stone disease.

Kidney stones are common in industrialized nations with an annual incidence of 0.5% to 1.9%.

SYMPTOMS:

- ❖ Pain is the leading symptom in 75% of cases.
- ❖ Fixed renal pain is located in the kidney region.
- ❖ Urteric colic is an agonizing pain passing from loin to the groin; coming on suddenly causing the patient to draw up his knees and roll about. It is often accompanied by vomiting and profuse sweating.
- ❖ Strangury is the passage of few drops of urine often blood stained after painful straining.
- ❖ Ureteric colic is often due to the stone entering the ureters. Also, it occurs when the stone is in the renal pelvis and temporarily blocks the passage of urine.
- ❖ Blood in urine (Heamaturia) is sometimes a leading and only symptom. It can occur during or after an attack of pain. Pyuria, which is infection of the kidney with pus in the urine, can also occur.

PHYSICAL SIGNS

(a) Tenderness at the *'renal angle'* posteriorly.

(b) Muscle rigidity over the kidney

(c) Swelling in the flank when there is hydronephrosis or pyonephrosis associated with renal calculus.

(d) Abdominal distension and diminished peristalsis may accompany ureteric colic.

TYPES OF RENAL CALCULI

Basically the renal stones can be divided into two major groups

I. Primary stones

Ii. Secondary stones.

(i) PRIMARY STONES

They appear in apparently healthy urinary tract without any antecedent inflammation.

(a) Calcium oxalate

(b) Uric acid calculi

(c) Cystine calculi

(d) Xanthine calculi

SECONDARY STONES:

They are usually formed as the result of inflammation.

(a) Triple phosphate

INVESTIGATIONS

(a) Blood examination

(b) Urinalysis

(c) Radiography -X-ray KUB

(d) Ultrasonography

(e) Computed tomography

(g) Cystoscopy

(h) Stone analysis

DIETARY MEASURES:

Fluid intake should be high at all times(5-6) litres.

Plantain pith juice to be taken.

Tender coconut water.

Foods not to be taken:

Milk.

Eggs.

Tomatoes.

Cauliflower.

Cabbage. Chicken.,meat, fish

CLINICAL STUDY

Clinical trial on Sarva Noi Linga Chenduram in the management of Kalladaippu (Renal calculi) for Lithontriptic activity got approved by institutional ethical committee, NIS on 24/12/2011. Approval no is NIS/IEC/2011/3/13a-24/12/2011.

Based on the protocol approved by IEC, NIS the study was conducted on Kalladaippu (Renal calculi) patients.

The study was conducted in National Institute of Siddha , Ayothidass Pandithar hospital, . Chennai -47.

Study type : pilot study

Sample size : 20 patients

SUBJECT SELECTION

Patients reporting at OPD of Ayothidoss Pandithar hospital with inclusion criteria were subjected to screening test & documented using screening proforma.

INCLUSION CRITERIA:

Age : 16-80 years

Sex : male and female

Weight : 45-85 kg

Symptoms:

- Colicky pain from loin to groin
- Burning micturition
- Frequency of micturition
- Dysuria
- Vomiting
- Nausea
- Fever
- Heamaturia

Any of 4 clinical symptoms

Patients who are willing to undergo X-ray KUB/U.S.G abdomen, haematological and urine investigations before and after treatment

Patients who are willing to attend OPD once in 7 days.

EXCLUSION CRITERIA:

- Renal failure
- Liver disorder
- Pregnancy and lactation
- Any other serious illness

WITHDRAWL CRITERIA:

- Development of any adverse reaction
- Occurrence of any other serious illness
- Non-co-operation of the patient

Trial drug : Sarva Noi Linga Chenduram.

Dose : 130 mg twice daily after food.

Vehicle : Mullangi (Raphanus sativus) juice.

Duration : 30 days

CONDUCT OF THE STUDY:

A number of 20 patients who satisfied the inclusion and exclusion criteria were admitted to the clinical trial. Patients informed consent was obtained. Routine haematological ,urine investigations along with U.S.G/KUB abdomen were assessed before and after treatment. Trial drug was issued to out patients once in 7 days for 30 days .Each time they were assessed clinically. For in- patients the trial drug were issued daily and they were monitored every day. Dietary advice was given. Haematological ,urine investigations and U.S.G abdomen were taken before and after treatment. Patients was informed to report about adverse effects if any.

Among 20 patients 11 patients are male 9 patients are female**.**

(trial drug 2 table 20,chart 1).

Among 20 patients ,

4 patients are in the age group of 20-30years.

11 patients are in the age group of 30-45years.

5 patients are in the age group of 45-60 years.**(** trial drug 2 table 20,bar diagram 5).

CLINICAL SYMPTOMS:

Among 20 patients included in clinical trial , 16 patients had loin pain radiating to groin,11 patients showed frequency of micturition and dysuria , 19 patients had burning micturition , 16 and 14 patients had nausea and vomiting respectively,6 patients had fever. 3 patients had oliguria. (trial drug 2 table 22)

PROGNOSIS :

During the treatment with Sarva Noi Linga Chenduram for the patients included in clinical trial 80% showed decrease in loin pain radiating to groin ,80% showed decrease in frequency of micturition and dysuria ,1% didn't show response for oliguria,70% showed decrease in burning micturition ,10% didn't show response for haematuria, 75% and 70% showed decrease in nausea and vomiting respectively**.(** trial drug 2 bar diagram 6 ,table 21).

There was a significant reduction in the size of renal calculi as per the U.S.G abdomen report after the treatment. (trial drug 2 ,table 22)

Symptoms were reduced which is statistically significant. (trial drug 2 table 23).

Reduction in the size of the calculi which is statistically significant.(trial drug 2 table 24)

Stones were expelled in 15 % of cases. Stone analysis was carried out .It reveals the presence of calcium, phosphrous and oxalate .(annexure 2).

No adverse reactions were estsblished during the study period.

DISCUSSION

The principle aim of this study was to assess the pre-clinical safety and efficacy and to evaluate the therapeutic efficacy of the drug Sarva Noi Linga Chenduram in the management of Kalladaippu (Renal calculi).

The literary evidence from the siddha text and modern science reviews supports the Lithontriptic activity of the drug.

Biochemical analysis:

The biochemical analysis of the drug reveals the presence of sulphate**,** borate.

AAS studies presented quantity of mercury and borate in "Sarva Noi Linga Chenduram" which are within normal limits.

Toxicological studies:

In acute toxicity study, the animals treated with "Sarva Noi Linga Chenduram" 1000mg/kg were showed tolerance with negligible toxic signs. Hence the one tenth of the dose was selected as median therapeutic dose for the further study.

Toxic effect was observed at 200mg/kg of Sarva Noi Linga Chenduram treated via oral route over a period of 28 days. So, it can be concluded that the Sarva Noi Linga Chenduram can be prescribed for therapeutic use in human with the dosage recommendations of upto maximum of 100mg/kg body weight p.o. for long term administrations the 20-30% of dose reduction is very essential to avoid organ damage.

Pharmacological studies:

Following ethylene glycol administration the excretion of calcium, oxalate, phosphate and protein were found to be increased in lithiatic group while in standard, test groups these levels were significantly decreased ($P<0.01$).

All the observations enabled us to confirm the inhibitory potential of Sarva Noi Linga Chenduram on ethylene glycol induced lithiasis.

The presented data indicate that administration of Sarva Noi Linga Chenduram to rats with ethylene glycol induced lithiasis reduced the formation of urinary stones, regarding lithontriptic activity of the drug " Sarva Noi Linga Chenduram"

The mechanism underlying this effect is still unknown, but is apparently related to diuresis and lowering of urinary concentrations of stone forming constituents. These effects could conclude the Lithontriptic property of Sarva Noi Linga Chenduram.

Clinical observation:

Clinical symptoms:

Among 20 patients included in clinical trial , 16 patients had loin pain radiating to groin,11 patients showed frequency of micturition and dysuria , 19 patients had burning micturition , 16 and 14 patients showed nausea and vomiting respectively,6 patients had fever. 3 patients showed oliguria.

Prognosis in symptoms:

Among the patients integrated in clinical trial 80% showed decrease in pain ,80% showed decrease in frequency of micturition and dysuria ,1% didn't show response for oliguria,70% showed decrease in burning micturition,10% didn't show response for haematuria, 75% and 70% showed decrease in nausea and vomiting respectively.

Although the drug produced some toxicity for higher dosage in animal model there were no toxic symptoms, adverse effects ,altered haematological investigations for the recommended dosage in clinical trial.

Bio-statistics:

In prognosis of symptoms Statistical analysis-paired 't' test "P" value showed 0.240 which is moderately significant and for size of renal calculi it showed 0.001 which is highly significant.

Siddha Aspect :

"நிலத்தி லெழுந்த பிணிநீங்காக் கிரந்தி
சலத்துடனே சூலைவெடிதானகற்றும்-பலத்ததாம்
சாதிலிங்கத் தின்குணத்தைச் சாற்றினேன் சன்னிமுதல்
ஓதுசுரம் போமே ஒளிந்து."

நிலத்தெழுந்த பிணி-பிருதிவி பூத உறுப்புகளில் உண்டாம் நோய்கள்;சலப்பிணி-அப்பு பூத உறுப்புகளில் உண்டாகும் நோய்கள்.

It is mentioned in the siddha text "அப்பு பூத உறுப்புகளில் உண்டாகும் நோய்களை நீக்கும்" for Lingam that means diseases related with fluids and also Venkaram has lithontriptic,diuretic activity.

According to AAS studies the the quantity of mercury in Sarva noi linga chenduram is 0.0146 ppm which is within normal range .Limit for mercury in drugs is 1 ppm.[31]. So the drug might not have been produced toxic effects during the treatment period.

As per the previous studies, mercurial compounds possess diuretic activity which supports lithontriptic activity of the drug.

An important reason for acute and chronic renal failure, includes both nephrolithiasis and urolithiasis. Medical management of lithiasis, today, includes lithotripsy and surgical procedures. Unfortunately, the underlying risk factors are not corrected by these techniques; hence there is a need to continue the medical supervision and therapy to prevent stone recurrence. The recurrence of urolithiasis represents a serious problem as patients who have formed one stone are more likely to form another. Not all standard pharmaceutical drugs used to cure urolithiasis are effective in all patients, and many have adverse effects that compromise their long-term use.

Hence Sarva Noi linga Chenduram can be a better choice of drug in the management of Kalladaippu(Renal calculi).

SUMMARY

- The literary evidence strongly supports the Lithontriptic activity of "Sarva noi linga chenduram".
- The drug "Sarva Noi Linga Chenduram" had been selected for this study to evaluate its Lithontriptic activity in the management of Kalladaippu (Renal calculi).
- Biochemical analysis of "Sarva Noi Linga Chenduram" drug reveals the presence of sulphate**,** borate.
- AAS studies presented quantity of mercury and borate in "Sarva Noi Linga Chenduram" which were within normal limits.
- In acute toxicity study, the animals treated with 1000mg/kg were showed tolerance with negligible toxic signs.
- In sub acute toxicity study toxic effect was observed at 200mg/kg of "Sarva Noi Linga Chenduram" treated via oral route over a period of 28 days. So, it can be concluded that the Sarva Noi Linga Chenduram can be prescribed for therapeutic use in human with the dosage recommendations of upto maximum of 100mg/kg body weight p.o. for long term administrations the 20-30% of dose reduction is very essential to avoid organ damage.
- Administration of Sarva Noi Linga Chenduram to rats with ethylene glycol induced urolithiasis reduced the formation of urinary stones.
- A number of 20 patients were recruited in clinical trial satisfying the inclusion criteria.

  The drug Sarva Noi Linga Chenduram was issued to the patients once in 7 days for 30 days.
- On account of clinical trial after treament with "Sarva Noi Linga Chenduram" 80% of patients showed decrease in pain ,80% showed decrease in frequency of micturition and dysuria, 1% didn't show response for oliguria,70% showed decrease in burning micturition ,10% didn't show response for haematuria, 75% and 70% showed decrease in nausea and vomiting respectively.

- There was a significant reduction in the size of renal calculi as per the U.S.G abdomen report after the treatment .Stones were expelled in 15 % of cases. Stone analysis was carried out .It reveals the presence of calcium, phosphrous and oxalate.

- In prognosis of symptoms Statistical analysis-paired 't' test "P" value showed 0.240 which is moderately significant and for size of renal calculi it showed 0.001 which is highly significant

- So the drug "Sarva Noi Linga Chenduram" is considered to be statistically significant in the management of kalladaippu.

- The drug "Sarva Noi Linga Chenduram" has

  lithontriptic Activity.

  Encouraging clinical results.

- From the clinical trial and statistical analysis it substantiates that the drug "Sarva Noi Linga Chenduram" is statistically significant on lithontriptic activity in the management of Kalladaippu (Renal calculi) in upcoming days.

CONCLUSION

- The literature and research journal review of the drug Sarva Noi linga Chenduram supports that it has lithontriptic activity.
- The safety studies (acute toxicity and repeated oral toxicity) studies conducted revealed that the trial drug Sarva Noi linga Chenduram is safe. Hence it can be reasonably assumed that the drug is safe for human use.
- The pharmacological study conducted in animal model showed significant **Lithontriptic activity.**
- Clinical study revealed the therapeutic efficacy of the trial drug by showing, reduction in symptoms and size of renal calculi significantly. There were no adverse reactions complained during the clinical trial.
- Hence, the drug **Sarva Noi linga Chenduram** can be used in the management of **Kalladaippu(Renal calculi)**

TABLE -1 DRUG -1 AMUKKARA KIZHANGU CHOORANAM QUALITATIVE ANALYSIS

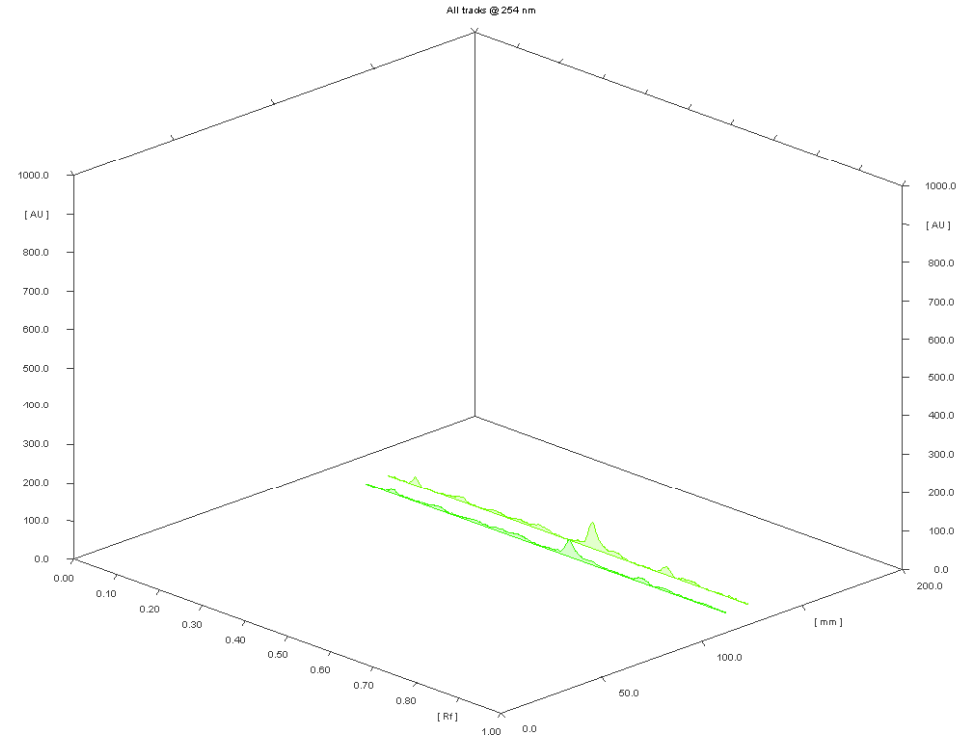
S.NO	PARAMETERS	RESULTS
1	Phosphate	Absent
2	Sulphate	absent
3	Magnesium	absent
4	Iron	absent
5	Amino acids	present
6	Starch	absent
7	Flavanoids	absent
8	Proteins	absent
9	Tannins	absent
10	Glycosides	present

TABLE -1 PHYSICAL PROPERTIES

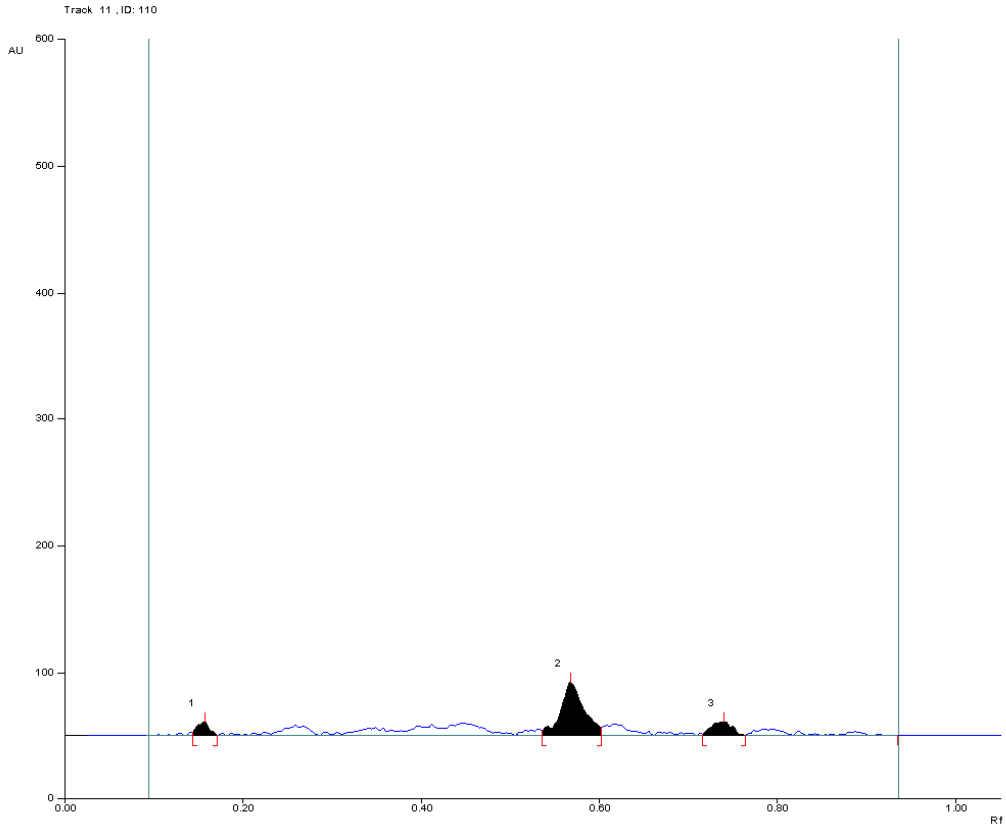
S.NO	CHARECTRISTIC TESTS	RESULTS
1	Ph	5.58
2	TOTAL ASH	0.33
3	WATER SOLUBLE ASH	0.01
4	ACID INSOLUBLE ASH	0.05

TABLE – 3 PREMILINARY ACID,BASIC RADICALS PHYTOCHEMICAL SCREENING

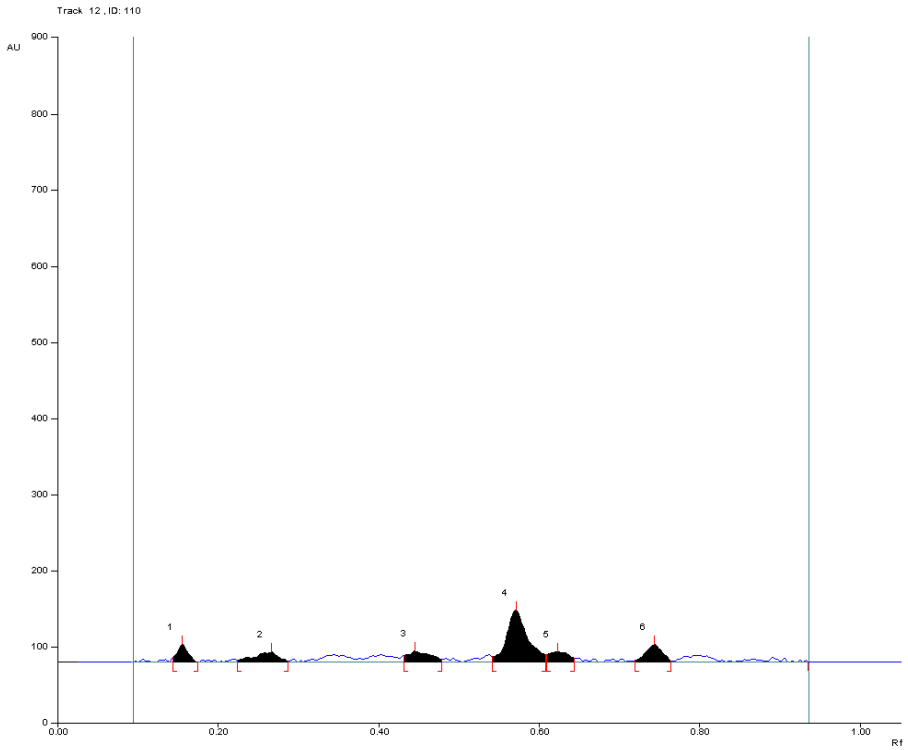
S.NO	CONSTITUENTS	AKC
1	Magnesium	Absent
2	Iron(ferric)	Absent
3	Iron (ferrous)	Present
4	Sulphate	Absent
5	Sodium	Absent
6	Starch	Absent
7	Sulphate	Absent
8	Phosphate	Absent



254nm 3D display graph 1

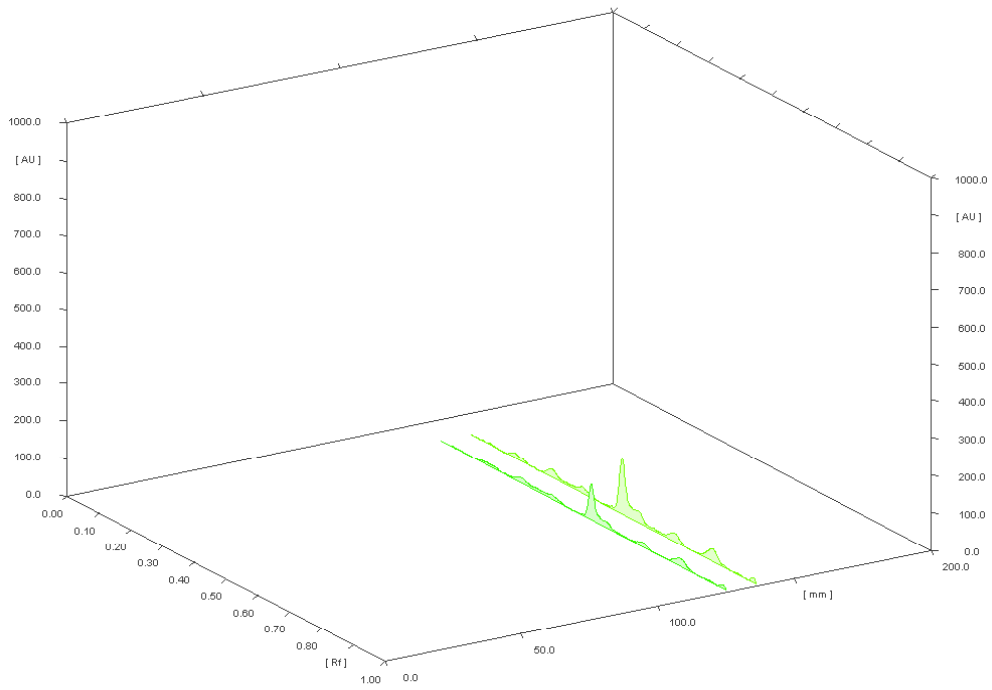


5µl (254nm) graph 2

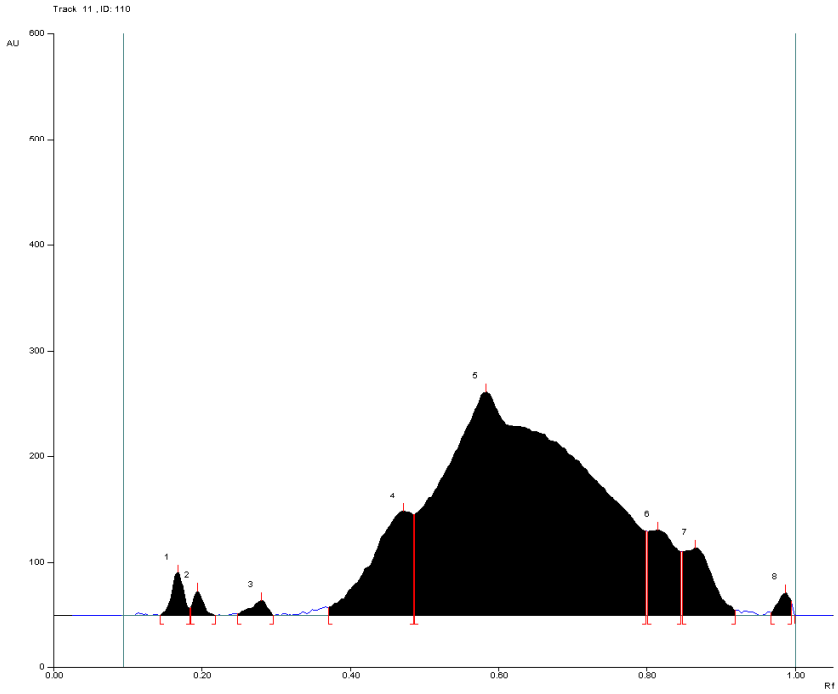


10 µl (254nm) graph 3

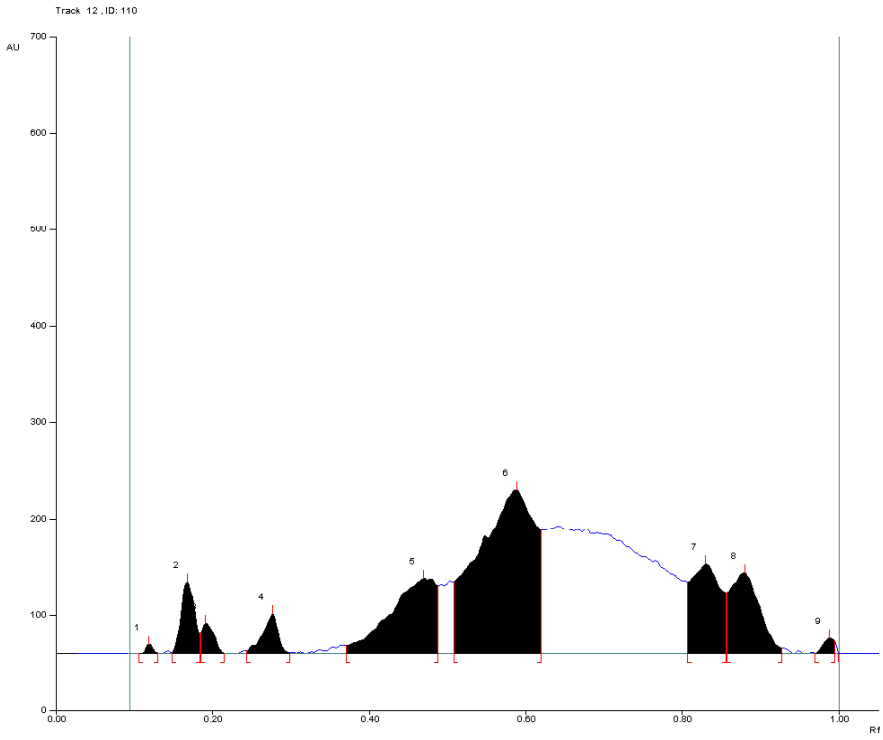
<u>298 nm 3D display</u>



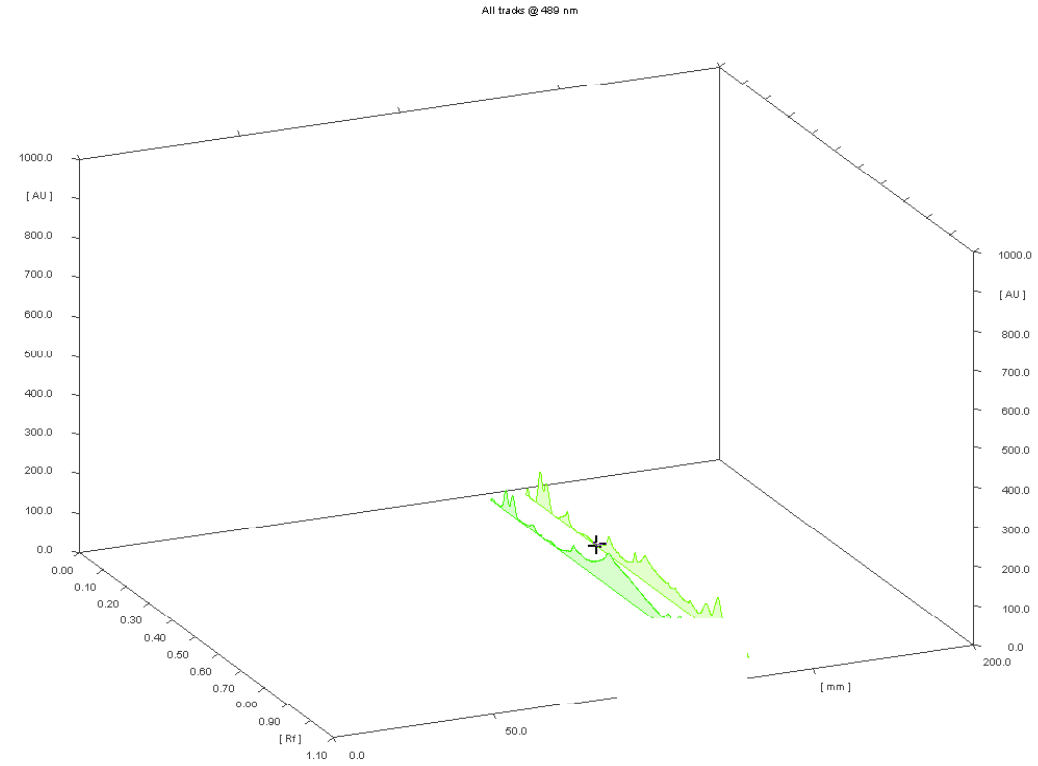
<u>Derivatisation (298nm) graph 4</u>



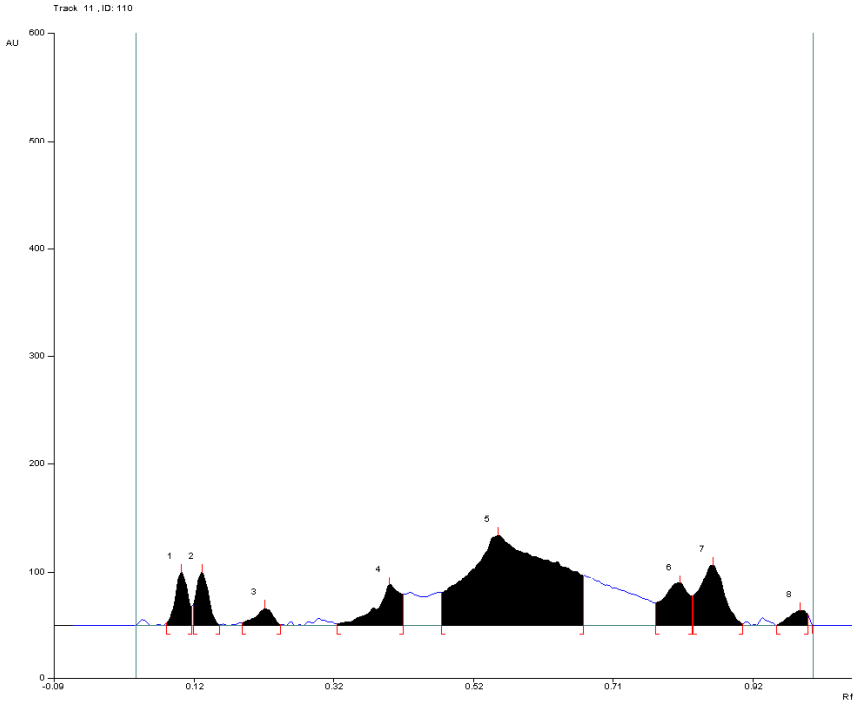
Derivatisation 5µl (298nm)graph 5



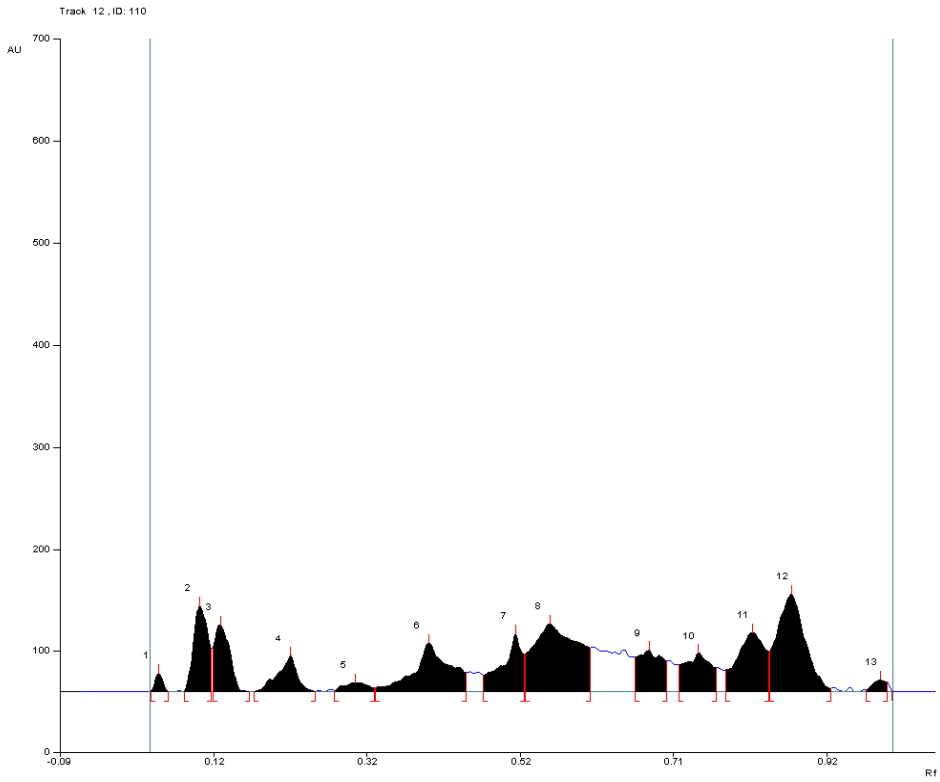
Derivatisation 10µl (298nm) graph 6



489 nm 3D display graph 7



Derivatisation 5µl (498nm) graph 8



Derivatisation 10µl (498nm) graph 9

TOXICITY STUDIES

TABLE- 4

Table 4: Dose finding experiment and its behavioral Signs of Toxicity

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	1000	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	2000	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	5000	+	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-

1. Alertness 2. Aggressiveness 3. Pile 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

Table 5. Body wt (g) of rats exposed to *Amukkara Kizhangu Chooranam* for 28days.

Dose (mg/kg/day)	Days				
	1	7	14	21	28
Control	114.21±5.12	118.97±6.15	121.25±5.46	126.48±6.18	128.04±5.49
100	118.35±4.22	123.10±5.11	123.14±5.82	125.52±6.02	126.44±6.00
200	117.18±5.10	119.05±6.45	122.00±5.43	124.12±6.10	126.30±7.02
400	115.10±5.24	118.02±5.88	120.10±6.33	121.78±6.41	125.14±6.37

Values are mean ± S.E.M. (Dunnet 't' test). $^{ns}P>0.05$. V*s.* Control group N=6.

Table 6. Food (g/day) intake of rats exposed to *Amukkara Kizhangu Chooranam* for 28days.

Dose (mg/kg/day)	Days (gms/rats)				
	1	7	14	21	28
Control	42.00±2.54	42.16±2.10	45.19±2.10	48.15±2.50	49.12±3.10
100	42.22±2.65	44.10±2.12	46.44±2.26	45.15±2.62	46.18±3.08
200	41.31±2.30	42.11±2.18	45.60±2.28	45.68±2.18	46.04±3.04
400	42.10±2.62	42.28±2.35	46.11±2.52	45.31±2.06	45.28±2.88

Values are mean ± S.E.M. (Dunnet 't' test). $^{ns}P>0.05$. V*s.* Control group N=6.

Table 7. Water (ml/day) intake of rats exposed to *Amukkara Kizhangu Chooranam* for 28days.

Dose (mg/kg/day)	Days(ml/rat)				
	1	7	14	21	28
Control	50.12±2.92	52.11±3.35	54.22±3.10	52.35±3.13	51.22±3.90
100	52.10±2.40	50.12±3.08	55.26±4.46	46.16±3.08	48.45±2.98
200	49.04±2.28	40.18±3.85	48.80±3.37	44.17±2.88	49.45±3.28
400	51.14±3.46	54.97±3.00	50.22±3.81	49.52±3.17	50.82±3.45

Values are mean ± S.E.M. (Dunnet 't' test). $^{ns}P>0.05$. V*s.* Control group N=6.

Table 8. Hematological parameters after 28days treatment with *Amukkara Kizhangu Chooranam* in rats.

Parameter	Control	100 mg/kg	200 mg/kg	400 mg/kg
Red blood cell (mm^3)	5.06±0.51	4.98±0.48	5.15±0.49	5.08±0.50
HB (%)	14.10±0.34	15.15±0.30	14.50±0.47	15.02±0.44
Leukocyte ($x10^3$/Cu.mm)	8.22±1.9	8.25±0.89	8.07±1.15	8.43±1.34
Platelets(K/µl)	448±23.10	496±32.24	487±32.20	495±30.46
MCV (gl)	55.48±4.80	52.12±4.28	55.00±4.20	54.10±4.16
N	15.45±1.38	15.20±1.17	41.80±0.86**	15.15±3.19
L	85.10±2.40	80.80±3.41	83.28±3.52	85.12±3.48
M	1.40±0.33	1.40±0.38	1.40±0.28	1.40±0.26
E	1.00±0.00	1.00±0.22	1.00±0.11	1.00±0.11
B	0±0.00	0±0.00	0±0.00	0±0.00
ESR(mm)	1±00	1±00	1±00	1±00
PCV	45.30±2.68	45.20±2.48	45.17±3.02	45.40±3.00

Values are mean ± S.E.M. (Dunnet 't' test). **P<0.01. V*s.* Control group N=6.

Table 9. Effect of treatment with *Amukkara Kizhangu Chooranam* biochemical parameters.

LFT

Dose (mg/kg)	Control	100 mg/kg	200 mg/kg	400 mg/kg
Total Bilirubin (mg/dL)	0.29±0.05	0.26±0.06	0.28±0.05	0.25±0.04
Bilirubin direct (mg/dL)	0.21±0.07	0.22±0.09	0.19±0.05	0.21±0.06
ALP (U/L)	102.14±10.17	104.14±11.02	116.30±10.10	114.2±10.32
SGOT (U/L)	116.20±6.10	115.17±6.50	110.85±5.98	116.04±6.78
SGPT(U/L)	36.04±2.04	34.81±3.00	35.08±2.15	36.60±2.17
Total Protein(g/dl)	6.02±1.30	6.10±0.10	7.50±0.25	8.12±0.40
Albumin(g/dl)	2.21±0.25	2.19±0.24	3.46±0.23**	3.12±0.11*
Globulin(g/dl)	4.02±0.17	5.12±0.20**	4.28±0.20	4.81±0.28*

Values are mean of 6 animals ± S.D

E.M. *P<0.05; **P<0.01. V*s.* Control group N=6.

Table-10 RFT

Dose (mg/kg)	Control	100 mg/kg	200 mg/kg	400 mg/kg
Urea(mg/dL)	5.42±1.68	4.30±2.16	5.4±2.04	5.81±1.32
Creatinine (mg/dL)	0.71±0.05	0.70±0.05	0.72±0.06	0.71±0.05
Uric acid (mg/dL)	3.61±0.14	4.10±0.18	4.16±0.16	4.06±0.14
Na m.mol	112.78±5.26	114.2±5.00	118.12±5.22	114.10±5.00
K m.mol	5.20±2.80	5.45±1.16	5.0±1.06	6.15±2.00
Cl m.mol	100.01±4.14	101.08±5.11	99.46±4.24	101.41±5.21

Values are mean ± S.E.M. ^{ns}P>0.05. V*s.* Control group N=6.

Table-11. Lipid Profile

Dose (mg/kg)	Control	100 mg/kg	200 mg/kg	400 mg/kg
Total cholestrol(mg/dL)	78.68±2.57	71.10±2.42	70.52±3.28	72.50±3.04
HDL(mg/dL)	123.02±2.74	113.25±2.78	124.00±3.45	123.20±2.44
LDL(mg/dL)	42.00±2.35	41.52±3.01	40.31±3.10	42.24±3.22
VLDL(mg/dl)	26.38±2.22	25.80±2.41	26.04±2.64	25.01±2.28
Triglycerides (mg/dl)	28.24±3.02	25.16±2.42	26.23±3.54	28.10±2.71
Blood glucose(mg/dl)	85.15±4.82	91.10±4.05	93.13±5.00	94.11±2.45

Values are mean ± S.E.M. $^{ns}P>0.05$. V*s.* Control group N=6.

Table-12 Urine Analysis

Parameters	Control	100 mg/kg	200 mg/kg	400 mg/kg
Colour	Yellow	Yellow	Yellow	Yellow
Transparency	Clear	Slightly turbid	Slightly cloudy	Slightly turbid
Specific gravity	1.010	1.010	1.010	1.010
PH	>7.2	>8.0	>7.5	>7.5
Protein	Nil	1+	1+	2+
Glucose	Nil	Nil	Nil	Trace
Bilirubin	-ve	-ve	-ve	-ve
Ketones	-ve	-ve	-ve	-ve
Blood	Absent	Absent	Absent	Absent
Urobilinogen	Normal	Normal	Normal	Normal
Pus cells	0-cells/HPF	1-cell/HPF	2-cells/HPF	1-cell/HPF
RBCs	Nil	Nil	0-1cells/HPF	Nil
Epithelial cells	Nil	1-cell/HPF	Nil	1-cell/HPF
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

Table 13. Effect of oral administration of *Amukkara Kizhangu Chooranam* on organ weight

Dose (mg/kg)	Control	100 mg/kg	200 mg/kg	400 mg/kg
Liver (g)	3.10±0.10	3.14±0.15	3.15±0.12	3.05±0.17
Heart (g)	0.32±0.04	0.32±0.05	0.35±0.04	0.35±0.04
Lung (g)	0.49±0.16	0.44±0.14	0.38±0.12	0.42±0.11
Spleen (g)	0.45±0.05	0.48±0.04	0.46±0.04	0.45±0.05
Ovary (g)	1.20±0.18	1.42±0.15	1.28±0.15	1.26±0.14
Testes (g)	2.18±0.14	2.45±0.22	2.40±0.25	2.41±0.21
Brain (g)	2.06±0.15	2.08±0.13	0.06±0.14**	2.03±0.14
Kidney (g)	0.83±0.04	0.81±0.04	0.80±0.04	0.82±0.05
Stomach (g)	1.16±0.12	1.24±0.10	1.18±0.11	1.15±0.12

Values are mean of 6 animals ± S.E.M. (Dunnet 't' test). **P<0.01 V*s.* Control group N=6.

PHARMACOLOGICAL STUDIES

Table 14*:* Effect of Amukkara Kizhangu Chooranam on body weight of Cholesterol rich diet induced hyperlipidemic rats

Groups	Body Weight (gm.)				
	Initial	**1st Week**	**2nd Week**	**3rd Week**	**4th Week**
Normal control	154.28±2.73	156.18±2.35	158.12±2.44	161.22±2.92	163.46±3.00
Hyperlipidemic Control	156.24±2.54	156.99±2.71	178.22±2.60**,a	196.84±2.48**,a	236.13±2.75**,a
AKC 100mg/kg	153.88±2.00	157.04±2.36	166.79±2.48b	182.40±3.22**,a	180.11±2.44**,a
AKC 200mg/kg	156.62±2.26	159.00±2.44	166.15±2.56b	180.12±2.82**,a	186.18±2.52**,a
Lovastatin	155.18±2.11	158.27±2.68	165.92±2.88a	179.37±2.46**,a	180.40±2.11**,a

Values are as mean ± SEM (n=6)
Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001
Comparison made between Group II Vs Group I
aP<0.001, bP<0.01, cP<0.05 compared between Group III, IV, V Vs Group II.

Table 15: Effect of Amukkara Kizhangu Chooranam on Lipid profiles of Cholesterol rich diet induced hyperlipidemic rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dg)
Normal control	122.15±4.96	110.34±4.88	55.46±2.46	48.18±4.10	23.85±2.83
Hyperlipidemic Control	179.46±5.13^{**,a}	285.17±4.10^{**,a}	42.18±2.30^{**,a}	87.34±4.15^{**,a}	62.10±3.06^{**,a}
AKC 100mg/kg	142.00±4.37^{*,a}	159.33±4.48^{**,a}	68.10±3.24^{*,a}	41.64±3.00^{a}	25.14±3.44^{a}
AKC 200mg/kg	138.81±3.48^{*,a}	147.10±3.52^{**,a}	44.65±2.88*	70.15±3.64^{**,b}	37.33±3.10^{*,a}
Lovastatin	126.77±2.87^{a}	138.98±3.12^{**,a}	58.79±3.00^{a}	44.52±3.74^{a}	31.64±2.46^{a}

Values are as mean ± SEM (n=6)

Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001

Comparison made between Group II Vs Group I

^{a}P<0.001, ^{b}P<0.01, ^{c}P<0.05 compared between Group III, IV, V Vs Group II.

Table 16: Effect of Amukkara Kizhangu Chooranam on atherogenic index and percentage protection of different groups.

Groups	Atherogenic Index(AI)	% Protection
Normal control	2.38±0.16	---
Hyperlipidemic Control	4.65±0.22**	---
AKC 100mg/kg	3.11±0.04^{**,a}	33.11
AKC 200mg/kg	3.48±0.04^{**,a}	25.16
Lovastatin	2.86±0.06^{**,a}	38.49

Table 17: Effect of Amukkara Kizhangu Chooranam on SGOT, SGPT Total protein, Urea and Blood glucose levels of Cholesterol rich diet induced hyperlipidemic rats on day 28.

Groups	SGOT(U/I)	SGPT(U/I)	Total Protein (gm/dl)	Urea (mg/dl)	Blood Glucose (mg/dl)
Normal control	166.21±4.80	61.16±2.72	5.99±0.32	42.04±0.92	84.87±1.52
Hyperlipidemic Control	234.48±5.50^{**,a}	130.40±4.56^{**,a}	7.22±0.30^{**,a}	26.11±0.98^{**,a}	92.56±1.88^{**,a}
AKC 100mg/kg	159.87±5.75^{a}	64.11±2.31^{a}	6.10±0.30^{a}	34.08±2.10^{**,a}	85.13±1.32^{a}
AKC 200mg/kg	198.40±5.44^{**,a}	101.02±2.64^{**,a}	6.98±0.28**	40.64±2.45^{a}	83.10±1.14^{a}
Lovastatin	172.15±5.62^{a}	72.46±2.87^{**,a}	6.23±0.31^{a}	36.98±2.00^{**,a}	82.36±1.18^{*,a}

Values are as mean ± SEM (n=6)

Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001

Comparison made between Group II Vs Group I

^{a}P<0.001, ^{b}P<0.01, ^{c}P<0.05 compared between Group III, IV, V Vs Group II

Table 18: Effect of Amukkara Kizhangu Chooranam on vital organ weights of Cholesterol rich diet induced hyperlipidemic rats on day 28.

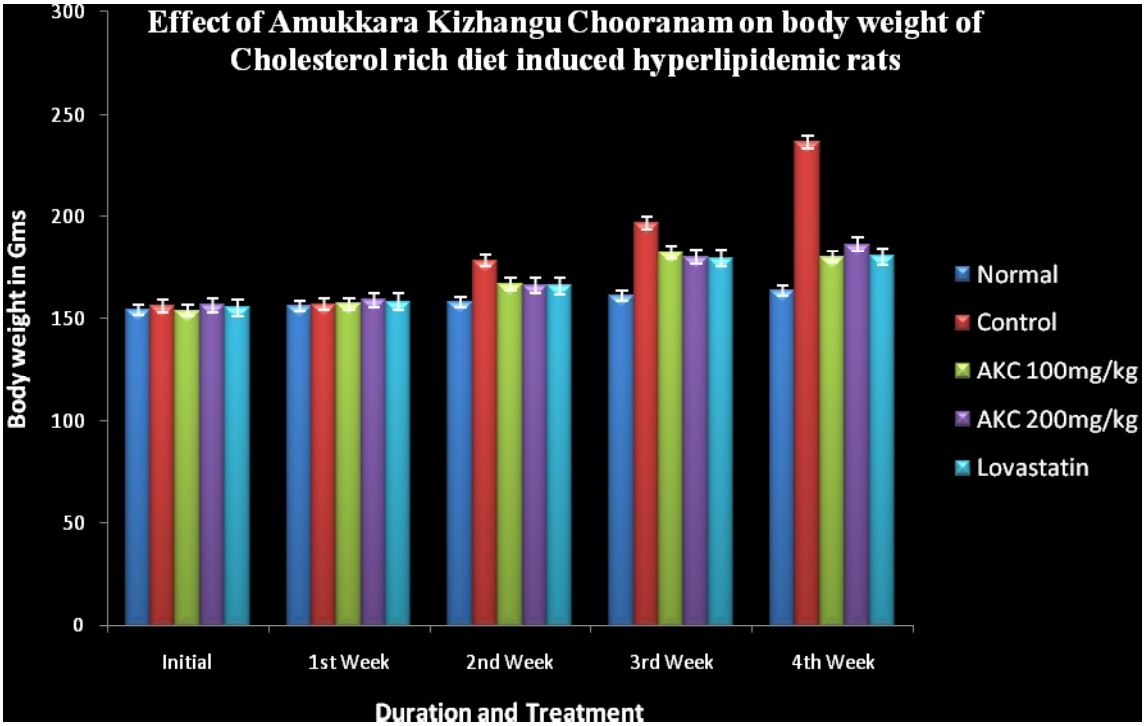
Groups	Liver(gm)	Heart(gm)	Kidney(gm)
Normal control	5.45±0.28	0.61±0.05	0.55±0.18
Hyperlipidemic Control	7.88±0.17^{**,a}	1.02±0.05^{**,a}	0.84±0.03^{**,a}
AKC 100mg/kg	5.82±0.15^{*,a}	0.58±0.10^{a}	0.58±0.02^{a}
AKC 200mg/kg	6.61±0.3^{**,a}	0.64±0.02^{a}	0.64±0.03^{a}
Lovastatin	5.92±0.03^{**,a}	0.62±0.02^{a}	0.60±0.14^{a}

Values are as mean ± SEM (n=6)

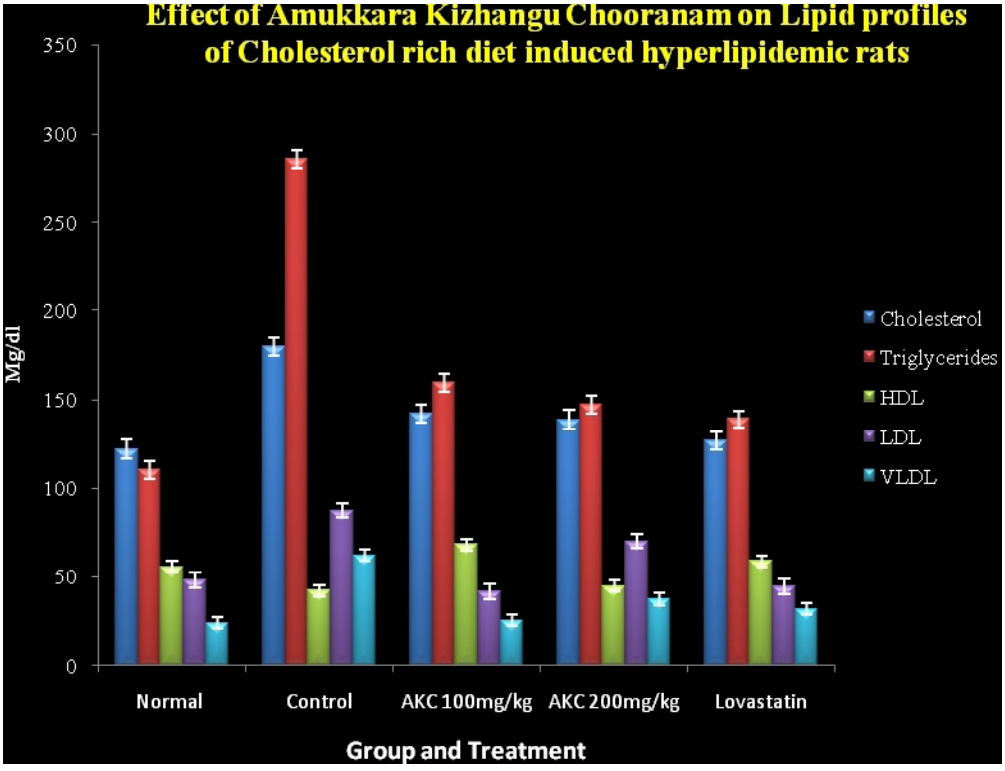
Values are statistically significant at *P<0.05, **P<0.01, ***P<0.001

Comparison made between Group II Vs Group I

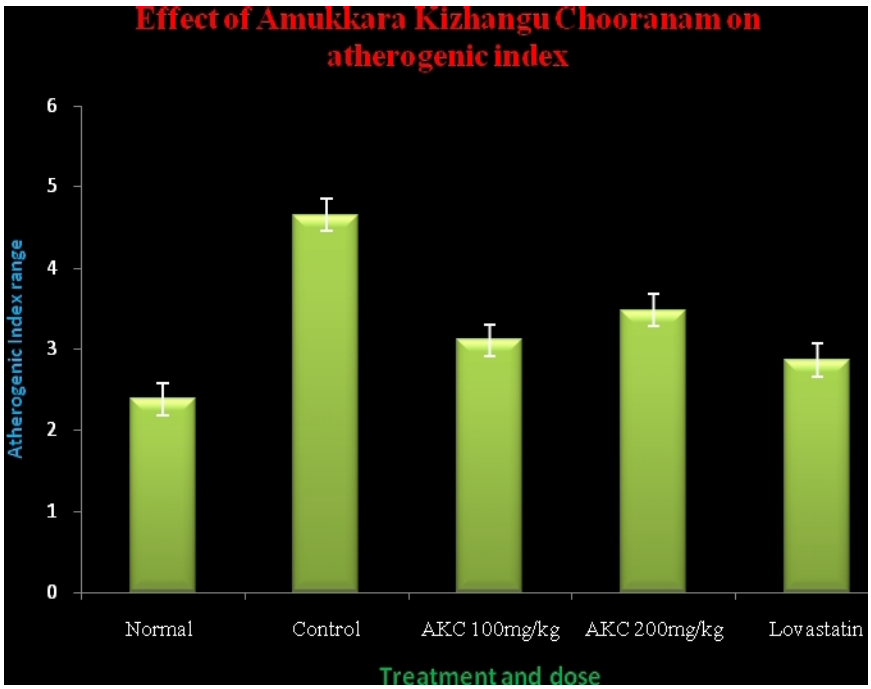
^{a}P<0.001, ^{b}P<0.01, ^{c}P<0.05 compared between Group III, IV, V Vs Group II.



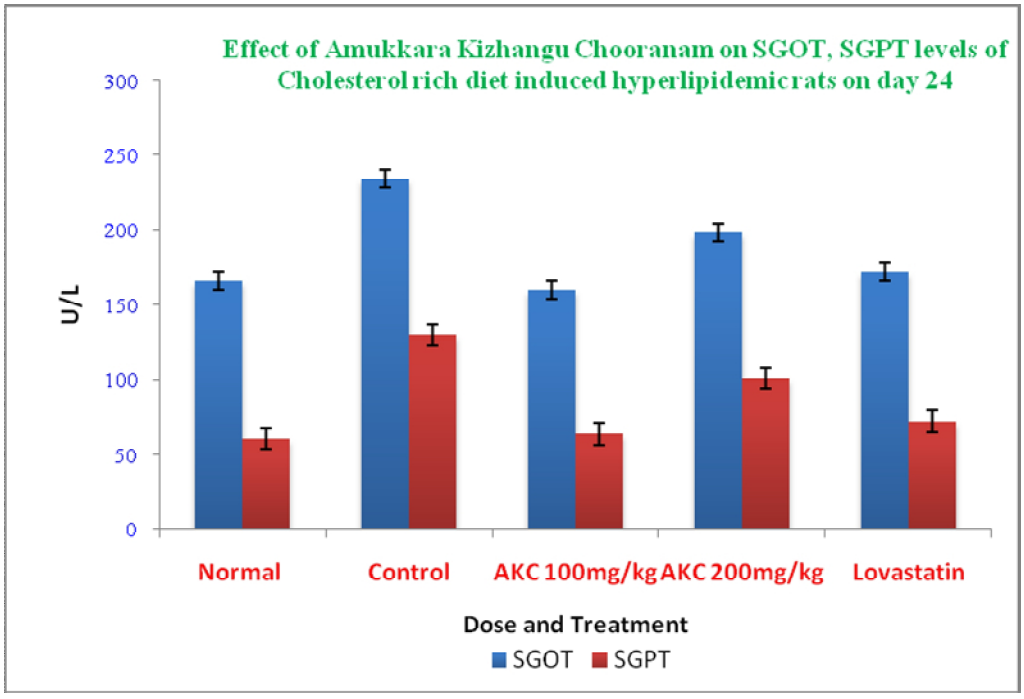
Bar diagram 1



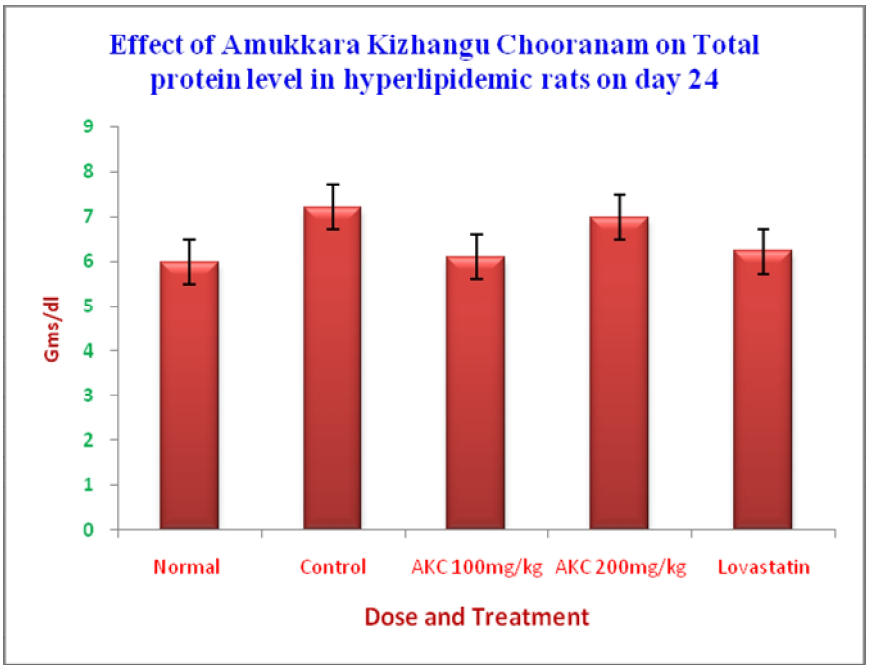
Bar diagram 2



Bar diagram 3



Bar diagram 4



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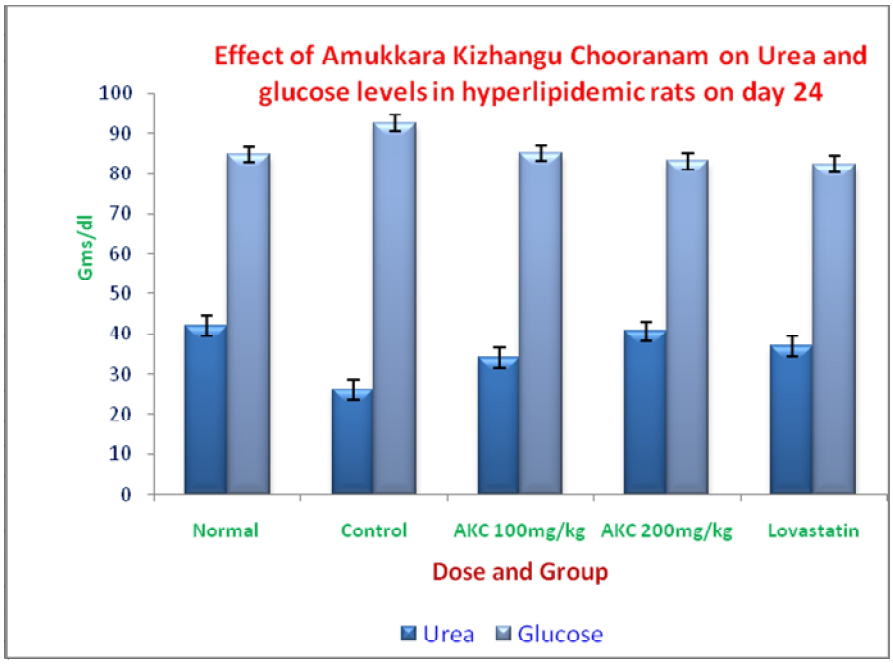
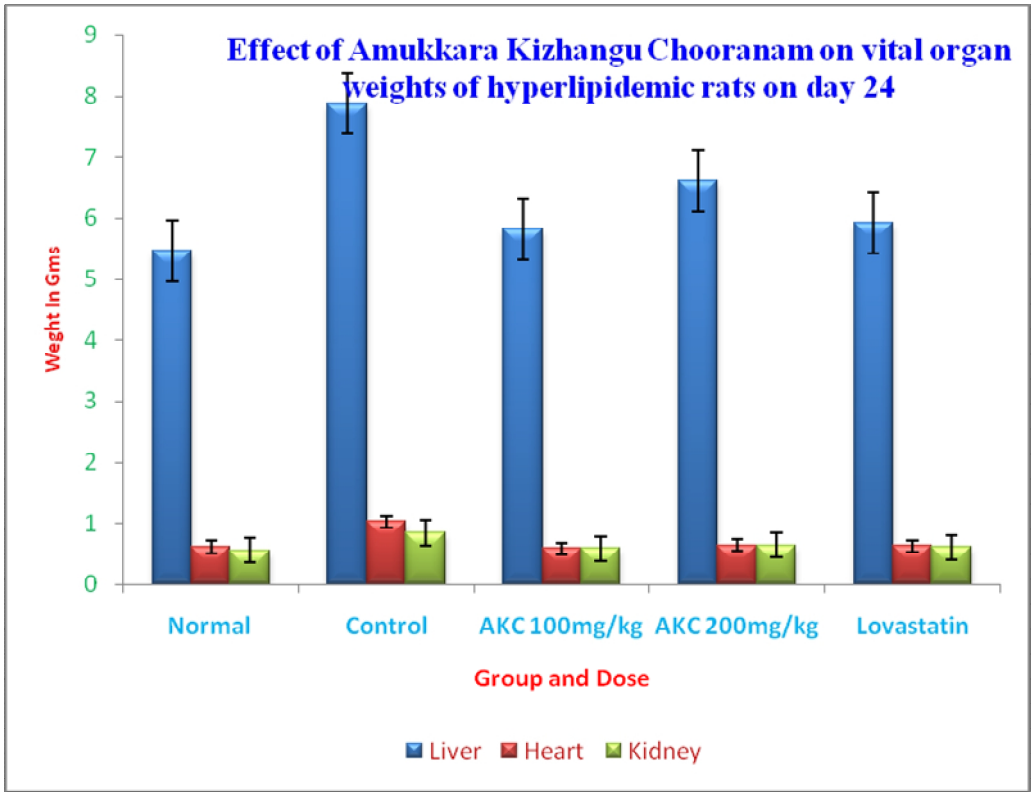


Bar diagram 6



Bar diagram 7

Table 19

CLINICAL STUDIES

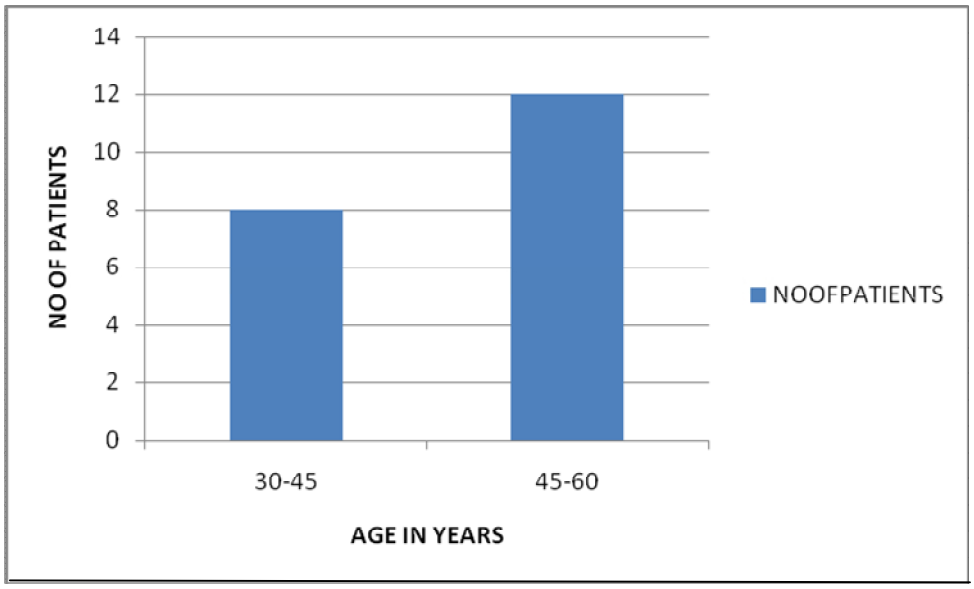
AGE WISE DISTRIB UTION FOR TRIAL DRUG 1

S.NO	AGE	NO OF PATIENTS	PERCENTAGE
1	30-45	8	40%
2	45-60	12	60%

Table 20 GENDER WISE DISTRIBUTION FOR TRIAL DRUG 1

S.NO	GENDER	NO OF PATIENTS	PERCENTAGE
1	MALE	9	45%
2	FEMALE	11	55%

AGE WISE DISTRIBUTION FOR TRIAL DRUG 1



Bar diagram 8

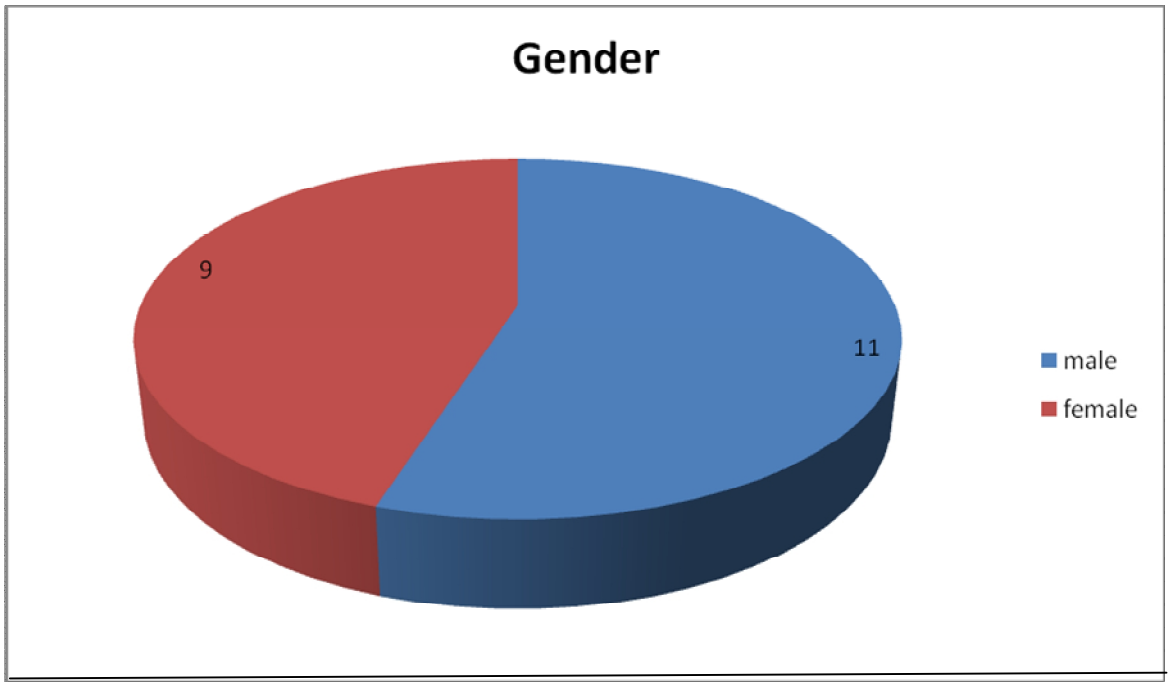


CHART 1

OPD NO	AGE	SEX	BTFBS	ATFBS	BTPPBS	ATPPBS	BT S.CHO	AT S.CHO	BTHDL	ATHDL	BTLDL	ATLDL	BTVLDL	ATVLDL	BTTGL	ATTGL
C83477	57	F	271	116	330	144	350	210	30	34	152	102	44	42	348	273
C85166	55	M	91	100	141	132	184	180	32	34	110	108	42	26	214	134
C85756	54	M	102	100	136	130	266	180	43	44	141	102	82	82	312	238
C86423	60	M	145	131	212	199	233	229	42	45	139	112	79	73	348	218
C8450	38	M	97	100	121	120	271	202	31	40	82	81	68	65	326	325
C87493	51	F	257	212	113	110	237	212	46	54	139	118	52	53	278	173
C87904	50	F	108	103	131	128	246	220	45	47	120	119	31	29	221	252
C88O95	57	F	111	106	179	122	205	176	35	42	141	172	39	36	328	260
C88994	56	F	122	108	141	120	174	161	29	32	99	56	68	45	340	330
C89385	50	M	138	112	202	182	234	254	40	52	134	129	64	63	320	398
C89311	42	F	90	108	105	103	276	194	36	44	109	92	32	31	236	257
C92412	41	M	142	128	132	131	265	200	39	35	158	89	55	45	278	195
C92933	40	F	76	82	138	136	264	229	40	41	146	178	46	54	232	176
C93005	45	F	123	140	244	238	203	195	38	35	117	101	50	35	252	179
C91627	35	M	101	88	121	101	369	300	45	39	201	125	27	49	137	147
B88419	55	M	121	122	131	136	192	192	36	38	93	94	62	56	314	284
U3205	36	F	111	105	137	128	248	226	49	46	163	167	32	24	160	121
D007816	54	F	157	95	216	123	256	204	45	39	116	100	50	32	253	159
C97096	58	F	108	96	115	117	237	210	42	38	136	100	47	47	237	237
D010184	42	M	164	139	179	175	192	201	38	40	100	102	48	26	243	132

LIPIDS AND BLOOD GLUCOSE LEVEL BEFORE AND AFTER TREATMENT FOR TRIAL DRUG 1-TABLE 21

BT- before treatment.
AT-after treatment.
FBS-fasting blood sugar.
PPBS- post prandial blood sugar.
S.CHO-serum cholesterol
HDL-high density lipoprotein.
LDL-low density lipo protein
VLDL-very low density lipo protein
TGL-Triglycerides.

Statistical Analysis: TRIAL DRUG 1 table 22

All collected data were entered into MS Excel software using different columns as variables and rows as patients. SPSS software was used to perform statistical analysis. Basic descriptive statistics include frequency distributions and cross-tabulations were performed. The quantity variables were expressed as Mean ± Standard Deviation and qualitative data as percentage. A probability value of <0.05 was considered to indicate as statistical significance. Paired 't' test was performed for determining the significance between before and after treatment.

S.NO	Obs	variable	Mean	Std. Deviation	t. value	p value
1	20	BT S.CHO	245.10	50.004	4.156	.001
		AT S.CHO	208.75	30.375		
2	20	BTHDL	39.05	5.726	-1.650	.115
		ATHDL	40.95	5.916		
3	20	BTLDL	129.80	28.050	2.722	.014
		ATLDL	112.35	30.432		
4	20	BTVLDL	50.90	15.650	2.244	.037
		ATVLDL	45.65	16.429		
5	20	BTTGL	268.85	60.796	3.624	.002
		ATTGL	224.40	74.826		

'p' value for S.T.CHOLESTEROL is 0.001 .TGL is 0.002 which is statistically significant.

SARVA NOI LINGACHENDURAM TRIAL DRUG 2

Table 1 QUALITATIVE ANALYSIS

S.NO	PARAMETERS	RESULTS
1	Sodium	Absent
2	Phosphate	Absent
3	Magnesium	Absent
4	Sulphate	Present
5	Iron	Absent
6	Chloride	Absent
7	Calcium	Absent
8	Borate	Absent

QUANTITATIVE ANALYSIS TRIAL DRUG 2 TABLE 2

S.NO	PARAMETERS	RESULTS	METHOD
1	Mercury	0.146	AAS
2	Sulphur	ND	AAS
3	Borate	2.26	AAS

PHYSICAL PROPERTIES TRIAL DRUG 2 TABLE 3

S.NO	CHARECTRISTIC TESTS	RESULTS
1	Ph	9.05
2	TOTAL ASH	0.41
3	WATER SOLUBLE ASH	0.35
4	ACID INSOLUBLE ASH	0.29

TABLE 4

PRELIMINARY ACID BASIC RADICALS SCREEENING TRIAL DRUG 2

S.NO	**CONSTITUENTS**	**SNLC**
1	**Magnesium**	**Absent**
2	**Iron(ferric)**	**Absent**
3	**Iron (ferrous)**	**Absent**
4	**Sulphate**	**Present**
5	**Sodium**	**Absent**
6	**Starch**	**Absent**
7	**Sulphate**	**Absent**
8	**Phosphate**	**Absent**

TOXICITY STUDIES

TRIAL DRUG 2

Table 5: Dose finding experiment and its behavioral Signs of Toxicity

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	500	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-
2	1000	+	+	-	+	+	+	-	+	+	+	-	-	-	+	-	-	-	+	+	-
3	2000	+	+	-	+	+	+	-	+	+	+	-	-	-	+	+	+	-	+	+	-

1. Alertness 2. Aggressiveness 3. Pile 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

Table 6. Body wt (g) of rats exposed to *Sarva Noi Linga Chendooram* for 28days.

Dose (mg/kg/day)	Days				
	1	7	14	21	28
Control	110.52±4.85	114.21±5.46	118.10±5.05	121.18±5.12	126.31±4.84
50	118.25±4.45	112.10±5.12	101.12±5.12	100.02±4.22*	108.20±5.01
100	115.33±5.14	110.22±5.00	104.02±5.13	108.17±4.22	110.10±5.11
200	116.46±5.28	109.10±4.72	110.12±5.00	110.48±5.10	110.10±4.10

Values are mean ± S.E.M. (Dunnet 't' test). *P>0.05; N=6.

Table 7. Food (g/day) intake of rats exposed to *Sarva Noi Linga Chendooram* for 28days.

Dose (mg/kg/day)	Days (gms/rats)				
	1	7	14	21	28
Control	40.00±2.25	43.16±2.12	45.10±2.46	44.64±2.58	45.15±2.15
50	45.30±2.41	44.10±2.18	44.20±2.30	45.14±2.29	46.00±4.02
100	43.35±2.10	45.42±2.63	45.15±2.34	44.25±2.18	45.14±3.00
200	44.15±2.22	45.24±2.55	45.34±2.52	45.43±2.60	45.40±2.48

Values are mean ± S.E.M. (Dunnet 't' test). $^{ns}P>0.05$. N=

Table 8. Water (ml/day) intake of rats exposed to *Sarva Noi Linga Chendooram* for 28days.

Dose (mg/kg/day)	Days(ml/rat)				
	1	7	14	21	28
Control	48.10±2.45	50.18±3.30	52.48±3.14	52.04±3.13	51.46±3.81
50	50.12±2.42	50.42±3.12	51.12±4.10	50.00±3.52	49.42±2.42
100	49.00±2.20	44.48±3.01	46.67±3.28	48.02±2.48	49.40±3.20
200	50.00±2.18	50.23±2.04	50.15±3.10	51.32±2.74	50.17±3.00

Values are mean ± S.E.M. (Dunnet 't' test). $^{ns}P>0.05$. N=6.

Table 9. Hematological parameters after 28days treatment with *Sarva Noi Linga* Chendooram.

Parameter	Control	50 mg/kg	100 mg/kg	200 mg/kg
Red blood cell (mm^3)	5.21±0.44	5.26±0.32	5.18±0.40	5.12±0.41
HB (%)	14.65±0.38	15.12±0.40	15.52±0.45	16.00±0.32
Leukocyte ($x10^3$/Cu.mm)	8.72±1.1	8.58±0.34	8.40±1.00	8.32±1.12
Platelets(K/µl)	448±25.00	456±30.41	476±22.14	481±23.04
MCV (gl)	52.21±4.26	51.34±4.26	52.10±4.71	53.12±4.20
N	15.14±1.42	15.42±1.19	14.22±0.50	14.87±1.14
L	84.12±2.54	83.92±2.48	82.00±2.75	83.21±2.92
M	1.50±0.38	1.42±0.40	1.41±0.32	1.42±0.36
E	1.01±0.10	1.00±0.21	1.00±0.18	1.00±0.14
B	0±0.00	0±0.00	0±0.00	0±0.00
ESR(mm)	1±00	1±00	1±00	1±00
PCV	46.30±2.45	46.42±2.81	45.75±3.00	45.66±2.38

Values are mean ± S.E.M. (Dunnet ‘t' test). *P<0.05; **P<0.01. N=6.

Table 10. Effect of treatment with *Sarva Noi Linga Chendooram on* biochemical parameters.

Dose (mg/kg)	Control	50 mg/kg	100 mg/kg	200 mg/kg
Total Bilirubin (mg/dL)	0.28±0.04	0.28±0.04	0.27±0.05	0.28±0.05
Bilirubin direct (mg/dL)	0.20±0.05	0.24±0.04	0.22±0.05	0.23±0.05
ALP (U/L)	102.10±10.00	104.42±9.20	110.18±10.05	112.32±10.27
SGOT (U/L)	114.05±4.14	112.10±4.00	110.42±4.44	112.10±5.85
SGPT(U/L)	34.10±2.66	35.14±3.15	35.04±2.18	35.62±2.14
Total Protein(g/dl)	6.34±1.42	6.70±0.14	7.11±0.29	7.10±0.27
Albumin(g/dl)	2.41±0.28	2.71±0.25	2.82±0.38	2.90±0.22
Globulin(g/dl)	4.18±0.20	5.30±0.24**	4.77±0.24	4.70±0.24

Values are mean ± S.E.M. (Dunnet 't' test). **P<0.01 Vs control group N=6.

Table-11 RFT

Dose (mg/kg)	Control	50 mg/kg	100 mg/kg	200 mg/kg
Urea (mg/dL)	4.56±1.88	5.44±1.25	5.78±1.22	5.74±1.70
Creatinine (mg/dL)	0.73±0.04	0.72±0.04	0.73±0.05	0.82±0.04
Uric acid (mg/dL)	3.52±0.15	4.12±0.18*	4.10±0.18	4.21±0.14*
Na m.mol	115.46±5.04	115.51±5.10	115.10±4.22	114.10±4.10
K m.mol	5.25±2.46	5.45±1.67	5.44±1.50	4.80±2.31
Cl m.mol	102.24±4.30	101.00±4.45	98.44±4.24	100.77±4.04

Values are mean ± S.E.M. (Dunnet 't' test). *P<0.05; Vs control group N=6.

Table-12. Lipid Profile

Dose (mg/kg)	Control	50 mg/kg	100 mg/kg	200 mg/kg
Total cholestrol(mg/dL)	80.11±2.80	79.14±2.37	78.78±3.02	76.99±2.90
HDL(mg/dL)	123.25±2.50	122.20±2.27	123.10±3.00	121.25±2.24
LDL(mg/dL)	42.12±2.55	42.24±2.80	43.00±2.88	42.62±2.04
VLDL(mg/dl)	26.10±2.40	27.38±2.14	26.10±2.48	25.40±2.25
Triglycerides (mg/dl)	27.24±2.62	26.28±2.28	27.20±3.00	28.08±2.55
Blood glucose (mg/dl)	92.40±4.50	94.24±3.15	94.22±3.00	95.28±2.42

Values are mean ± S.E.M. (Dunnet 't' test). ^{ns}P>0.05. Vs control group N=6.

Table-13 Urine Analysis

Parameters	Control	50 mg/kg	100 mg/kg	200 mg/kg
Colour	Yellow	Reddish Yellow	Reddish Brown	Reddish Brown
Transparency	Clear	Slightly turbid	Turbid	Turbid
Specific gravity	1.010	1.010	1.010	1.010
PH	>7.2	>7.4	>7.2	>8.4
Protein	Nil	1+	1+	2+
Glucose	Nil	Nil	Nil	Trace
Bilirubin	-ve	-ve	-ve	-ve
Ketones	-ve	-ve	-ve	+ve
Blood	Absent	Absent	Absent	Absent
Urobilinogen	Normal	Normal	Normal	Normal
Pus cells	0-cells/HPF	1-cell/HPF	2-cells/HPF	1-cell/HPF
RBCs	Nil	Nil	0-1cells/HPF	Nil
Epithelial cells	Nil	1-cell/HPF	Nil	1-cell/HPF
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

Table 14. Effect of oral administration of *Sarva Noi Linga Chendooram* on organ weight

Dose (mg/kg)	Control	50 mg/kg	100 mg/kg	200 mg/kg
Liver (g)	3.10±0.10	3.14±0.12	3.12±0.10	3.08±0.12
Heart (g)	0.32±0.04	0.31±0.05	0.30±0.04	0.32±0.04
Lung (g)	0.44±0.12	0.45±0.12	0.43±0.10	0.42±0.10
Spleen (g)	0.45±0.05	0.46±0.05	0.46±0.05	0.46±0.05
Ovary (g)	1.43±0.12	1.44±0.12	1.45±0.14	1.46±0.12
Testes (g)	2.12±0.12	2.10±0.17	2.12±0.12	2.20±0.14
Brain (g)	2.11±0.10	2.15±0.12	2.16±0.14	2.12±0.15
Kidney (g)	0.80±0.05	0.78±0.05	0.81±0.05	0.82±0.05
Stomach (g)	1.14±0.12	1.15±0.11	1.12±0.13	1.14±0.12

Values are mean ± S.E.M. (Dunnet 't' test). $^{ns}P>0.05$. Vs control group N=6.

PHARMACOLOGICAL STUDIES

Table 15: Diuretic activity of Sarva noi linga chendooram in rats

Group	Treatment and Dose	Volume of Urine (ml/4hrs)	Sodium (mMol/l)	Potassium (mMol/l)	Chloride (mMol/l)
I	Saline (10ml/ kg)	0.86±0.17	79.8±7.5	61.0±5.5	95.6±9.0
II	SNLC (50 mg/ kg)	0.94±0.15[a]	105.2±9.5	83.4±7.2	116.1±12.4
III	SNLC (100 mg/ kg)	1.35±0.14[a]	110.4±8.1	92.1±6.0[*]	128.6±7.8
IV	Frusemide (20 mg/ kg)	4.12±0.24	128.6±5.2	107.5±8.1	144.2±10.3

All values are expressed as mean ±S.E.M for six rats in each group.

Comparisons made between [*]$p<0.05$; T_1, T_2 V_s normal control.; [a]$p<0.001$ T_1, T_2 V_s Standard.

Table 16: Estimation of Urinary Electrolytes of Normal and Urolithiatic Rats.

S.No	Group & Drug Treatment	Estimation of Urinary Electrolytes		
		Oxalate(mg/dl)	**Calcium(mg/dl)**	**Phosphate(mg/dl)**
1	Normal control (Saline)	0.38±0.04	2.74±0.15	3.45±0.05
2	Calculi induced(0.75% EG)	$2.13\pm0.06^{©}$	$9.21\pm0.43^{©}$	$8.16\pm0.10^{©}$
3	Standard (Cystone 750 mg/kg)	1.22 ± 0.07^{x}	3.38 ± 0.23^{x}	3.92 ± 0.07^{x}
4	T_1 (SNLC 50 mg/kg)	$0.751\pm0.23^{***}$	$5.79\pm0.10^{***}$	$5.11\pm0.09^{a,***}$
5	T_2 (SNLC 100 mg/kg)	$0.448\pm0.12^{b,***}$	$4.36\pm0.14^{a,***}$	$4.32\pm0.08^{c,***}$

All values are expressed as mean ±S.E.M for six rats in each group.

Comparisons made between

$^{a}p<0.001, ^{b}p<0.01, ^{c}p,<0.05$; T_1, T_2 V_s Standard.

$^{***}p<0.001, ^{**}p<0.01, ^{*}p<0.05$; T_1, T_2 V_s Calculi induced.

$^{©}p<0.001$, $^{\alpha}p<0.01, @p<0.05$; Calculi induced V_s normal control.

$^{x}p<0.001, ^{y}p<0.01, ^{z}p,<0.05$; Calculi induced V_s Standard., One-way ANOVA followed by Tukey test.

Table 17: Estimation of Kidney Homogenate Electrolytes of Normal And Urolithiatic Rats.

S.No	Group &Drug Treatment	Estimation of Kidney Homogenate Parameters		
		Oxalate(mg/dl)	Calcium(mg/dl)	Phosphate(mg/dl)
1	Normal (Saline)	0.186±0.03	3.431±0.28	2.65±0.05
2	Positive control (0.75% EG)	1.742±0.09$^{©}$	6.024±0.20$^{©}$	4.10±0.14$^{©}$
3	Standard (Cystone 750 mg/kg)	0.446±0.05^{x}	4.326±0.19^{x}	3.20±0.08^{x}
4	T_1 (SNLC 50 mg/kg)	0.689±0.05***	5.452±0.26^{c}	3.88±0.12^{a}
5	T_2 (SNLC 100 mg/kg)	0.575±0.06***	4.234±0.18***	3.01±0.09***

All values are expressed as mean ±S.E.M for six rats in each group.

Comparisons made between

$^{a}p<0.001$,$^{b}p<0.01$,$^{c}p,<0.05$; T_1,T_2V_s Standard.

$^{***}p<0.001$,$^{**}p<0.01$,$^{*}p<0.05$; T_1,T_2V_s Calculi induced.

$^{©}p<0.001$,$^{\alpha}p<0.01$,@$p<0.05$; Calculi induced V_s normal control.

$^{x}p<0.001$,$^{y}p<0.01$,$^{z}p,<0.05$; Calculi induced V_s Standard., One-way ANOVA followed by Tukey test.

Table 18: Estimation of Serum Parameters of Normal and Urolithiatic Rats.

S.No	Group & Drug Treatment	Estimation of Serum Parameters		
		BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
1	Normal (Saline)	18.256±0.32	0.682±0.05	4.99±0.05
2	Positive control (0.75% EG)	27.109±0.36$^{©}$	0.902±0.05$^{@}$	6.72±0.09$^{©}$
3	Standard (Cystone 750 mg/kg)	22.754±0.44^{x}	0.918±0.04	5.56±0.07^{x}
4	T_1 (SNLC 50 mg/kg)	34.022±0.65^{a,***}	1.226±0.05^{b,**}	6.04±0.09^{b,***}
5	T_2 (SNLC 100 mg/kg)	30.239±0.48^{a,***}	1.101±0.07	6.12±0.08^{a,***}

All values are expressed as mean ±S.E.M for six rats in each group.

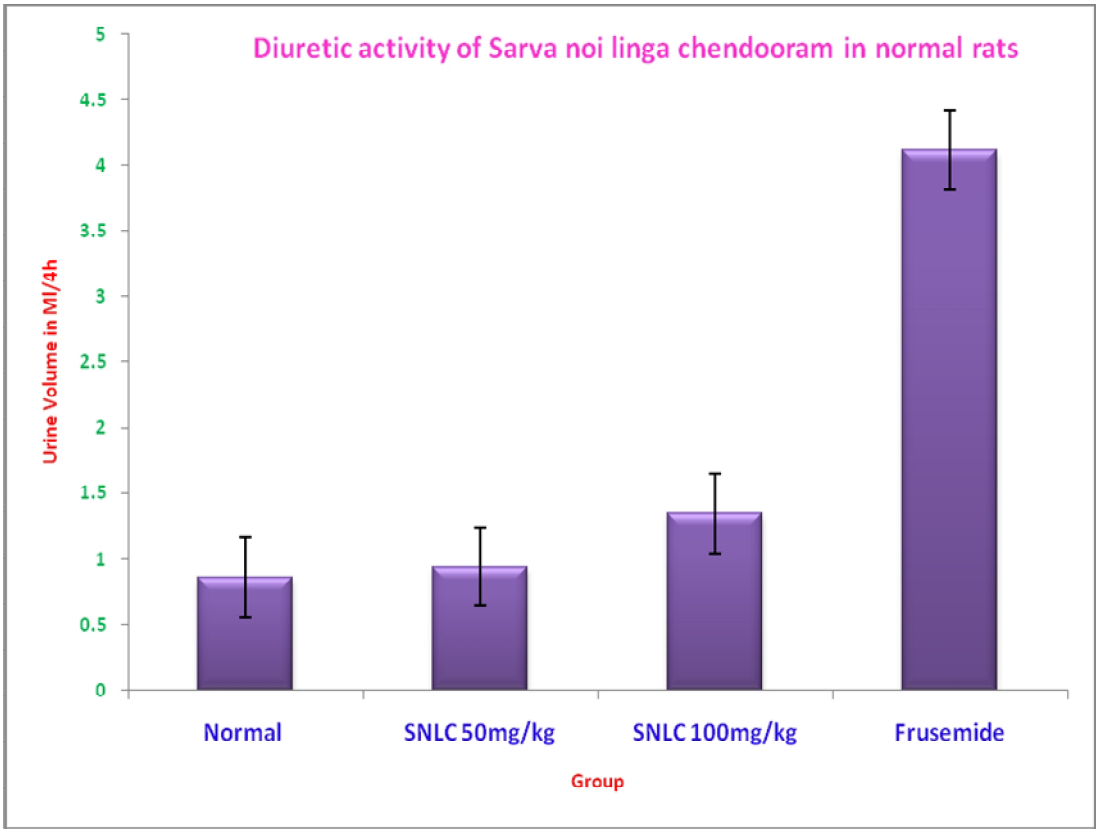
Comparisons made between

$^{a}p<0.001$,$^{b}p<0.01$,$^{c}p,<0.05$; T_1,T_2 V_s Standard.

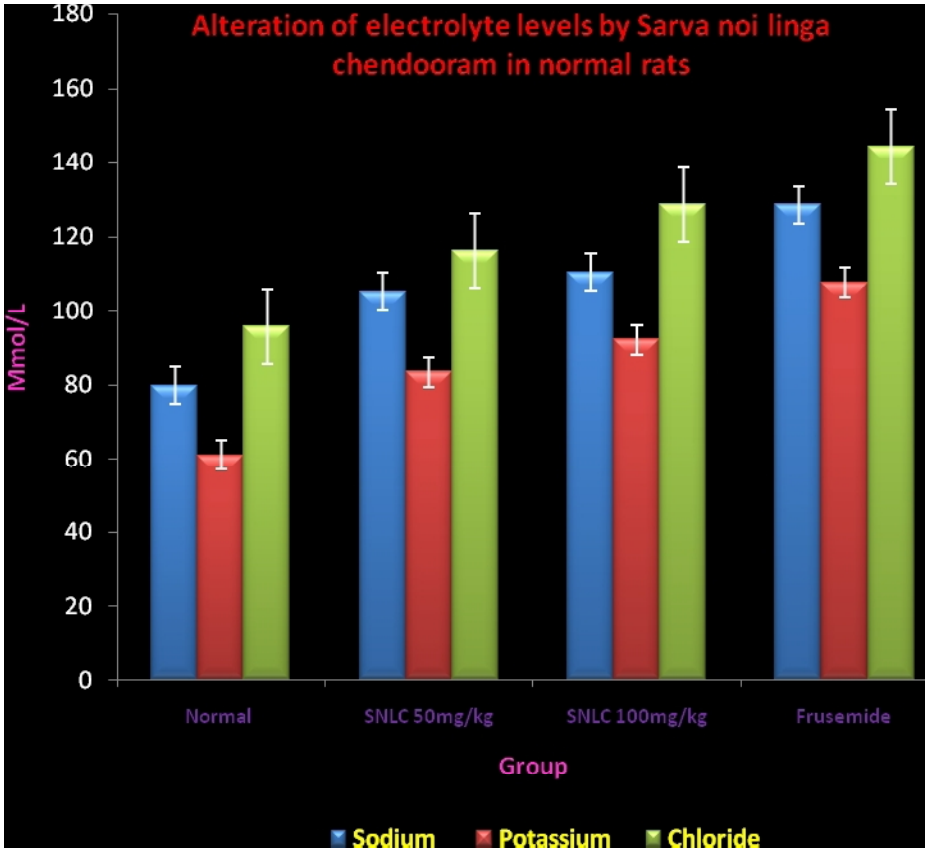
$^{***}p<0.001$,$^{**}p<0.01$,$^{*}p<0.05$; T_1,T_2 V_s Calculi induced.

$^{©}p<0.001$,$^{\alpha}p<0.01$,@$p<0.05$; Calculi induced V_s normal control.

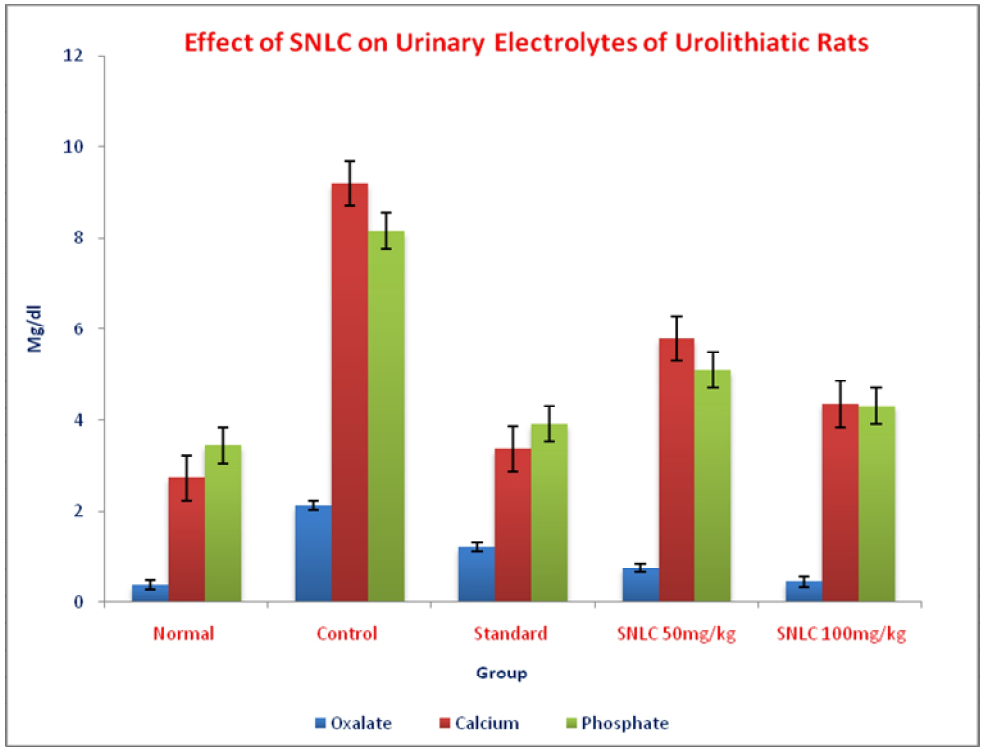
$^{x}p<0.001$,$^{y}p<0.01$,$^{z}p,<0.05$; Calculi induced V_s Standard., One-way ANOVA followed by Tukey test.



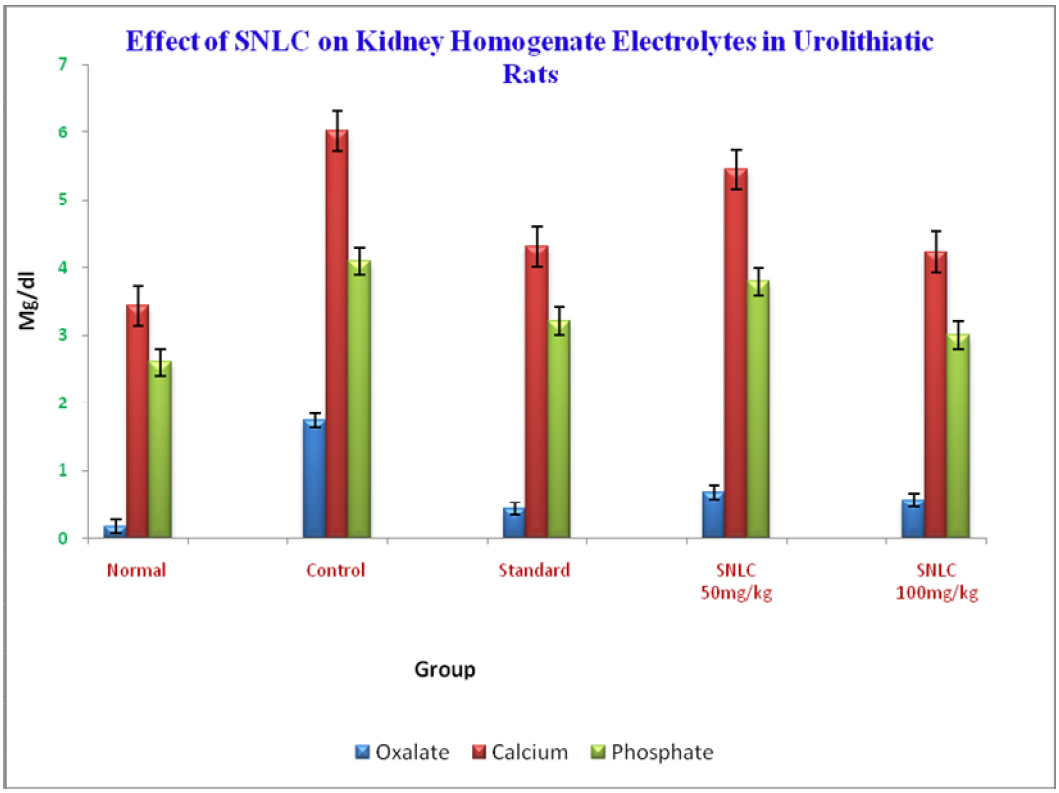
TRIAL DRUG 2 Bar diagram 1



TRIAL DRUG 2 Bar diagram 2



TRIAL DRUG 2 Bar diagram 3



TRIAL DRUG 2 Bar diagram 4

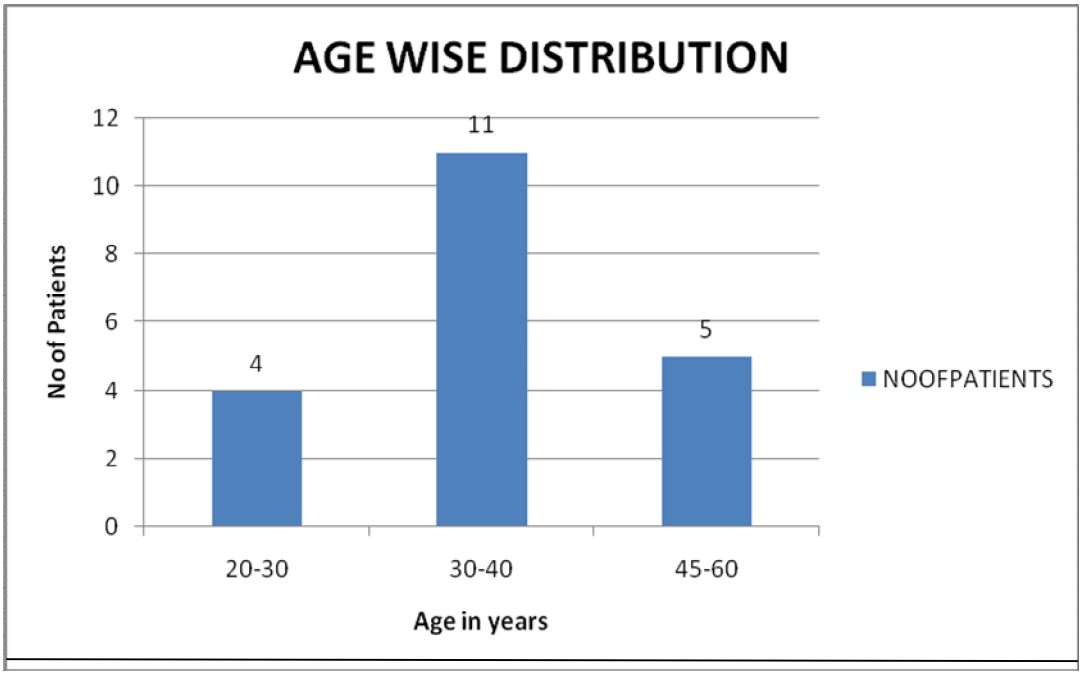
AGE WISE DISTRIBUTION TRIAL DRUG 2

TABLE 19

S.NO	AGE WISE DISTRIBUTION	NO OF PATIENTS
1	20-30	4
2	30-45	11
3	45-60	5

GENDER WISE DISTRIBUTION TRIAL DRUG 2
TABLE 20

S.NO	GENDER	NO OF PATIENTS	PERCENTAGE
1	MALE	11	55%
2	FEMALE	9	45%



Bar diagram 5 **TRIAL DRUG 2**

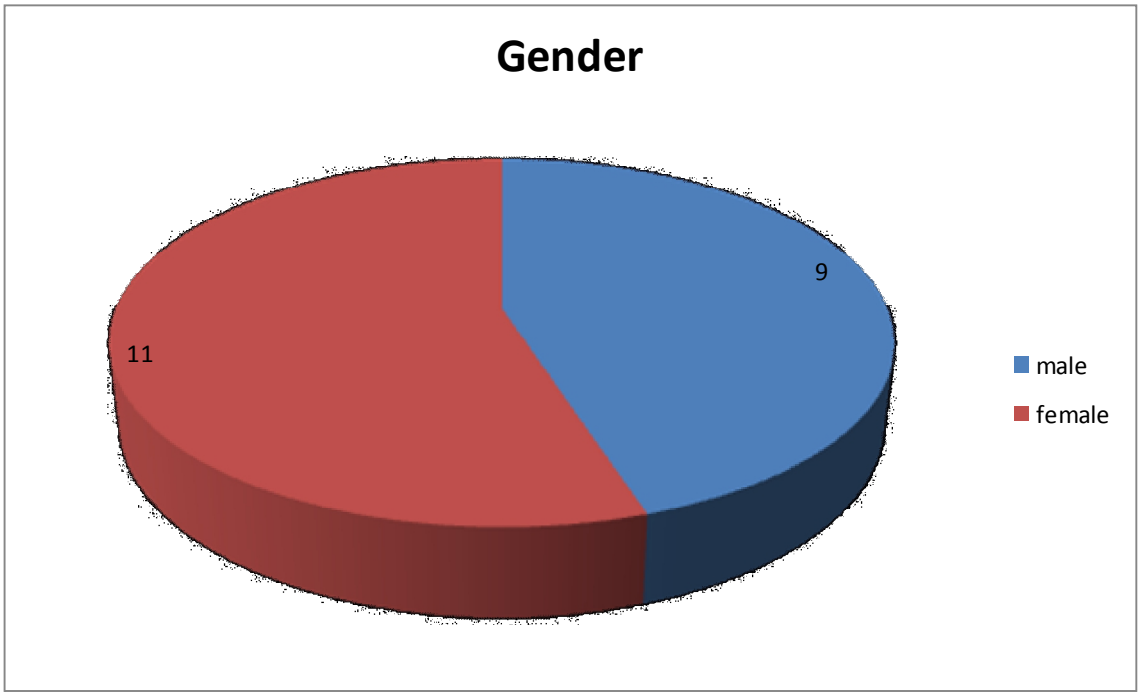


Chart 1 **TRIAL DRUG 2**

PROGNOSIS IN SYMPTOMS Of RENAL CALCULI TRIAL DRUG 2

TABLE 21

		No of patients	
S.NO	SYMPTOMS	BT	AT (prognosis)
1	Pain	16	13
2	Frequency of micturition	11	9
3	Dysuria	11	9
4	Oliguria	3	1
5	Burning micturition	19	14
6	Haematuria	6	4
7	Nausea	16	15
8	Vomiting	14	12
9	Fever	6	6

S.NO	OPD/IPD	AGE	SEX	BTRTKCAL size in mm	ATRKCAL size in mm	BTLTKCAL size in mm	ATLTKCAL size in mm
1	C80114	36	F	2-3	0	2-3	4.5
2	C79916	46	M	7	5	0	0
3	C81077	51	F	6.6,2.9	1.16	4.2	3.7
4	C81387	37	M	7 , 5	4,7,4,3.8	6	5
5	C85062	36	M	0	0	4.3	3.2
6	C86007	35	M	7	3	9	6
7	C87501	45	F	5.3	3.3	5	4
8	C79067	29	M	9,7.5.	7	10	9
9	C89477	55	F	6	5	6	0
10	C66643	25	M	0	0	9.9	6
11	C89987	29	F	4,6	2,3	4,2	0
12	C89500	27	F	5.3	5	4.2	4.2
13	C21280	31	F	3.5	0	0	0
14	C87193	35	F	0	5	4.2	0
15	5017	45	M	0	0	6.1	0
16	C77638	45	M	6	0	0	0
17	C88424	38	M	10	0	9	6
18	C79579	36	M	0	0	5,4	5,4
19	C83911	35	F	10	6	0	0
20	C89260	52	M	4.6	7	6	5

SIZE OF RENAL CALCULI BEFORE AND AFTER TREATMENT TABLE 22

BTRTKCAL –before treatment right kidney calculus

ATRKCAL- after treatment right kidney calculus

BTLTKCAL-before treatment left kidney calculus

ATLTKCAL- After treatment left kidney calculus

All collected data were entered into MS Excel software using different columns as variables and rows as patients. SPSS software was used to perform statistical analysis. Basic descriptive statistics include frequency distributions and cross-tabulations were performed. The quantity variables were expressed as Mean ± Standard Deviation and qualitative data as percentage. A probability value of <0.05 was considered to indicate as statistical significance. Paired 't' test was performed for determining the significance between before and after treatment

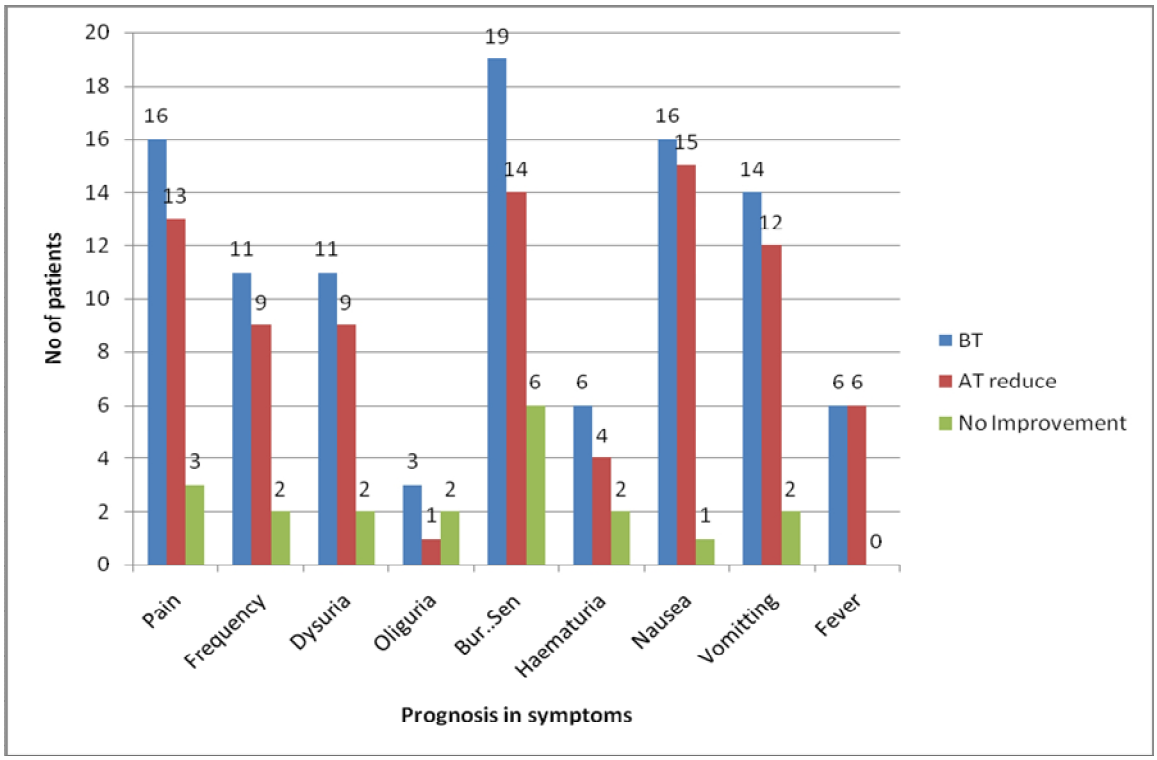
TRIAL DRUG 2 TABLE 23

Prognosis in symptoms of renal calculi						
variable		Obs	Mean	Std. Deviation	t value	P value
sym	BT	20	4.75	1.118	10.556	0.001
	AT	20	0.90	1.071		

Trial drug 2 table 24

Reduction in size of renal calculi						
variable		Obs	Mean	Std. Deviation	t value	P value
size	BT	20	10.930000	5.83610	4.856	<0.0001
	AT	20	6.488000	4.69628		

BAR DIAGRAM -6 TRIAL DRUG -2



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

69, Anna Salai, Guindy, Chennai-600 032

This Certificate is awarded to ~~Mr/Ms~~/Dr. A. PUNITHA

for participating as a ~~Resource Person~~ / Delegate in the VII Workshop

on **"Research Methodology & Biostatistics"**

for AYUSH Post-Graduates & Researchers

organized by the Department of Siddha

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

from 6th Feb. 2012 to 10th Feb. 2012.

DR. MAYILVAHANAN NATARAJAN
M.S.Orth. M.Ch.Orth. (L'pool) Ph.D. (Orth. Onco.) F.R.C.S. (Eng) D.Sc.
7th VICE CHANCELLOR

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Name: Dr. A. PUNITHA Reg No: 32101705

Title: Pre-clinical and clinical study on "AMMUKKARA KIZHANGU CHOORNAM" for HYPOLIPIDAEMIC ACTIVITY" in the management of Athimetham [Hyperlipidaemia]

No. NIS/IEC/2011/3/136 - 24/12/2011

DECSION

Opinion of the Institutional Ethics Committee – Please Check one
✓ Approval _____ Modifications required prior to approval (Please specify one space below) _____ Disapproval Date of review: _____ K. Manimah (DR.K.MANICKAVASAKAM) Member Secretary Signed: _____ (Please print name) Dr.V.SUBRAMANIAN Chair Person (Please delee as appropriate, Chairperson, Secretary)
Modifications needed Modification given to candidate

The research proponent is hereby informed that the Institutional Ethics Committee will require the following:

1. All adverse drug reactions (ADRs) that are both serious and unexpected to be reported promptly to the IEC within 7 working days
2. The progress report to be submitted to the IEC atleast annually
3. Upon completion of the study, a final study status report needs to submitted to the IEC

IAEC PROTOCOL NO: 1248/ac/09/CPCSEA/A-138/2011.

CERTIFICATE 20/12/2011

This is certify that the project title. Pre-clinical & clinical study on "AMUKKARA CHOORNAM KIZHANGU" for "HYPOLIPIDAEMIC ACTIVITY" in the management of Vathimedham (Hyperlipidaemia) **has been approved by the IAEC.**

Prof. Dr. K. Manickavasakam
Name of Chairman/Member Secretary IAEC:

Dr. B. Jayachandran Dare
Name of CPCSEA nominee:

Signature with date

K. Manickam

Chairman/Member Secretary of IAEC:

CPCSEA nominee:

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

NATIONAL INSTITUTE OF SIDDHA

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Name: A. PUNITHA Reg.No: 32101705

Title: Pre-clinical and Clinical study on "SARVA NOI LINGA CHENDURAM" for LITHONTRIPTIC ACTIVITY" in the management of Kalladaippu [Renal calculus]

No. NIS/IEC/2011/3/13a - 24/12/2011

DECSION

Opinion of the Institutional Ethics Committee – Please Check one
✓ Approval _____ Modifications required prior to approval (Please specify one space below) _____ Disapproval Date of review: _____ Signed: _____ (Please print name) Dr.V.SUBRAMANIAN Chair Person (Please delee as appropriate, Chairperson, Secretary)
Modifications needed Modification given to candidate
The research proponent is hereby informed that the Institutional Ethics Committee will require the following: 1. All adverse drug reactions (ADRs) that are both serious and unexpected to be reported promptly to the IEC within 7 working days 2. The progress report to be submitted to the IEC atleast annually 3. Upon completion of the study, a final study status report needs to submitted to the IEC

K. Manickavasakam
(Dr. K. MANICKAVASAKAM)
Member Secretary

IAEC PROTOCOL NO: IIHS/ac/09/CPCSEA/A-13A/2011.

20/12/2011

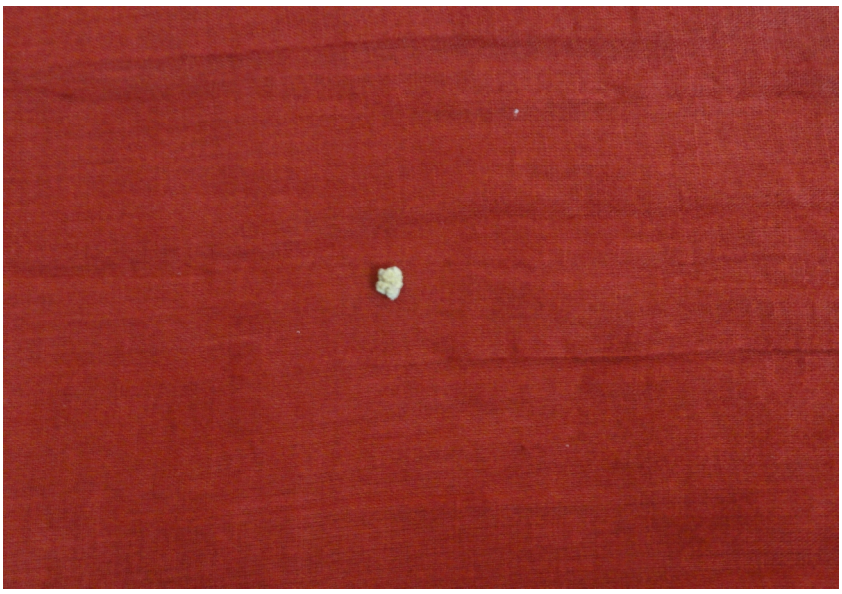
CERTIFICATE

This is certify that the project title. Pre-clinical & clinical study on "SARVA NOI HINGA CHENDURAM" for "LITHONTRIPTIC ACTIVITY" in the management of kallandaippu (Renal calculus) **has been approved by the IAEC.**

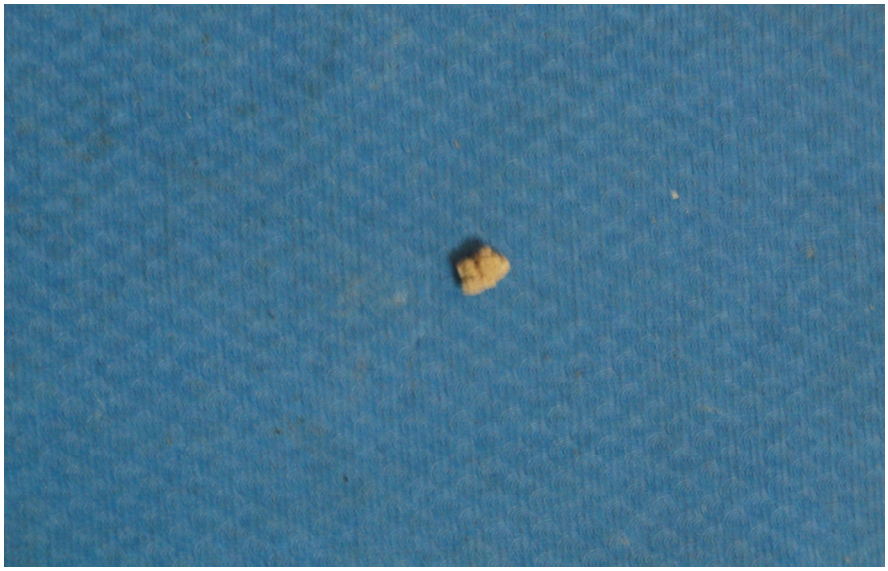
Prof. Dr. K. Marichavasakam	Dr. B. Jayachandran Dare
Name of Chairman/Member Secretary IAEC:	**Name of CPCSEA nominee:**
Signature with date	
K. Thamizholi	
Chairman/Member Secretary of IAEC:	**CPCSEA nominee:**

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

OPD NO: C 79067 29/M



OPD NO :C88424 38/M



எ.பி. கேர் பரிசோதனை நிலையம்
AB Care Diagnostics Centre

எண். 2, வேளச்சேரி மெயின் ரோடு, கேம்ப் ரோடு ஜங்சன்
(பாரதி பள்ளி சந்து, குப்தாபவன் அருகில்)
சேலையூர், சென்னை - 73.
Phone : 32427159, Cell : 9500082696

எண். 43/22, கலைஞர் நெடுஞ்சாலை,
சீனிவாசன் நகர், புதிய பெருங்களத்தூர்,
சென்னை - 600 063.
Phone : 80562 72561

Working Hours : Week Days : 6.30 a.m. to 9.00 pm Sunday : 6.30 a.m. to 1.30 p.m.
(HOUSE calls undertaken by prior appointment)

Report

URINARY BLADDER

The Urinary bladder is adequately distended. No calculus / mass seen within. The wall appears normal.

UTERUS

The Uterus is normal in size and measures 6.8 x 3.3 x 3.1 cm. Endometrial thickness measures 7.0 mm. Myometrial and endometrial echoes are homogenous. No focal lesion seen. Cervix appears normal.

OVARIES

Both ovaries are normal. Right ovary measures 3.8 x 2.0 cm.
Left ovary measures 3.5 x 2.2 cm.

There is no evidence of free fluid in the abdomen and pelvis.

IMPRESSION:

- *RIGHT RENAL CALCULUS.*
- *NORMAL SONOGRAPHIC STUDY OF REST OF THE ABDOMEN AND PELVIC ORGANS*

Please correlate

DR.P.BHASKAR, MBBS.
Sonologist

Get Well Soon

எ.பி. கேர் பரிசோதனை நிலையம்
AB Care Diagnostics Centre

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Phone : 80562 72561

Working Hours : Week Days : 6.30 a.m. to 9.00 pm Sunday : 6.30 a.m. to 1.30 p.m.
(HOUSE calls undertaken by prior appointment)

Report

PATIENTS NAME	MRS.JEBAVIJILA	AGE	31 Yrs
CR.NO	1023	SEX	FEMALE
REF CONSULTANT	SELF	DATE	11.08.2012

USG – ABDOMEN & PELVIS

LIVER

The liver is normal in size, shape and shows normal parenchymal echoes. No focal or diffuse lesion seen. Both intra and extra hepatic biliary radicals appear normal. The Portal vein is of normal caliber. The common bile duct is normal in size. No CBD calculus seen.

GALL BLADDER

The gall bladder is adequately distended. Wall thickness is normal. No calculus seen.

PANCREAS

Normal in size, shape and echotexture. No focal or diffuse lesion seen. No dilatation of pancreatic duct seen.

SPLEEN

Normal in size and shows homogeneous normal echo texture. No focal lesion seen.

KIDNEYS

Right Kidney is normal in size and shape. The cortical thickness and echogenicity appear normal. The pelvicalyceal system is not dilated. No mass seen. **Evidence of calculus measures 3.5 mm noted in the mid pole.**

Left Kidney is normal in size and shape. The cortical thickness and echogenicity appear normal. The pelvicalyceal system is not dilated. No calculus / mass seen.

Measurement: Rt.Kidney 9.5 x 4.8 cm
Lt.Kidney 11.0 x 5.4 cm

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சேலையூர், சென்னை - 73.
Phone : 32427159, Cell : 9500082696

எண். 43/22, கலைஞர் நெடுஞ்சாலை,
சீனிவாசன் நகர், புதிய பெருங்களத்தூர்,
சென்னை - 600 063.
Phone : 80562 72561

Working Hours : Week Days : 6.30 a.m. to 9.00 pm Sunday : 6.30 a.m. to 1.30 p.m.

(HOUSE calls undertaken by prior appointment)

Report

URINARY BLADDER

The urinary blladder is adequately distended. No calculus / mass seen within. The wall appears normal.

UTERUS

The Uterus is normal in size and measures 6.8x3.3x2.0 cm.Endometrial thickness measures 7.0mm.Mymometrial and endometrial echoes are homogenous.No focal lesion seen. Cervix appears normal.

OVARES

Both overies are normal R.O measuring 3.8x2.0cm
L.O measuring 3.5x2.2cm

No free fluid or mass or cyst seen.
Aorta and IVC appear normal. Retroperitoneum appears normal.

IMPRESSION:

- NORMAL SONOGRAPHY STUDY OF ABDOMEN AND PELVIS ORGAN.

For clinical correlation

DR.P.BHASKAR.MBBS.,
Sonologist.

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எ.பி. கேர் பரிசோதனை நிலையம்

AB Care Diagnostics Centre

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Phone : 80562 72561

Working Hours : Week Days : 6.30 a.m. to 9.00 pm Sunday : 6.30 a.m. to 1.30 p.m.
(HOUSE calls undertaken by prior appointment)

Report

PATIENTS NAME :MRS. JABAVIJILA AGE: 31 / F

REF CONSULTANT : PUNITHA DATE:17.09.2012

USG -ABDOMEN &PELVIS

LIVER

The liver is normal in size, shape and shows increased parenchymal echoes. No focal or diffuse lesion seen. Both intra and extra hepatic biliary radicals normal. The portal vein is of normal caliber. The common bile duct is normal in size. No CBD calculus seen.

GALL BLADER.

The gall bladder is adequately distended. Wall thickness is normal. No calculus seen.

PANCREAS

Normal in size, shape and echotexture. No focal or diffuse lesion seen. No dilatation of pancreatic duct seen.

SPLEEN

Normal in size and shows homogeneous normal echo texture. No focal lesion seen.

KIDNEYS

Right kidney is normal in size and shape. The cortical thickness and echogenicity appear normal. The pelvicalyceal system is not dilated. No calculus / mass seen.

Left kidney is normal in size and shape. The cortical thickness and echogenicity appear normal. The pelvicalyceal system is not dilated. No calculus / mass seen.

Measurement : Rt Kidney 9.5 x 4.8 cm
Lt Kidney 11.0 x 5.4 cm

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Name : Mr.Muruganandham	Date: 03.08.2012
Age : 45Y / M	ID/AS/TBM/US/ 2961
Ref.By.: Dr.A.Punitha.,	

Ultrasound Abdomen

Liver:

Is enlarged in size and measures 17.1 cms shows homogenous increased in echo texture. Intrahepatic biliary radicles, portal vein, hepatic veins and IVC appear normal.

Gall Bladder:

Is adequately distended. No calculus or internal echoes are seen.
Wall thickness is normal. The CBD is not dilated.

Pancreas:

Appears normal in size and shows uniform echo texture.
The pancreatic duct is normal. No calcifications are seen.

Spleen:

Appears enlarged in size and measures 14.4 cms it shows uniform echo texture.

Kidneys:

RT.Kidney measures 10.8 x 5.6 cms.
Multiple calculi measuring less than 6mm noted in the renal field of the right kidney.
LT.Kidney measures 11.5 x 6.3 cms.
Renal cortical echoes and Cortico medullary differentiation are normal on both sides. Pelvicalyceal system on both sides appears normal.

Bladder:

Is normal in contour. No intraluminal echoes are seen.
No calculus or diverticulum is seen.

Note : This imaging modality is having its own limitations. Hence this report should be correlated with clinical features and other parameters.

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AN ISO 9001 ORGANISATION

Name : Mr.Muruganandham	Date: 03.08.2012
Age : 45Y / M	ID/AS/TBM/US/ 2961
Ref.By.: Dr.A.Punitha.,	

Ultrasound Abdomen

Prostate:

Appears normal in size and it shows uniform echo texture.
Measures 3.1 x 2.4 x 2.9 cms. Volume- 12.0 cc.

RIF and Retroperitoneum:

Appear normal. No retroperitoneal lymphadenopathy.
The psoas appears normal. No free fluid.

Impression:

- *Right renal calculi.*

-Suggested clinical correlation.

Dr.Chitra Vishwesh.,
Sonologist.

LPAUK : 766, P.H.Road, Chennai-10. Ph : 4399 2900. Mob.: 99401 10501.
ADAPALANI : 60, 100 Feet Road, Chennai-26. Ph : 4399 2992. Mob.: 99401 10502.
WARPET : 17, C.V.Raman Road, Chennai-18. Ph : 4399 2939. Mob.: 99400 22558.
NDIARPET : 622 T.H.Road, Chennai-81. Ph: 4345 2121. Mob.: 99401 10505.
ERAMBUR : 49/50, Paper Mills Road, Chennai-11. Ph : 26706622. Mob.: 95000 76590.
• TAMBARAM : 116, Ezhumalai St., Mudichur Rd., Chennai-45. Ph : 22261944.
• VELACHERI : 3, Ist Main Road, Vijai Nagar, Chennai - 42. Mob.: 99400 75351.
• ANNA NAGAR : Aarthi Diagnostics, 116/1, "S" Block, 6th Main Road, CHENNAI - 40. Ph : 26208166, 26208177. Mobile : 96770 66661.

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Patient : MR. MURUGANANTHAM
Ref.By : DR. A. PUNITHA.

Age/Sex : 45 Yrs / M
Date : 21.09.2012

ULTRASONOGRAPHY REPORT – ABDOMEN / MALE

LIVER :
Normal in size and echo pattern, measures 160.0 mm
(normal size for body mass) . No focal or diffuse pathology.
CBD and IHBR appear normal. Portal vein is normal.

GALL BLADDER :
Adequately distended. Wall is normal.
No calculus / sludge / polyp.

PANCREAS :
Normal in size & echo pattern. Pancreatic duct is not dilated.
No focal / diffuse pathology.

SPLEEN :
Normal in size and measures 115.0 mm.

KIDNEYS :
Right kidney measures 106.5 x 50.7 mm.
Cortical echoes are normal. No focal lesion.
Collecting system is normal. No evidence of calculus.

Left kidney measures 112.5 x 60.9 mm.
Cortical echoes are normal. No focal lesion.
Collecting system is normal. No evidence of calculus.

URINARY BLADDER:
Distended. Wall is normal. Bladder wall thickness 3.5 mm.
No abnormal intraluminal echoes.

PROSTATE :
Prostate appear normal in size.
It measures 34.8 x 34.8 23.7 mm. Wt. 15.0 gms.

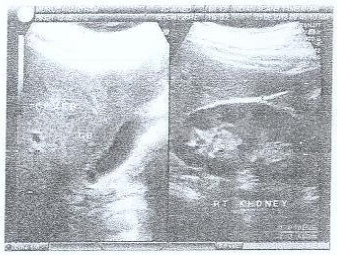
PERITONEUM:
No evidence of ascites.

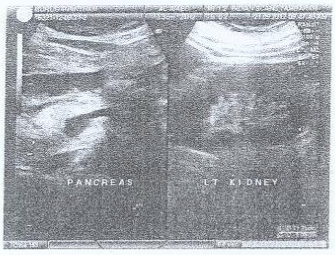
AORTIC & IVC:
Normal in calibre. No demonstrable para aortic nodes.

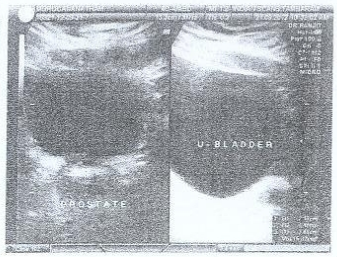
RIGHT ILIAC FOSSA:
No ultra sonographically demonstrable pathology or tenderness.

IMPRESSION :

• NORMAL SONOGRAPHIC STUDY OF ABDOMEN .







DR. RANJIT.
Consultant Radiologist.

No. 7/9, Duraisamy Pillai Street, West Tambaram, Chennai - 45. Ph : 22262428, 22261473

24 HOURS EMERGENCY SERVICE • AMBULANCE SERVICE AVAILABLE ON REQUEST

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Name : Mr.P.Thiyagarajan	Date: 07.08.2012
Age : 45Y / M	ID/AS/TBM/US/ 3047
Ref.By.: National Institute Of Siddha.,	

Ultrasound Abdomen

Liver:
Is normal in size and shows uniform echo texture. Intrahepatic biliary radicles, portal vein, hepatic veins and IVC appear normal.

Gall Bladder:
Is adequately distended. No calculus or internal echoes are seen.
Wall thickness is normal. The CBD is not dilated.

Pancreas:
Appears normal in size and shows uniform echo texture.
The pancreatic duct is normal. No calcifications are seen.

Spleen:
Appears mildly enlarged in size and measures 10.0 x 5.3 cms it shows uniform echo texture.

Kidneys:
RT.Kidney measures 9.5 x 5.3 cms.
LT.Kidney measures 9.9 x 5.7 cms.
Multiple micro calculi are noted in the renal field of the left kidney.
Largest calculus measures 6.1 mm noted in the mid pole of the left kidney.
Renal cortical echoes and Cortico medullary differentiation are normal on both sides. Pelvicalyceal system on both sides appears normal.

Bladder:
Is normal in contour. No intraluminal echoes are seen.
No calculus or diverticulum is seen.

Note : This imaging modality is having its own limitations. Hence this report should be correlated with clinical features and other parameters.

AARTHI SCANS

AARTHI SCANS™
AN ISO 9001 ORGANISATION

Name : Mr.P.Thiyagarajan	Date: 07.08.2012
Age : 45Y / M	ID/AS/TBM/US/ 3047
Ref.By.: National Institute Of Siddha.,	

Ultrasound Abdomen

Prostate:
Appears normal in size and it shows uniform echo texture.
Measures 3.1 × 3.5 × 2.6 cms. Volume- 15.2 cc.

RIF and Retroperitoneum:
Appear normal. No retroperitoneal lymphadenopathy.
The psoas appears normal. No free fluid.

Impression:

- *Left renal calculi.*
- *No evidence of hydronephrosis.*

-Suggested clinical correlation.

Dr.Naveen.Y.G MDRD.,
Consultant Radiologist.

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• Computerised ECG • Treadmill • PFT • Digital X-Ray • Laboratory • Sonomammography • 4D Scan • EEG • Digital Mammogram

Patient : MR. THYAGARAJAN
Ref By : DR. A. PUNITHA,

Age/Sex : 45 Yrs / M
Date : 09.09.2012

ULTRASONOGRAPHY REPORT – ABDOMEN / MALE

LIVER :
Normal in size and echo pattern. No focal or diffuse pathology.
CBD and IHBR appear normal. Portal vein is normal.

GALL BLADDER :
Adequately distended. Wall is normal.
No calculus / sludge / polyp.

PANCREAS :
Normal in size & echo pattern. Pancreatic duct is not dilated.
No focal / diffuse pathology.

SPLEEN :
Normal in size and measures 91.0 mm.

KIDNEYS :
Right kidney measures 89.6 x 49.5 mm.
Cortical echoes are normal. No focal lesion.
Collecting system is normal. No evidence of calculus.

Left kidney measures 87.7 x 50.2 mm.
Cortical echoes are normal. No focal lesion.
Collecting system is normal. No evidence of calculus.

URINARY BLADDER:
Distended. Wall is normal. Bladder wall thickness 3.5 mm.
No abnormal intraluminal echoes.

PROSTATE :
Prostate appear normal in size.
It measures 38.8 x 28.3 x 23.8 mm. Wt. 13.6 gms.
No focal lesion.

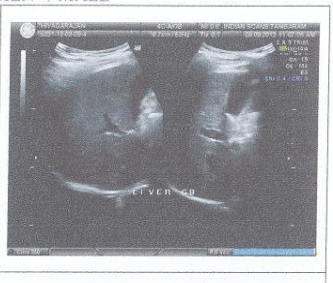
PERITONEUM:
No evidence of ascites.

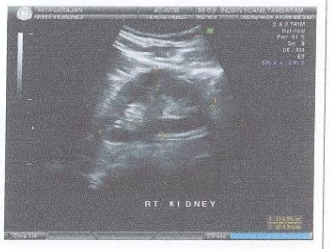
AORTIC & IVC:
Normal in calibre. No demonstrable para aortic nodes.

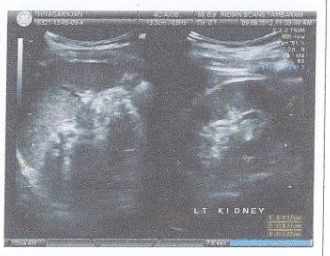
RIGHT ILIAC FOSSA:
No ultra sonographically demonstrable pathology or tenderness.

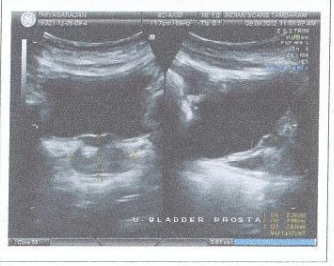
IMPRESSION :

NORMAL SONOGRAPHIC STUDY OF ABDOMEN.









DR. NISCHAL, MD., RD
Consultant Radiologist.

No. 7/9, Duraisamy Pillai Street, West Tambaram, Chennai - 45. Ph : 22262428, 22261473

24 HOURS EMERGENCY SERVICE ◆ AMBULANCE SERVICE AVAILABLE ON REQUEST

HYPER LIPIDEMIA INVESTIGATIONS BEFORE TREATMENT

s.no	OPD/IPD	AGE	SEX	Hb	TC	DC			ESR		B.sugar		LFT									b.urea	s.crea	urine		
							L	E	1/2hr	1	FBS	PPBS	s.bilrubin			SGOT	SGPT	SALKPHO	S.T PROT	s.alb	glob			sug	alb	dep
													total	direct	indir											
1	C83477	57	F	12.3	7,600	53	45	2	10	26	271	330	0.3	0.1	0.2	29	30	159	6.7	4.2	2.5	18	0.6	nil	nil	nil
2	C85166	55	M	15.9	8400	64	30	6	2	4	91	141	0.6	0.2	0.4	27	28	177	6.9	3.9	3	22	0.4	nil	nil	nil
3	C85756	54	M	13.9	7400	45	42	3	4	8	102	136	0.7	0.3	0.4	36	20	417	6.4	4	2.4	26	0.3	nil	nil	nil
4	C86423	60	M	13.8	7200	66	32	2	2	6	145	212	0.6	0.1	0.2	23	24	210	6.6	4.9	1.7	27	0.8	nil	nil	nil
5	C84500	38	M	14.1	10700	44	53	3	4	10	97	121	0.5	0.2	0.2	38	49	237	6.8	3.9	2.9	16	0.6	nil	nil	nil
6	C87493	51	F	13.1	7600	65	31	4	3	6	257	113	0.4	0.1	0.2	26	32	219	6.8	3.2	3	28	0.7	nil	nil	nil
7	C87904	50	F	13.6	7600	57	34	9	2	7	108	131	0.6	0.3	0.1	32	36	214	6	3	2	32	0.4	nil	nil	nil
8	C88O95	57	F	13.3	10000	60	38	2	10	40	111	179	0.8	0.3	0.4	35	28	188	6.6	3.8	2.7	23	0.3	nil	nil	nil
9	C88994	56	F	13.9	11200	72	26	2	12	26	122	141	0.5	0.2	0.3	20	21	176	7.4	4.2	3.2	26	0.6	nil	nil	nil
10	C89385	50	M	13.5	8100	60	37	3	7	14	138	202	0.6	0.4	0.3	21	223	213	6.9	5	1.9	26	0.4	nil	nil	nil
11	C89311	42	F	13.5	7100	52	40	8	4	8	90	105	0.6	0.4	0.3	32	33	246	6.3	3.8	2.6	34	0.3	nil	nil	nil
12	C92412	41	M	14.1	8900	55	42	3	6	14	142	132	0.7	0.3	0.2	33	37	179	7.5	3.5	2.8	24	0.6	nil	nil	nil
13	C92933	40	F	13.7	10900	60	38	2	12	36	76	138	0.5	0.2	0.4	34	36	198	7	5	2.8	18	0.4	nil	nil	nil
14	C93005	45	F	11.4	8000	55	40	5	4	12	123	244	0.5	0.2	0.3	35	37	178	7.5	5.5	2	31	0.7	nil	nil	nil
15	C91627	35	M	16.2	11900	61	35	4	2	4	101	121	0.6	0.4	0.2	15	16	171	6.6	4.2	2.4	25	0.4	nil	nil	nil
16	B88419	55	M	14.2	7200	66	30	3	4	8	121	131	0.4	0.3	0.2	32	28	143	6.5	5	4.2	26	0.6	nil	nil	nil
17	U3205	36	F	13.6	1200	56	41	3	8	3	6	137	0.3	0.2	0.7	33	30	156	7.5	4	3.8	28	0.3	nil	nil	nil
18	D007816	54	F	12	9900	65	32	3	4	10	157	216	0.2	0.3	0.4	32	33	242	6.2	4	2.2	32	0.6	nil	nil	nil
19	C97096	58	F	13.9	9400	58	38	4	4	22	108	115	0.5	0.3	0.3	34	36	233	244	4	3	37	0.9	nil	nil	nil
20	D010184	42	M	13.4	7000	50	40	10	2	6	164	179	0.7	0.3	0.4	20	22	179	6.6	4.3	2.3	38	0.5	nil	nil	nil

HYPERLIPIDEMIA INVESTIGATIONS AFTER TREATMENT

s.no	OPD/IPD	AGE	SEX	Hb	TC	DC			ESR		B.sugar		LFT									B.UREA	S.CREAT	URINE		
						P	L	E	1/2hr	1	FBS	PPBS	s.bilrubin			SGOT	SGPT	SALK PHO	S.T PROT	s.alb	S.GLOB			SUG	ALB	DEP
													total	direct	indir											
1	C83477	57	F	11	6800	64	34	2	6	14	116	144	0.4	0.2	0.2	21	22	176	5.1	2.6	2.5	24	0.7	NIL	NIL	NIL
2	C85166	55	M	16.5	8500	65	33	2	8	16	100	132	0.4	0.2	0.2	21	22	176	7.5	4.5	3.5	18	0.8	NIL	NIL	NIL
3	C85756	54	M	13	9200	60	35	5	2	6	100	130	0.7	0.3	0.4	20	21	145	6.9	4.9	2	16	0.5	NIL	NIL	NIL
4	C86423	60	M	14.6	7400	62	30	8	2	6	131	199	0.5	0.3	0.2	23	24	210	6.6	4.9	1.7	27	0.8	NIL	NIL	NIL
5	C84500	38	M	13	7200	58	36	6	12	24	100	120	0.6	0.2	0.4	13	16	134	5.2	3.2	2	20	0.6	NIL	NIL	NIL
6	C87493	51	F	10	8600	65	34	1	8	28	212	110	0.4	0.2	0.2	38	49	237	6.8	3.9	2.9	16	0.6	NIL	NIL	NIL
7	C87904	50	F	11.5	7700	70	28	2	4	12	103	128	0.5	0.3	0.2	32	33	184	7.3	5.6	1.7	14	0.5	NIL	NIL	NIL
8	C88O95	57	F	10.3	6000	60	35	5	6	30	106	122	0.6	0.3	0.3	32	33	214	6.2	4.1	2.1	18	0.5	NIL	NIL	NIL
9	C88994	56	F	11.2	5300	58	30	12	4	16	108	120	0.5	0.2	0.3	20	21	176	7.4	4.2	3.2	26	0.6	NIL	NIL	NIL
10	C89385	50	M	14	9400	75	23	2	6	14	112	182	0.6	0.4	0.2	15	17	141	5.1	3	2.1	23	0.6	NIL	NIL	NIL
11	C89311	42	F	10	6400	61	35	4	6	20	108	103	0.5	0.3	0.2	21	23	213	6	3	3	30	1.1	NIL	NIL	NIL
12	C92412	41	M	10	5900	56	40	4	6	12	128	131	0.7	0.4	0.3	12	14	145	5.6	2.9	2.7	0.5	3.2	NIL	NIL	NIL
13	C92933	40	F	11.5	6000	71	25	4	10	20	82	136	0.4	0.2	0.2	28	29	179	7.5	5.5	2	28	0.6	NIL	NIL	NIL
14	C93005	45	F	10.8	7400	65	26	9	6	14	140	238	0.5	0.3	0.2	19	21	179	6.5	4	2.5	18	0.5	NIL	NIL	NIL
15	C91627	35	M	11	6800	58	36	6	8	24	88	101	0.3	0.2	0.1	16	18	159	7.5	4.5	3	29	1	NIL	NIL	NIL
16	B88419	55	M	12.3	5600	74	20	6	9	26	122	136	0.5	0.3	0.2	35	37	175	7.5	5.5	2	15	0.4	NIL	NIL	NIL
17	U3205	36	F	11	6000	76	20	4	7	28	105	128	16	0.6	0.4	20	21	125	6.4	4.2	2.2	16	0.6	NIL	NIL	NIL
18	D007816	54	F	10	7800	59	44	7	2	8	95	123	0.5	0.3	0.2	29	30	216	6.9	3.5	3.4	17	0.6	NIL	NIL	NIL
19	C97096	58	F	11	5600	53	45	2	10	26	96	117	0.5	0.2	0.3	12	14	181	7	5.6	7.4	20	0.6	NIL	NIL	NIL
20	D010184	42	M	14	8000	64	30	6	6	4	139	175	0.5	0.3	0.2	21	23	136	6.5	4.2	2.3	21	0.6	NIL	NIL	NIL

PROGNOSIS IN SYMPTOMS OF RENAL CALCULI

OPD/IPD	AGE	SEX	BT pain	ATpain	BTFRE	ATFRE	BTDY	ATDYS	BTOLIG	ATOLIG	BTBURN	ATBURN	BTHAE	ATHAE	BTNAU	ATNAU	BTVOM	ATVOM	BTFEVER	ATFEVER
C80114	36	F	YES	NO	YES	NO	YES	NO	NO	NO	YES	NO	NO	NO	YES	NO	YES	NO	NO	NO
C79916	46	M	YES	NO	NO	NO	NO	NO	NO	NO	YES	NO	YES	NO	YES	NO	NO	NO	NO	NO
C81077	51	F	YES	NO	NO	NO	YES	YES	YES	NO	YES	NO	NO	NO	YES	NO	NO	NO	NO	NO
C81387	37	M	YES	NO	YES	NO	YES	NO	NO	NO	YES	NO	YES	NO	NO	NO	NO	NO	NO	NO
C85062	36	M	YES	YES	YES	YES	NO	NO	NO	NO	YES	NO	NO	NO	YES	NO	NO	NO	YES	NO
C86007	35	M	YES	NO	NO	NO	NO	NO	NO	NO	YES	NO	NO	NO	YES	NO	YES	YES	NO	NO
C87501	45	F	NO	NO	YES	NO	NO	NO	NO	NO	YES	YES	NO	NO	YES	NO	YES	NO	NO	NO
C79067	29	M	YES	YES	NO	NO	YES	YES	NO	YES	YES	YES	YES	YES	YES	NO	NO	NO	NO	NO
C89477	55	F	NO	NO	yes	NO	YES	NO	YES	NO	YES	NO	YES	NO	NO	NO	YES	NO	YES	NO
C66643	25	M	YES	YES	NO	NO	YES	NO	YES	NO	YES	YES	NO	NO	NO	NO	NO	NO	NO	NO
C89987	29	F	YES	NO	NO	YES	YES	NO	NO	NO	YES	YES	NO	NO	YES	NO	NO	NO	NO	NO
C89500	27	F	YES	NO	YES	NO	NO	NO	NO	NO	NO	NO	NO	YES	YES	NO	NO	NO	YES	NO
C21280	31	F	YES	NO	YES	NO	NO	NO	NO	NO	YES	NO	NO	NO	YES	NO	NO	NO	NO	NO
C87193	35	F	YES	NO	NO	NO	NO	NO	NO	NO	YES	YES	YES	NO	YES	NO	YES	NO	NO	NO
5017	45	M	NO	NO	YES	NO	YES	NO	NO	NO	YES	NO	NO	NO	NO	NO	NO	NO	YES	NO
C77638	45	M	NO	NO	YES	NO	YES	NO	NO	NO	YES	NO	NO	NO	YES	NO	NO	NO	YES	NO
C88424	38	M	YES	NO	YES	NO	YES	NO	NO	NO	YES	NO	YES	NO	YES	NO	YES	YES	YES	NO
C79579	36	M	YES	NO	NO	NO	NO	NO	NO	NO	YES	YES	NO	NO	YES	YES	YES	NO	NO	NO
C83911	35	F	YES	NO	NO	NO	YES	NO	NO	NO	YES	NO	NO	NO	YES	NO	NO	NO	NO	NO
C89260	52	M	YES	YES	YES	NO	NO	NO	NO	NO	YES	NO	NO	NO	YES	NO	NO	NO	NO	NO

RENAL CALCULI INVESTIGATIONS BEFORE TREATMENT

s.no	OPD/ IPD	A	S	Hb	TC	DC			ESR		LFT											Blood sug		B.U	CREA	urine		
						P	L	E	1/2hr	1	Bilirubin			Sgot	Sgpt	S.alk	t.pro	s.alb	s.glob	s.cal	s.pho	SU.A	FBS	PPBS			sug	alb	dep
											T	D	I.D															
1	C80114	36	F	11.8	7600	60	34	6	10	22	0.4	0.3	0.2	25	26	128	6	4.2	1.8	11	3.6	2.5	105	130	19	0.4	nil	Nil	nil
2	C79916	46	M	14.6	6700	45	48	7	2	4	0.4	0.2	0.2	14	16	102	6.9	4	2.9	11	3.2	5.8	111	145	22	0.6	Nil	Nil	Nil
3	C81077	51	F	12.4	8000	62	32	6	2	4	0.5	0.2	0.3	29	30	216	6.9	3.5	3.4	12	3.4	4.2	96	126	34	0.4	Nil	Nil	Nil
4	C81387	37	M	15.1	8700	70	24	6	4	16	0.5	0.2	0.3	12	14	181	7	5.6	7.4	11	3.8	6.1	98	134	28	0.3	Nil	Nil	cao
5	C85062	36	M	14.5	6700	57	36	5	12	26	0.5	0.2	0.3	26	36	195	6.6	4.5	2.1	11	3	5	87	111	22	0.4	Nil	Nil	Nil
6	C86007	35	M	14.7	8100	62	35	3	2	4	0.5	0.2	0.3	33	35	176	7.6	5	2.6	11	4.1	4.8	99	254	24	0.2	Nil	Nil	Nil
7	C87501	45	F	12.9	10900	73	23	3	10	26	0.5	0.2	0.3	20	24	216	6.6	4.4	3.2	9	4.1	7	103	135	32	0.4	Nil	Nil	P
8	C79067	29	M	15.2	8000	60	35	5	4	10	0.4	0.2	0.2	27	32	199	6.5	4.3	3.3	9	3.6	3.9	100	135	26	0.5	Nil	Nil	Nil
9	C89477	55	F	10	8000	62	35	3	6	12	0.8	0.6	0.2	32	30	200	7	5	2	9	4	2.7	80	110	22	0.5	Nil	Nil	cao
10	C66643	25	M	10	7200	68	30	2	4	12	0.5	0.3	0.2	38	28	194	7.5	4.2	3.3	10	3.5	5.1	99	135	38	0.2	Nil	Nil	Nil
11	C89987	29	F	13.3	7200	70	27	3	4	10	0.5	0.2	0.3	14	15	165	7.2	5.2	2	11	3.2	6	137	154	15	0.4	Nil	Nil	Nil
12	C89500	27	F	12.6	12300	65	30	5	2	6	0.4	0.2	0.2	27	28	181	7.4	5.3	2.1	10	3	6.2	96	132	19	0.6	Nil	nil	Nil
13	C21280	31	F	13.7	11800	56	28	14	2	4	0.8	0.3	0.5	18	20	140	5.5	3.2	2.3	10	3	5.4	97	114	19	0.5	Nil	Nil	Nil
14	C87193	35	F	13	8400	65	34	1	4	12	0.6	0.4	0.3	34	26	156	6.3	4	2.8	9	3.2	3.2	122	145	24	0.5	Nil	Nil	Nil
15	5017	45	M	14	7700	58	40	2	2	6	0.6	0.5	0.5	38	30	278	7.1	4.1	3.8	8.6	3.1	6.6	101	133	24	0.5	Nil	Nil	Nil
16	C77638	45	M	14.6	8700	64	35	1	4	8	0.8	0.6	0.2	23	30	176	6.6	4.8	3.1	11	3	5.3	102	135	22	0.6	Nil	Nil	Nil
17	C88424	38	M	13	7500	62	37	1	2	10	0.5	0.2	0.3	28	32	146	6.3	4	4.2	11	3.8	4.2	90	120	20	0.8	Nil	Nil	cao
18	C79579	36	M	16.3	7300	53	41	6	2	5	0.5	0.3	0.2	24	26	149	7	4	3	11	4	6.1	167	387	18	0.5	Nil	Nil	Nil
19	C83911	35	F	8.6	6600	64	34	2	2	10	0.8	0.4	0.2	33	23	150	7.5	4.2	3.3	10	4.2	5	100	140	25	0.9	Nil	Nil	Nil
20	C89260	52	M	13	6400	70	28	2	6	18	0.6	0.3	0.3	30	27	154	7	4	3	9.5	11	4	90	110	31	0.9	nil	nil	nil

RENAL CALCULI INVESTIGATIONS AFTER TREATMENT

s.no	OPD/IPD	A	S	Hb	TC	DC			ESR		LFT								s.ca	s.p	U.A	B.glucose		urea	crea	urine		
						P	L	E	1/2	1	Bilirubin			SGOT	SGPT	s.alk	t.pro	s.alb	s.glob				FBS	PPB			sug	alb	dep
											T	D	I.D															
																										Nil	Nil	nil
1	C80114	36	F	12.4	6800	52	42	6	2	4	0.5	0.2	0.3	24	25	152	6.7	4.6	2.1	9	4	5	104	127	18	0.4	Nil	Nil	nil
2	C79916	46	M	15.3	7600	45	30	15	4	8	0.9	0.3	0.6	46	39	198	6.7	3.9	2.8	11	3.4	4	99	114	14	0.4	Nil	Nil	nil
3	C81077	51	F	16.3	7300	53	41	6	2	4	0.5	0.3	0.2	24	26	149	7	4	3	11	5	4	87	130	18	0.5	Nil	nil	nil
4	C81387	37	M	15	8700	68	26	6	6	10	0.6	0.3	0.3	15	18	190	7.2	5.1	2.9	9	4	6	101	135	22	0.6	Nil	nil	Nil
5	C85062	36	M	15	6800	57	36	5	12	20	0.7	0.4	0.3	28	38	198	6.6	4.5	2.1	11	3.5	7	82	115	25	0.6	Nil	nil	Nil
6	C86007	35	M	13	10000	72	24	2	10	14	0.6	0.4	0.2	20	24	176	6.8	4.3	2.5	8	3.8	5	89	124	23	0.8	Nil	nil	Nil
7	C87501	45	F	13.5	7200	68	24	8	8	16	0.8	0.6	0.2	32	35	168	7	5	2	7	3.9	4	90	130	20	0.9	Nil	nil	nil
8	C79067	29	M	12	6800	65	32	3	6	12	0.8	0.4	0.4	32	33	195	7.6	4.3	3.3	7.5	3.4	4.5	100	140	34	1.1	Nil	nil	nil
9	C89477	55	F	11	6500	69	56	6	6	14	0.7	0.4	0.3	37	23	164	7.8	5	3.8	8	3.7	5	111	132	20	0.9	Nil	nil	nil
10	C66643	25	M	12.6	8000	60	38	2	8	16	1	0.8	0.2	29	34	172	6.4	4.2	2.2	8.9	4.1	5.2	99	129	29	0.6	Nil	nil	nil
11	C89987	29	F	14	6900	62	32	6	12	18	0.9	0.5	0.4	45	35	192	6.8	4	2.8	9.2	4.5	5.4	90	123	21	0.9	Nil	nil	nil
12	C89500	27	F	14	7200	70	23	7	14	22	0.7	0.4	0.3	36	26	180	8	4	4	10	5.1	3.9	94	139	29	0.6	Nil	Nil	nil
13	C21280	31	F	11	5400	65	32	3	6	18	0.3	0.1	0.2	27	16	200	9.9	3.9	3	8.6	3	3.2	82	123	34	1.1	nil	Nil	Nil
14	C87193	35	F	14	6200	69	27	4	2	6	0.6	0.4	0.2	20	24	96	6.7	4.2	4.3	8.4	3.8	2.8	94	134	20	0.3	Nil	nil	Nil
15	5017	45	M	13.2	7400	50	44	6	4	8	0.7	0.3	0.4	21	18	125	7.5	5	2.5	9.2	4	2.9	102	145	36	0.8	Nil	Nil	Nil
16	C77638	45	M	10.9	5800	53	45	2	2	4	0.4	0.2	0.2	38	34	156	6.4	4	2.4	10	4.2	4.3	90	134	38	0.9	Nil	Nil	Nil
17	C88424	38	M	11.6	6100	64	30	6	4	10	0.5	0.2	0.4	36	22	164	5.1	2.6	2.1	9.7	3.3	6.1	99	128	24	1.2	Nil	Nil	Nil
18	C79579	36	M	13.4	7300	62	34	4	10	12	0.6	0.2	0.4	29	26	148	6.9	4.9	2	8.9	4	5.8	111	146	28	0.7	Nil	Nil	Nil
19	C83911	35	F	15	5500	45	42	3	6	18	0.4	0.2	0.2	23	22	132	5.2	3.2	2	10	3.8	3.2	87	138	37	1	Nil	Nil	Nil
20	C89260	52	M	16	60000	71	24	5	8	10	0.6	0.3	0.3	34	28	199	6.6	4.9	1.7	10	3.3	4.3	90	148	25	0.7	nil	nil	nil

CERTIFICATE

This is to certify that the project title: Pre clinical study on Amukkara kizhangu choornam for hypolipidemic activity in the management of athimetham (Hyperlipidemia)" has been approved by the IAEC with the reference number. XIII/VELS/PCOL/36/2000/CPCSEA/IAEC/08.08.12

Name of Member Secretary IAEC:

Dr. J. Anbu

Name of CPCSEA nominee:

Dr. K. Sadhasivan Pillai

Signature with date

08/08/12

Member Secretary of IAEC

Dr. J.ANBU, M.Pharm., Ph.D., D.M.L.T.,MBA.
Professor & Head
Department of Pharmacology & Toxicology
School of Pharmaceutical Sciences
Vels University
Pallavaram, Chennai-600 117.

CERTIFICATE

This is to certify that the project title: "Preclinical study on sarva noi linga chenduram for lithotriptic activity in the management of kalladaippu (Urolithiasis)." has been approved by the IAEC with the reference number. XIII/VELS/PCOL/37/2000/CPCSEA/IAEC/08.08.12

Name of Member Secretary IAEC:

Dr. J. Anbu

Name of CPCSEA nominee:

Dr. K. Sadhasivan Pillai

Signature with date

J. Anbu 08/08/12

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SREE METRO DIAGNOSTIC CENTRE

A new era in reference laboratory

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Name : P0037635 **Mr. BALASUPRAMANIYAN (M)**

SID.No.: **013967**

Ref.by : **Dr.A. PUNITHA.M.D.,(S).**

Sample Dt: 17/09/2012
Report Dt: 21/09/2012
Time : 19:12:53
Page No : 1

Test	Result	Reference Value

LABORATORY REPORT

URINE - BIOCHEMISTRY

STONE ANALYSIS

Calcium	: PRESENT	
Carbonate	: ABSENT	
Phosphorous	: PRESENT	
Oxalate	: PRESENT	
Uric Acid	: ABSENT	
Cystine	: ABSENT	

P. Kalidasan. M.Sc., M.L.T.,
Lab Incharge

Dr. M. BALAMURUGAN. M.D., (PATHO)
Consultant Pathologist

* End Of Report *

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E-mail : sreemetrolab@gmail.com
website :www.sreemetrodiagnosticcentre.com

Name : P0037637 **Mr. SAKTHIVEL (38/M)**

SID.No.: **013969**

Ref.by : **Dr.A. PUNITHA.M.D.,(S).**

Sample Dt: 17/09/2012
Report Dt: 21/09/2012
Time : 19:12:59
Page No : 1

Test	Result	Reference Value

LABORATORY REPORT

URINE - BIOCHEMISTRY

STONE ANALYSIS

Calcium	: PRESENT	
Carbonate	: ABSENT	
Phosphorous	: PRESENT	
Oxalate	: PRESENT	
Uric Acid	: PRESENT	
Cystine	: ABSENT	

P. Kalidasan. M.Sc., M.L.T.,
Lab Incharge

Dr. M. BALAMURUGAN. M.D., (PATHO)
Consultant Pathologist

* End Of Report *

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