PRECLINICAL SAFETY EVALUATION OF

KOTHANTHI THALAGA PARPAM

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Chennai – 47.

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**Preclinical safety evaluation of "*KOTHANTHI THALAGA PARPAM*"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. R. Madhavan, M.D(S). Ph.D., Department of Nanju Maruthuvam, National Institute of Siddha, Chennai -47**, and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or another similar title.

Date: **Signature of the Candidate**

Place: Chennai **Dr. R. Aarthi**

BONAFIDE CERTIFICATE

Certified that I have gone through the dissertation submitted by **Dr. R.AARTHI, (Reg. No: 321516201)** a student of final year M.D(s), Branch-VI, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai-47, and the dissertation work has been carried out by the individual only. This dissertation does not represent or reproduce the dissertation submitted and approved earlier.

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1.INTRODUCTION

Siddha medicine is a form of south Indian traditional medicine which is popular in ancient India. These systems providing preventive, promotive, curative, rejuvenating and rehabilitative health care by adopting scientific and holistic approach.

Nowadays, many people are choosing Siddha medicines to improve theirhealth conditions or as curative substance either alone or in combination with others.[1]Traditional Medicine has played an important role in meeting the demands of primary health care in many developing countries and its use has expanded widely in many developed countries.[2]

The raw materials used in siddha medicines are plants, metals, minerals & animal origin. The extensive use of Herbo mineral formulations is considered as high order. The preparation of majority of medicine involves tedious processes which resulting physio-chemical transformation of particle size, chemical composition, which regulate the bio-mechanism of the drug in body.

Arsenic (As) is a highly toxic metalloid. Metalloid is intermediate state between those of metals and non-metals. As is commonly known as a poison. Only a few people know that as has also been widely used in medicine.

The international agency for research on cancer (IARC) has recognized arsenic as an element with carcinogenic effect evidenced by epidemiological studies and also used in the treatment of neoplastic diseases[3].

“All substances are poison; there is none, which is not a poison”

“There is no such thing as poison; It all depend on the dose”

-Paracelsus

The right dose differentiates a poison from a remedy[4].

In Tamil text “**Shiva Purana**” parkadal kadaithal- churning the milky ocean initially, came poison and then came many virtuous things.

In siddha system, there are some highly poisonous substances and heavy metals are used for preparation of drugs. They are purified by specific suththi methods. suththi is the technical method to reduce or remove the toxic effect of these minerals.

There are several suththi methods mentioned in siddha literature it may or may not be a single process. the technical expertise (suththi) of neutralization of the poisonous quality of them is highly advanced and marvelous. After neutralizing by various processes, they are used for treatment[5].

Unique preparation of the siddha system of medicine like Parpam, Chendhooram, Chunnam, Kattu, Paddangam, are life saving and miracle nano medicine which were prepared by the siddhars on the basis of nano technology[6].

"In **Thirumanthiram** Siddhar Thirumoolar mentioned about the atomic theory in 7th thanthiram verse, 2011 which is led to the nano science. In many places he used the word "ANU" which refers to atom.

"அணுவில் அணுவினை ஆதிப் பிரானை
அணுவில் அணுவினை ஆயிரங் கூறிட்டு
அணுவில் அணுவை அணுக வல்லார்கட்கு
அணுவில் அணுவை அணுகலு மாமே."

(திருமந்திரம்1971)

In siddha system of medicine is commonly used formulations are Parpam (minerals/metallic oxides), Chendhooram (mineral/metallic sulphides), Chunnam (caustic or major oxides) and Pathangam(sublimation). Among them Parpam and Chendhooram type of medicines are widely used, having potential therapeutic values[7].

World Health Organization (WHO) stresses the importance of the qualitative and quantitative methods for characterizing the samples, quantification of the biomarkers and/ or chemical markers and the fingerprint profiles. Therefore, a multidimensional approach is essential for standardization of traditional medicine[8].

Parpam is one of the 32 types of internal medicines in siddha system of medicine. parpam is an oxidized product of metals or minerals, which are free from metallic residue of its parent substances.It is considered to be a very potent form of drug having extensive shelf life up to 100 years and it provides speedy recovery from many of the chronic illnesses[9].

Kothanthi thalaga parpam, a Herbo -mineral drug consists of heavy metal thalagam and nayuruvi sambal. It is used to cure various ailments like Swasakaasam, Irumal, suram[10].

Kothanthi thalaga parpam is used in clinical practice for the management of kabam related disorders (Respiratory disorders) cough, bronchial asthma & all type of fever. this particular drug specifically acts on respiratory system. In pandemic situation like covid 19, and acute respiratory syndrome like diseaseses may treat with kothanthi

thalaga parpam. but there is less scientific background for standardization and safety of it till now.

Toxicological screening is very important for the extension of therapeutic potential of *kothanthi thalaga parpam*. This study is aimed to do safety profile of *kothanthathi thalaga parpam* for global acceptance through toxicity study in acute and 28 days repeated dose toxicity study in Wister rats.

2. AIM & OBJECTIVE

AIM:

To evaluate the safety profile of **"*Kothanthi Thalaga Parpam"*** in Wister albino rats as per OECD guidelines (423&407)

BJECTIVE:

- Collect the literature review of ingredients of test drug.
- Collection and identification of the test drug.
- Purification and preparation of medicine as per – literature.
- To analyse the Physicochemical and biochemical properties of the test drug.
- To analyze quantitative and qualitative test of test drug by ICP-OES, SEM-EDS, XRD.
- To study Acute toxicity profile of drug as per OECD guideline 423.
- To study Sub acute toxicity profile of drug as per OECD guideline 407.

3.LITERATURE REVIEW

3.1.தாளகம்

வேறுபெயர்:

கோதந்தம், மால்தேவி, அரிதாரம், அரிதளம், பீதகி, ஆலம்பி, பிஞ்சனம், பழுப்பு, மாலம், கால்புத்தி, மஞ்சள்வர்ணி. பொன்வர்ணி,

வகைகள்:

சிவந்த அரிதாரம், மடல் அரிதாரம், பொன் அரிதாரம், கரட்டுத் தாளகம்

செய்கை:

உடல்தேற்றி, சுரமகற்றி, கோழையகற்றி, வாந்தியுண்டாக்கி, நச்சரி

பொதுக்குணம்:

“தாளகத்தின் பேருரைக்கத் தாலுகவுள் நோய்குட்டம்

நீளக் குளிர்காய்ச்சல் நீடுகபம் - நாளங்கொள்

துட்டப் பறங்கிப்புண் சூழழுகண் மண்டைநோய்

கிட்டப் படுபமா கிளந்து”.

தாளகத்தினால் நாக்கு, கபாலம்இவைகளைப் பற்றியநோய், குட்டம், குளிர்சுரம், கபம், மூத்திரநாளத்தைப் பற்றிய பறங்கிப்புண், அழுகண், மண்டைநோய் முதலியன நீங்கும்.

சிவந்த அரிதாரம்:

“சிவந்தவரி தாரமது செஞ்சிலைப்போற் காட்டும்

உவந்ததனை யுண்முறையோ டுண்டால் - இவர்ந்தாழ்

சுரங்குளிர்மா வாதமுடற் சூலைநமை குட்டம்

இரங்குமென நாளும் இசை".

சிவந்த அரிதாரத்தால் சுரத்துடன் கூடிய குளிர், பெரிய வாதநோய், உடலில் குத்தல்,

நமைச்சல், குட்டம் முதலியன நீங்கும்.

மடல் அரிதாரம்:

"மடலரி தாரத்தில் வருங்கர டிரண்டும்

உடல்வி டங்களைப் போக்கிடும் உண்மை"

மடல் அரிதாரத்தினால் ஈளை, இருமல், கோழை, மலம் நீங்கும். இது வன்மையுள்ள இளைப்பு நோய், கரப்பான், ஆறாதபுண் இவற்றையும் போக்கும்.

பொன் அரிதாரம்:

"மந்தாரத் தாலே வளருஞ் சுவாசநோய்

உந்திவரு தீச்சுரநோய் ஓடுங்காண் - முந்து

தொனிக்கயஞ்செய் யாங்கடியுந் தோற் குட்டம் ஏகுந்

தனிப்பொன் அரிதாரத் தால்".

பொன் அரிதாரத்தால் சுவாசகாசம், கொடியசுரம், இளைப்பு, செய்யாங்கடி, தோலைப்பற்றிய குட்டம் முதலியன நீங்கும்.

சுத்தி:

❖ தாளகத்தை பணம் போல வெட்டி சீலையில் முடிந்து கோமயம், காடி, சுண்ணநீர், பூசணிக்காய்நீர், ஆவின்பால், அரசம்பட்டை கியாழம் இவைகள் ஒவ்வொன்றிலும் தனித்தனியாய் ஊறவைத்து தோலாந்திரமாக நீர் முக்கால் பாகம் சுண்டும் வரை அவித்து எடுக்கச் சுத்தியாகும்.ஒரு பலம் தாளகத்திற்கு ஒவ்வொறு படி நீர் பொருள் எடுத்துக்கொள்ள வேண்டும்.

❖ ஒரு பலம் தாளகக் கட்டியை எடுத்து சுண்ணாம்புக் கல்லின் இடையில் வைத்து பனங்கள்ளிணால் 10 தரத்துக்கு குறையாமல் தாளித்து எடுத்துக் கழுவி உலர்த்திக் கொள்ளவும்.

❖ தாளகத்தை சன்னமாக வெட்டி இரட்டை மடிப்பு சீலையில் கட்டி கோமூத்திரம், அரிசிகழுவிய நீர், புளித்த காடி இவைகளில் ஒன்றால் 3 நாட்கள் தோலாந்திரமாய் கமலாக்கினிக்கொண்டு எரிக்க சுத்தியமாகும்.

❖ அமுரி ஒரு படியில் குப்பைமேனிச்சாறு கால்படி, கற்சுண்ணம் கால்படி கலந்து அடுப்பேற்றி தாளகத்தை கிழிகட்டி தோலாந்திரமாய் எடுத்துக் கொள்ள சுத்தியமாகும்.

❖ தாளகத்தை சுண்ணத்திலிட்டு கழுதைநீரிட்டு தாளித்து எடுக்க சுத்தியமாகும்.

பயன்கள்:

❖ தாளகம் சுரத்தையும், தோலைப்பற்றிய பிணிகளையும் போக்கி, உடலுக்கு வன்மை, அழுகு, ஆயுள், முதலியவற்றைக் கொடுக்கும்.

❖ தாளகத்தை இரசம், நாபி முதலியவற்றுடன் கலந்து மருந்தாக்கி சுரத்திற்குக் கொடுப்பதுண்டு.

❖ தோற்பிணிகளை நீக்க வெளிபிரயோகத்திற்காக செய்யும் மருந்துகளில் சேரும் உரோமத்தை நீக்க இது சுண்ணாம்புடண் கலந்து பயன்படுத்துவது வழக்கம்.

தாளகத்தின் நஞ்சிக் குறிகுணங்கள்:

தாளகம் விடமித்தால் நகக்கண்களில் இரத்தம் சுவறதல், பக்குக்கட்டிப் புண்ணாகிச் சீழ் வடிதல், வயிற்றில் எரிச்சல், குரல் மாறல், மூக்கிலிருந்து இரத்தம் பாய்தல், அருசி, உணவு வேண்டாமை, தலையில் நமை உண்டாகி மயிர்முனை சிவத்தல், மேல்சுவாசத்தை கிளப்பிப் பந்திக்கச் செய்தல், பிரமை, அடிவயிற்றில் வீக்கசாயலைக் காட்டல் , இடை, பக்கம் இவைகளில் தீராத வலி ஆகிய குறிகுணங்கள் உண்டாகும்.

நஞ்சு முறிவு:

❖ சித்திரமூலவேர்பட்டை, மிளகு, கறியுப்பு இவைகளில் முதல் இரண்டும் கால் பலம், மூண்றாவது 1/8 பலமும் கூட்டி, முன்னிரண்டை குடிநீரிட்டு கறியுப்பைச் சேர்த்துக் காலை மாலைகளில் நோயின் விடத் தன்மைக்கு ஏற்ப அரைமண்டலமாவது முக்கால் அல்லது ஒரு மண்டலமாவது உட்கொள்ளத் தீரும்.

❖ நீலிவேர், ஆவாரம்பூ, வெட்டிவேர், சீரகம், மாதுளைவித்து, தென்னங்குரும்பை, காசினிவேர் இவைகளை வகைக்கு கால்பலம் கூட்டிக் குடிநீர் செய்து அருந்த தாளகத்தால் விளையும் நஞ்சு தீரும்.[11]

தாளகம் சேரும் பிறமருந்துகள்:

❖ **சயகுலாந்தக செந்தூரம்**[12]

அனுபானம்: தேன், திரிகடுகு

தீரும் நோய்கள்: சயம், காசம், சுவாசம், கபநோய்கள்

❖ **இரசகந்தி மெழுகு**[12]

அளவு: சுண்டைக்காயளவு

தீரும் நோய்கள்: சூலை, விப்புருதி, புலுவெட்டு, கண்டமாலை, சிரங்கு, கிரந்தி, புண், புற்று, பவுத்திரம், மூலம், குட்டம், சொறி, இரணம், வெள்ளை, கருமேகம், வாதம், சயம், குன்மம்.

❖ **சூலைகுடாரம்**[12]

அனுபானம்: இஞ்சிசாறு

தீரும் நோய்கள்: குன்மம், சூலை.

❖ **சஞ்சீவி மாத்திரை**[12]

அளவு: குண்றிமணி

அனுபானம்: சாதிக்காய், குடிநீர், சிறுநீர், பழச்சாறு, நொச்சிச் சாறு, வெள்ளாட்டுப் பால்.

தீரும் நோய்கள்: மூலம், வாயு, பாம்புகடி, குன்மம், சூலை, கயம்.

❖ **பிரம்மானந்த பைரவம்**[12]

அளவு: மிளகளவு

அனுபானம்: தேன், இஞ்சிசாறு

தீரும் நோய்கள்: சுரம், சன்னி.

❖ **திரிமூர்த்தி செந்தூரம்**[13]

அளவு: ½ முதல் 1 குண்றிமணி அளவு

அனுபானம்: தேன், நெய், வெண்ணெய், தாளிசபத்திரி இளகம், பஞ்சதீபக்கினி இளகம், கூழ்ப்பாண்ட இளகம்.

தீரும் நோய்கள்: கழிச்சல், கபநோய்கள், மூலம், சுரம், வயிற்றுவலி

❖ **கனகலிங்க மெழுகு**[13]

அளவு: ½ முதல் 1 குண்றிமணி அளவு

அனுபானம்: பனைவெல்லம், சர்க்கரை, பாலேடு, வெண்ணெய்

தீரும் நோய்கள்: வெட்டை, புண்கள், சூலை, வாயு, சூதகவாயு, வயிற்றுவலி, சன்னிபாதம், முடக்குவாதம், நீர்க்கடுப்பு, பிலவை, 7 வகை வலிப்பு.

❖ **வான்மெழுகு**[12]

அளவு: உளுந்தளவு

அனுபானம்: பனைவெல்லம்.

தீரும் நோய்கள்: கபவாதசன்னி, 8 வகைசுரம், காசம், சோகை, சூலை, இரணம், ஈளை, தோடம், 8 வகைகுன்மம், சூதகக்கட்டு, சோகை, காமாலை, பவுத்திரம், கல்லடைப்பு, நீரிழிவு.

❖ **கஸ்தூரிக் கருப்பு**[12]

அளவு: ½ முதல் 1 குண்றிமணி அளவு

அனுபானம்: தேன், முலைப்பால், இஞ்சிச்சாறு.

தீரும் நோய்கள்: சளி, சுரம், இருமல், இரைப்பு.

❖ **பஞ்சமுக செந்தூரம்**[13]

அளவு: ½ முதல் 1 அரிசி அளவு

அனுபானம்: தேன், இளகங்கள், பனைவெல்லம்.

தீரும் நோய்கள்: சுரம், இருமல், இரைப்பு, சன்னி, குன்மம்.

MODERN ASPECT OF YELLOW ARSENIC TRISULPHIDE

OTHER NAMES:

Arsenic trisulphide

Orgiment Sulphuret of arsenic

Chemical formula: $As:S_1$.

Group	:	Sulphides.
Geo.Name	:	Orpiment
Chem.Name	:	Arsenic Trisulphide
Formula	:	As_2S_3
		Sulphur- 39.0
		Arsenic- 61.0
Colour	:	Lemon yellow of several shades
Streak	:	Pale yellow
Diaphaniety	:	Sub transparent to sub translucent
pH	:	1.5 – 2
Luster	:	Pearly – Sub metallic
Cleavage	:	Highly perfect
Fracture	:	Sectile[14]

Crystallizes is the monoclinic system. Crystal rare commonly met foliated. Mica like, Granular or the earthy mass. Origin low temperature hydro thermal. It commonly associated with realgar. Other associated minerals include stibnite, native assenic, calcite, barite and gypsum.

APPEARANCE:

It is a bright yellow solid Insoluble in water.

It also occurs as the mineral orpiment (Latin: auripigment), which has been used as a pigment called King's yellow.[15]

HABIT:

Rare crystals are small, tabular, or prismatic, often poorly formed. Columnar or foliated aggregates are common.[16]

STRUCTURE AND COMPOSITION:

AsS3 pyramids share edges, producing 6-member rings. Crumpled layers of rings are stacked on top of each other.

RELATED MINERALS:

Getchellite, AsSb{3} is similar in structure to orpi ment. Realgar, ASS, is closely related in composition.[16]

VERNACULAR NAMES:

(Sans. Mah. & Ben. - Haritala. Eng.-Orpiment; Yellow sulphuret of arsenic, Yellow Arsenic trisulphide. Hind.& Duk.-Haratala. Arab-Ursanigum. Pers-Zarneik-zard. Guj-Aratal. Tam. -Arridaram; Yellikud pashanam. Tel. -Daddipashanum. Can. & Kon.-Ardala. Burm.-Hsaydan Shwaywa. Sinh.-Aridala) is found native in China and Persia.[17]

- Assam - Haritala
- Hindi - Haratal
- Kannada -Harital, Ardal
- Malayalam - Aritalam
- Gujarati - Aradal, Hartal
- Marathi - Harital
- Tamil - Aridaram
- Telugu - Doddipashanam
- Bengali - Harital
- English - Orpiment, Yellow Arsenic[18]

DESCRIPTION AND OCCURRENCE:

It is golden yellow coloured, hard substance. The layers were found to form a hard mass. It has soft surface. It is a chemical compound of arsenic and sulphur. The name orpiment originated from Latin word Ori pigment. These kings yellow have 69% of arsenic and 31% of sulphur[19]. The chief sources of Arsenic trisulphide are Iran and Italy and it is also found in Burma and China. Countries like China, Italy, and Cicely have the mines of Orpiment. Now a days it is available all over the world[20].

Orpiment is found in volcanic environments, often together with other arsenic sulfides, mainly reafgar. It is sometimes found in low-temperature hydrothermal veins,

together with some other sulfide and sulfo salt minerals.[1]

PROPERTIES:

- **Molecular formula:** As2S3
- **Molar Mass:** 246 g/mol
- **Appearance:** Yellow-Orange monoclonic crystals
- **Odour:** Odourless
- **Density:** 3.43 at 68° F
- **Melting point:** 572 °C
- **Boiling point:** 707 °C
- **Solubility:** Soluble in alkalies, carbonates, alkali sulphides and slowly solublein hot HCl[20]

SYNTHETIC PREPARATION:

As;S; forms when aqueous solutions containing As (III) are treated with H.S. Arsenic was in the past analyzed and assayed by this reaction, which results in the precipitation of As_2S_1, which is then weighed. AsS) can even be precipitated in 6M HCL. As_2S; is so insoluble that it is not toxic.[15]

BIOLOGICAL ROLE OF YELLOW ARSENIC IN HUMAN BODIES:

It could play a role in the development of diabetes, cancer, vascular disease and lung disease.

The Food and Drug Administration says that long-term exposure to high levels of arsenic is associated with higher rates of skin cancer, bladder cancer and lung cancer, as well as heart disease.[15]

TOXICOKINETICS OF ARSENIC TRISULPHIDE:ABSORPTION:

Arsenic is absorbed through all routes including oral, inhalation and cutaneous. Average arsenic consumption unknowingly, per day ranges from ½-1 mg (contained in food and water). Arsenic is well absorbed from GIT, respiratory tract and skin. Upon ingestion, it is bound to the protein segment of Hb and α-globulins[21].

DISTRIBUTION:

Once absorbed, arsenic is immediately circulated to all the organs and tissues. Initially, arsenic is found maximally in the liver (in fatal cases >1mg %) followed by kidneys and spleen. In case if person survives, it can be found in muscles for days, in bones and in the keratin tissue, hair, nails, and skin for quite a long time. Ordinarily, the

hair contains < 2 parts/million arsenic. inorganic arsenic can cross the placenta[22].

EXCRETION:

It is excreted mainly by the kidneys, but some part through faeces, bile, sweat, milk, nails, and hair. Arsenic excreted mainly by kidney as methylated Arsenic. Mostly found in urine within ½ an hour of ingestion, then elimination is continuous for about 10-12 days[21].

MECHANISM OF ACTION:

Arsenic inhibits cellular respiration by uncoupling mitochondrial oxidative phosphorylation by combining with the sulfhydryl groups of mitochondrial enzymes, especially pyruvate dehydrogenase and certain phosphatases. So, conversion of pyruvate to acetyl CoA is decreased, citric acid cycle activity is decreased and the production of cellular ATP is decreased. It inhibits cellular glucose uptake, gluconeogenesis, fatty acid oxidation and further production of acetyl CoA. Locally, itcauses irritation of the mucous membranes, and remotely, depression of the nervous system. Arsenic is a carcinogenic substance since lung, skin, and bladder (transitional cell) carcinoma has been observed in populations with multiple exposures.

FATAL DOSE:

Toxicity in Children - 2 mg/kg of arsenic trioxide Toxicity in Adults - 120-250mg/kg of arsenic trioxide

FATAL PERIOD: 1-2 days[23]

TOLERANCE:

Some people take arsenic daily as an aphrodisiac, and they acquire tolerance up to 0.3 or more than one dose. Such people are known as Arsenophagists[21].

ARSENIC POISONING:

ACUTE ARSENIC POISONING:

- Arsenic, in the form of AS2O3 used to be a common cause of poisoning becauseit is readily available. Particular it is tasteless and has the appearance of sugar.
- Gastrointestinal discomfort is usually experienced within an hour after intake ofthe arsenicals. Although it may be delayed as much as12 hours, after oral ingestion, if food is in stomach.
- Burning lips, constriction of the throat and difficulty in swallowing may be thefirst symptoms followed by excruciating gastric pain, projectile vomiting

and severe diarrhoea.

- Oliguria with proteinuria and haematuria is usually present. Eventually anuria may occur
- The patient often complains marked skeletal muscle cramps and severe thirst. As the loss of fluids proceed, symptoms of shock appear.
- Hypoxic convulsion may occur terminally then coma and death can occur withinan hour. But usual interval is 24 hours.

CHRONIC ARSENIC POISONING:

- The most common early sign of arsenic poisoning is seen in muscle of the neck,eyelids, nipples and axillae.
- Hyperkeratosis weaknesses and aching, skin pigmentation specially.
- Gastro - intestinal involvement is less prominent in chronic exposures.
- Other signs and symptoms that should arose suspicion of arsenic poisoninginclude garlic odour of the breath and perspiration.
- Excessive salivation and sweating stomatitis.
- Generalized itching, sorethroat, coryza, lacrimation, numbness, burning ortingling of the extremities.
- Dermatitis, vitiligo and alopecia, poisoning may begin insidiously with symptoms of weakness, longer anorexia, occasional nausea and vomiting, diarrhoea or constipation.
- Eventually the cirrhosis of liver may occur form the hepato-toxic action.
- Peripheral neuritis results in motor and sensory paralysis of the extremitiesusually the legs are more severely affected than the arms.
- The bone marrow is seriously injured by arsenic. There is severe explosion ofall hematological pathways may be affected and causes hyperkeratosis.
- Eventually those actions proceed to atrophy, degeneration and there is possibleto develop cancer.
- Skin eruptions are common in patients who received inorganic arsenic medication[22].

TREATMENT:

ACUTE ARSENIC POISONING TREATMENT:

Gastric Lavage- the stomach should be repeatedly washout with warm water and milk to remove arsenic particles adherent to the mucous membrane of the stomach

Then freshly precipitated hydrated ferric oxide orally in small dose convert toxic arsenic to non-toxic ferric arsenite. If ferric oxide cannot be quickly prepared, calcined magnesia or charcoal may be substituted, Butter and greasysubstances are useful to prevent absorption

The systemic effects should be treated by intramuscular injection of B.A.L. (Dimercaprol) in an oily solution, Parenteral fluid should be administered to counteract dehydration, glucose to combat liver damage, sodium bicarbonate to regulate acid-base balance, Hemodialysis or exchange transfusion may be givenin cases of renal failure.

CHRONIC ARSENIC POISONING TREATMENT:

- The treatment of chronic poisoning consists of removal of the patient from further exposure
- Dimercaprol (B.A.L.) has greatly improved the prognosis, Vitamin B-complex,Intravenous sodium thiosulphate is useful, Complete recovery may require sixmonths to one year[25].

ANTIDOTE:

Common treatment for arsenic poison:

There is no specific method used to treat arsenic poisoning. The best way to treat the condition is to eliminate arsenic exposure. Full recovery may not happen for weeks or months. It all depends on how long you have been exposed.

Vitamin E and selenium supplements have been used as alternative remedies to limit the effect of arsenic exposure

Pottassium supplemental decreases the risk of experienceing a life-threatening heart rhythm problem from arsenic trioxide.

Chelation:

Dimercaprol and dimercaptosuceinic acid are chelating agents that sequester

the arsenic away from blood proteins and are used in treating acute poisioning.[15]

SCIENTIFIC VALIDATION OF YELLOW ARSENIC TRISULPHIDE:

❖ **Antimicrobial effect:**

Garg Lokesh Kumar et al evaluated the antimicrobial activity of Hartala Bhasma against gram positive, gram negative bacteria. Hartala Bhasma has an effective antimicrobial activity against Streptococcus pneumoniae, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in Kirby- Bauer disc diffusion method and Stoke's disc diffusion method and Dilution methods like Broth dilution method & Agar dilution method. 250mg/ml concentration of Hartala Bhasma was more effective on gram + ve bacteria[26].

❖ **Anti-convulsant Activity:**

Ajit Narayan KS et al demonstrated anticonvulsant action of Haratala Bhasma by In vivo method conducted on Albino rats[27].

❖ **Elemental analysis of Yellow Arsenic Trisulphide:**

Athimeena et al observed the change in elemental nature of unpurified and purified Thalagam using atomic absorption spectrometry (AAS) methods of analysis. Biochemical analysis for the basic radicals reveals that both unpurified and purified Thalagam contains ferrous iron, Potassium, Calcium, Arsenic,Mercury and Lead. At the same time Calcium contains only in purified samples.Unpurified and purified Thalagam contains fluoride & oxalate. Results of elemental analysis reveals the absence of cadmium in the purified sample and also significant decrease in the level of arsenic in purified sample. It was concluded that the purification process had significantly reduced the level of arsenic and also notable level of calcium observed exclusively in purified form[28].

நாயுருவி – Nayuruvi[29]

***Achvranthes aspera*, Linn.**

வேறு பெயர்: -

அபமார்க்கி, காஞ்சரி, சனம், சிகிசிரம், கதிரி, சரமஞ்சரி, சிறுகடலாடி, சுவானம், அபாமார்க்கம், நாய்குருவி, கிருஷ்ணபன்னி சகரிகம், செகரிகம், கேசரிகம், சேகரிகம், கொட்டாவி,விட்டிறுக்கி, சேகரி,நாயரஞ்சி, மாமுனி

Eng. Rough chaff or Prickly chaff, Flower Plant
Sans. Apamarga
Tel. Uttareni
Hind. Chir-chi
Mal. Kadalad
Kan. Uttaranee

இ஦்து இந்தியாவில் எங்கும் வளரக்கூடிய பூண்டு, இதில் செந்நாயுருவி என்றொரு வகையுமுள்ளது. இது "படருக்கி" என்றும் வழங்கும்.

ப-உ: செடி

சுவை-கைப்பு, துவர்ப்பு, கார்ப்பு, தன்மை-வெப்பம், பிரிவு-கார்ப்பு.

செய்கை:

துவர்ப்பி	*ஸங்கோசனகாரி*	Astringent
சிறுநீர்ப்பெருக்கி	*மூத்திரவர்த்தினி*	Diuretic
உடற்றேற்றி	*வியதாபேதகாரி*	Alterative

முறைவெப்பகற்றி *முறைவியாதிரோதி* Antiperiodic

குணம்:

வேரினால் அழகுண்டாகும். இலை, கீழ்வாய்க் குருதிப் போக்கையும், கழிச்சல், ஐயநோய், வியர்வை, வெள்ளை இவைகளையும் போக்கும் அரிசி பசியைப் போக்கும். முழுச் செடி, பிள்ளை பெற்றவர்களுக்குண்டாகும் அழுக்குத் தடையைப் போக்கும்; வங்ககத்தை சிந்தூரமாக்கும்.

மலிகாரங் கைப்புள்ள அபமார்க்கி யின்வேரால் வசிய முண்டாம்,

இலைமூல உதிரமந்தம் பேதிகபம் வியர்வுந்தி யிறங்கு மேகம்

மலையேறும் படிபுரியு முள்ளரிசி பசிமாற்றும் வனச மூலம்

பலமாதர்க் குள்ளழுக்கை நீக்குவங்கச் சிந்தூரம் பண்ணுமாதோ

செந்நாயுருவி

இதனால், வீக்கம், பாண்டு, காமாலை இவை நீங்கும், சூதகத்தைத் தடை செய்யாது வழக்கப்படி உண்டாகும்.

ஒதமுறு சோபை யுயர்பாண்டு வைப்போக்குந்

தீதறுகா மாலைநோய் தீர்க்குமினார்-சூதகநீர்

பொய்ப்புறுகா லத்தனைப் பொங்குவிக்குங் காரமொடு

கைப்புறுசெந் நாயுருவி காண்.

குறிப்பு: இவ்விரு வகைகளிலும் செந்நாயுருவி சிறப்புடையது.

வ-கு: வேர், செடியைக் குடி நீரிட்டு 19 மி.லி - 35 மி.லி வரையிலும் கொடுத்துவர, சிறுநீரைப் பெருக்கித் தள்ளும்.

வேர் ஊறல் நீர் - வயிற்று நோய்க்கு நன்று.

இலைச்சாறு, வயிற்றுவலி, சொறி இவைகளை நீக்கும்.

இலையை அரைத்து வெல்லமும் சேர்த்து அல்லது இலையுடன் மிளகு பூண்டு சேர்த்தரைத்து, சுரத்துக்குக் கொடுக்கலாம்.

இலையை நீர் விட்டரைத்துச் சிறு கடி நஞ்சுகளுக்குப் பூசலாம்.

இலைச் சாற்றை வெயிலில் வைத்து வற்றச்செய்து மெழுகு போலாக்கி அத்துடன் சிறிது அபின் சேர்த்துக் கொறுக்குப் புண்ணிற் போடப்போம்.

வேர்ப் பொடியுடன் சிறிது மிளகு பொடியும், தேனும் செர்த்துக் கொடுக்க இருமல் நீங்கும்.

வேர்ச்சாம்பல் 325 மி.கிராம் எடை வெல்லத்தில் கொடுக்க, எளிதில் பிரசவம் உண்டாகும்.

பச்சை வேரால் பல் துலக்கிவர, பல் அழுக்குகள் நீங்கி அழகுண்டாகும்.

நாயுருவி சாம்பல் தெளிநீர் 4000 கிராம் எடை, நாயுருவி சாம்பல் 250 கிராம், நல்லெண்ணெய் சேர் இவைகளைச் சேர்த்துக் காய்ச்சி வடித்துக் கொள்ளவும் இத்தைலம் செவிடு, காதிற்சீழ் முதலியவைகளை நீக்கும். மூக்கில் விட, மூக்கினின்று வடியும் குருதி போம்.

இச்சாம்பலினின்றும் மிக்க காராமான செயநீர் எடுக்கலாம். வங்கத்தை மடிக்கும் மூலிகைகளில் இ.ுதும் ஒன்று.

நாயுருவி விதையை அரிசி கழுவிய நீருடன் உட்கொண்டு வந்தால் மூலம் நீங்கும்.

விதையைப் பாயசங்களில் சேர்த்து உட்கொள்ள, மூளை நோய்களை நீக்கும். மற்றும் இதனால் அடியிற்கண்ட பலன்களைப் பெறலாம்.

சோம்புகின்ற மண்டுரஞ் சுத்தபொடி நாயுருவிச்
சாம்பல் மிளகுபொடி தான்சரியாய் - தேம்பிடவே
இட்டமுடன் கூடி யிதனிற் கழஞ்சுகொள்ள
அட்டகுன்ம மோடு மலைந்து

(பொ-ள்): நாயுருவிச் சாம்பல், மண்டுரப்பொடி, மிளகு இம் மூன்றையும் ஒரு அளவாகக் கூட்டி ஒரு கழஞ்சளவு கொள்ள, எண்வகைக்குன்ம நோய்களும் போம்.

நாயுருவி முற்றினதாய் நறுக்கி மிக்க
நற்பசுவின் கோமயத்திற் போட்டுப் பாண்டம்
வாயிலிட்டுப் பூப்புடத்திற் றயிலம் வாங்கி
வைத்ததனை வெற்றிலையில் மென்று தின்ன
நோயிற்பெரு மந்தார சுவாச காசம்
துணிக்கிருமல் ஈளைகண்டத் துடிப்பு நீங்கும்

காயமதைப் பலக்கவைக்குஞ் சித்தர் சொன்னார்

கருணையிது தப்பாது கண்டு தேறே

முற்றிய நாயுருவியை நறுக்கிப் பசு நீரில் ஊரப்போட்டு உலர்த்தி,குழித்தைலம் வடித்து,அதில் ஒன்றிரண்டு துளி வெற்றிலையில் பூசித்தின்ன,இருமல்,ஈளை,இரைப்பு நோய்களைப் போக்கி உடற்கு ஊடந்தரும்.

ACHYRANTHES ASPERA,Linn.

(N.O.-Amarantaceae)

Sans: Aghata.; Khara: manjari; Apamarga, Eng: Rough Chaff tree; Prickly chaff-flower. mon. Ben: Apang, Burm: Kune-la, Duk: Agari, Guj: Safed Aghedo, Mah. Aghada; Pan dhara-aghada, Tel: Uttaraene; Antisha; Apamargamu, Hind: Latjira; Chirchira, Tam: Shiru-kadaladi; Nayuruvi, Mal: Kada ladi; Katalati, Can, and Kon: Uttaranee, Arab:Atkumah, Pers: Khare-vazhun, Gwalior: Adharajhada, Punj: Kutri.[30]

Scientific taxonomic classification:[31]

Kingdom - Plantae

Subkingdom – Tracheobinota Super

Division – Spermatophyta

Division – Mangoliophyta

Class – Mangoliophsida

Subclass – Caryophyllidae

Order – Caryophyllales

Family – Amaranthaceae

Genus – Achyranthes

Species - aspera

Habitat: Small herb found all over India.

Parts Used: Herb, leaves, seeds and root.

Constituents: Fruit contains a large percentage of alkaline ashcontaining potash.

Action:

Astringent,

Diuretic,

Alterative and antiperiodic;

Purgative.

Emmenagogue

Preparations:

Decoction and infusion of leaves;

Khar prepared by incineration of the plant;

Powder of root, paste and medicated oil.

Uses:

- Decoction (2 oz. of the plant in 11/2 pints of water boiled for 20 minutes to half an hour and then strained) is a good diuretic found efficacious in renal dropsies "and general anasarea; one to two ounces of the mixture is given two or three times daily"; [1]
- This leaf juice is also useful in stomach ache and bowel complaints, piles, boils, skin eruptions etc.; in large doses it produces abortion or labour pains.
- "A decoction of powdered leaves with honey or sugar candy, is useful in the early stages of diarrhoea and dysentery.
- " Fresh leaves ground into a paste with jaggery or mixed with black pepper and garlic and made into pills are used as antiperiodic especially in quartan fevers;
- Leaves rubbed into a paste with water are applied with much benefit to bites of poisonous insects, wasps, bees etc.
- Fresh juice of the leaves thickened into an extract by exposure to the sun and mixed with a little opium is an efficacious application to primary syphilitic sores.
- An Infusion of the root is given as a mild astringent in bowel com plaints.
- The decoction of the plant is useful in pneumonia and renal dropsy while the juice of the plant is used in ophthalmia and dysentery.
- leaves are used as a cure for gonorrhoea, and excessive perspiration, their extract is used for leprosy and the heated sap for tetanus.
- This root is astringent, the paste is applied to clear opacity of cornea, and to wounds as an haemostatic. The root is also reported to be useful in cancer.
- A decoction of the root is used for stomach troubles and an aqueous extract for stones in the bladder. The flowers, ground and mixed with curd and sugar, are given for menorrhagia. The flower tops are stated to be employed for the treatment of rabies.

- Powdered seeds are soaked in butter milk and given for biliousness. The seeds are said to be emetic and used in hydrophobia (Nadkarni, 1954; Chopra et al., 1956; Wealth of India, 1985).

PHARMACOGNOSTIC STUDIES DONE ON *ACHYRANTHUS ASPERA*[32]

The detailed Pharmacognostic studies of the stem, leaf and root of the plant has been studied by Prasad and Bhattacharya (1961).

The root has single layered epidermis, followed by 2-5 layers of parenchymatous cortex including a distinct endodermis which shows casparian dots on the radial walls. The pericycle is represented by single layer of cells enclosing a diarch stele. As the secondary growth takes place a small central cylinder of wood, surrounded by phloem in produced

The cork cambium arises in the pericycle and produces 3-5 layers of cork cells on the outer side and 3-6 layers of phelloderm cells on the inner side. Small Ares of secondary cambium originate in the secondary phloem and subsequently in the ground tissue of the phelloderm. A continuous ring of vascular tissue surrounding the central xylem cylinder is formed in some cases, while in most cases a discontinuous ring joining the inner cylinder is formed.

The stem has a distinct pith while it is absent in the root (Prasad and Bhattacharya, 1961).

The seeds available in the market are actually the dry indehiscent fruits enclosed within a husk of two splinescent bracteoles and 5 scarious perianth leaves. The true seed contains a dicotylednous curved embryo embedded in a hard horny endosperm (Prasad and Bhattacharya, 1961)

PHARMACOLOGICAL AND BIOLOGICAL STUDIES

A. aspera Linn. General pharmacological studies of the plant did not elicit any exciting activity; However, the antifertility activity needs to be looked into. The plant seems to lower lipids

Antifertility

The alkaloidal fraction obtained from the alcoholic extract of the root bark inhibited the response of oxytocin in isolated rat uterus. This fraction did not inhibit the

responses to serotonin and acetylcholine in rat uterus and to histamine in guinea pig uterus (Gupta and Khanijo. 1970).

Abortifacient activity

Abortifacient activity in rats in a dose of 50 mg/ kg. The extract did not show toxicity in the acute and chronic toxicity tests. No teratogenicity was observed (Pakrashi and Bhattacharya, 1977).

Antitumor

In an antitumor evaluation of some plants, a sterol glycoside (mp 280-85°C) isolated from the plant did not show any antitumor activity against P-388 tumor in mice (In dap et al., 1986).

Anticancerous activity

Rishi khant singh et al, to summarize, *Achyranthes aspera* L., a medicinal plant, inhibited the proliferation of Dalton's Lymphoma cells by inducing apoptosis. In this study, we specifically reported that AAML promoted mitochondrial apoptotic cascade in DL cells were mediated by suppressed PKCα signaling pathway. Meanwhile, the *in vivo* study also augmented the antitumor effect of AAML by increasing the survival time, restoring hematological parameters, attenuating angiogenesis and metastasis in DL induced mice.[33]

Anti-proliferative and anti-cancerous property

R. Subburayan et al analyzed the *in vitro* anti-proliferative activity of LE on a panel of cancer cell lines selected from the list of NCI60 tumor cell line maintained by the National Cancer Institute (Shoemaker, 2006). This is the first documented study of this kind for this plant. Our results demonstrated that LE has well defined differential, time and dose dependent anti-proliferative activity on a panel of cancer cell lines.[34]

Antimalarial activity

Mary matawal mankilik et al, concluded, the extract displayed good anti-malarial activity in the mice model, which could be due to secondary metabolites present. Extract was safe. It also prolonged survival time of the mice, prevented weight loss, and temperature reduction. This promising result from the crude extract could confirm the traditional medicinal claim for the use of the plant against malaria in Nigeria. However, further studies are required to determine the mechanism of action and characterization of its active anti-plasmodial components.[35]

Immunostimulant

Praveenkumar srinivastava et, al concluded Natural products are having a great importance in ancient traditional medicine systems. Herbs are the natural drugs used to regain the alterations made in normal physiological system by foreign organisms or by any malfunctioning of the body. From different literature and review it has been seen that plant Achyranthes aspera is a resuscitative plant due to its large number of medicinal properties and having medicinally important chemicals like ecdysterone, n-hexacos-17- enoic, spinasterol, achyranthine, betaine, pentatriaontane, hexatriacontane, tritriacontane, Hydroquinone, p-benzoquinone, spathulenol, nerol, asarone, and essential fatty acids (EFAs). The plant shows many pharmacological activities like spermicidal, anti-allergic, cardiovascular, nephroprotective, antiparasitic, anti-inflammatory, hypoglyceamic, analgesic, hepatoprotective potency, inhibit leukocyte infiltration (particularly eosinophils and neutrophils), antiperiodic, antimicrobial, purgative, antipyretic and are used in various types of gastric disorders. Thus, Achyranthes aspera is quite promising as a multipurpose medicinal agent and further clinical trials should be performed to prove its efficacy. So the present review substantiates the long standing believe that Achyranthes aspera has medicinal properties. Being a weed, this can prove to be an elixir to the common man. Further research can be directed at elucidating the active components as well as the pathways threw which they act[36.]

Anthelmintic activity:

Esther et al present study, it can be concluded that the whole plant extracts of A. aspera (aqueous, ethyl acetate and ethanol) has potent anthelmintic activity when compared with the standard drug, albendazole. Further studies using animal models are required to establish the pharmacological rationale for the same. Besides, identification and isolation of active principles responsible for this activity has to be done to formulate the extract into suitable dosage forms. [37]

Spermicidal Activity:

Extracts from roots of Achyranthes aspera have been reported to possess spermicidal activity in human and rat sperm, as studied by Paul et al. (2010). Study was made on hydroethanolic, n-hexane and chloroform extracts, which were found to be most effective for sperm immobilization, sperm viability, acrosome status, 5'-nucleotidase activity and nuclear chromatin decondensation. Vasudeva and Sharma

(2006) reported the ethanolic extract of the root of Achyranthes aspera shows post coital antifertility activity in female 126 Saba Hasan albino rats. According to their study, the extract exhibited 83.3% anti-implantation activity when given orally at 200 mg/kg body weight. [38]

Antiparasitic Activity:

Ethyl acetate extracts of A. Aspera have been proved to contain anti parasitic activity by Zahir et al. (2009). It has been studied that dried leaf, flower and seed extract of A. Aspera are active against the larvae of cattle tick Rhipicephalus (Boophilus)microplus (Acari:lxodidae),sheep internal parasite Paramphistomum cervi. [38]

Hypoglyceamic and Cancer Chemo preventive Activity:

Aqueous methanolic extract of the whole plant have been shown to possess hypoglycaemic activity by Akhtar and Iqbal (1991). Methanolic extracts from leaves of Achyranthes aspera have been proved to have cancer preventive action on Epstein-Barr virus early antigen activation induced by tumor promoter 12-O-tetradecanoylphorbol-13-acetate in Raji cells, as reported by Chakraborty et al. (2002).[38]

Hepatoprotective Activity:

Bafna and Mishra (2004) reported that the methanolic extract of the aerial parts of Achyranthes aspera shows hepatoprotective activity on rifampicin induced hepatotoxicity in albino rats. Methanolic extract showed dose dependent decrease in the levels of SGPT, SGOT, ALKP and total bilirubin. [38]

Anti-inflammatory, anti-arthritic and Anti-oxidant activity:

Alcoholic extract of the roots of Achyranthes aspera, was found to exhibit anti-inflammatory activity in Wistar rats using carrageenan-induced paw edema method and cotton pellet granuloma test, as studied by Vijaya Kumar et al. (2009). Gayathri et al. (2009) also reported antioxidant activity on leaves and roots.[38]

Nephroprotective Activity:

Methanolic extract of the whole plant of Achyranthes aspera was shown to produce nephroprotective activity against lead acetate induced nephrotoxicity in male albino rats, as reported by Jayakumar et al. (2009). Anti-depressant Activity: Barua et al. (2009) showed that Methanolic extract of the leaves of Achyranthes aspera shows

anti-depressant effect in mice and rats using forced swimming test in mice and rats and tail suspension test in rats.[38]

Cardiovascular Activity:

Achyranthine, a water-soluble alkaloid isolated from Achyranthes aspera, decreased blood pressure and heart rate, dilated blood vessels, and increased the rate and amplitude of respiration in dogs and frogs. The contractile effect of the alkaloid at 0.5 mg/ml on frog rectus abdominal muscle was less than that of acetylcholine (0.1 mg/ml), and its spasmogenic effect was not blocked by tubocurarine.[38]

Bronchoprotective Activity:

Ethanolic extract of Achyranthes aspera shows bronchoprotective effect in toluene diisocyanate (TDI) induced occupational asthma in Wistar rats as reported by Goyal et al. (2007). The total and differential leucocytes were counted in blood and bronchoalveolar (BAL) fluid. Liver homogenate was utilized for assessment of oxidative stress and lung histological examination was performed to investigate the inflammatory status of airway. The results suggest that Achyranthes aspera treated rats did not show any airway abnormality. [38]

Anti-allergic and Wound Healing Activity:

Datir et al. (2009) reported that the petroleum ether extract (200 mg/kg, i.p.) of the plant shows significant antiallergic activity in both milk induced leukocytosis and milk induced eosinophilia in mice. Thus, the antiallergic activity of A. aspera may be due to the presence of steroids. Thus, these steroids present in the plant may be responsible for the antiallergic activity. Edwin et al. (2008) investigated the ethanolic and aqueous extracts of leaves of Achyranthes aspera for wound healing activity.[38]

CLINICAL STUDIES

A. aspera Linn.

The plant was subjected to wide clinical evaluation with special reference to its use in leprosy, bronchial asthma and fistula-in-ano. Diuretic activity could not be confirmed.

Fistula-in-ano

There have been a number of studies on the use of "Kshaarasootra' (a medicated thread prepared by coating the latex of Euphorbia neriifolia, alkaline powder of A. aspera and Curcuma longa) in the treatment of fistula-in-ano.

The studies revealed that the long-term use of Kshaarasootra' was quite effective in treatment of various fistulous tracks (Deshpande and Sharma, 1973, 1976: Deshpande et al., 1966, 1975; Raghavaiah, 1976: Gangasatyam. 1981: Varshney and Tyagi, 1991). The standardization of 'Kshaaraspotra', the methods of identification and assay of the individual constituents were studied (Dwivedi et al., 1991; Sharma et al., 1994, 1995).

The Indian Council of Medical Research has carried out a multicentric randomized controlled trial to evaluate the efficacy of 'Kshaarasootra' in the management of fistula in-ano (265 patients) in comparison with the conventional surgery (237 patients). The trial was carried out at Bombay, Chandigarh, New Delhi and Wardha.

The results have revealed that the long term outcome with 'Kshaarasootra' (recurrence 4 percent). was better than with the surgery (recurrence 11 percent), although the initial healing time was longer (8 wk with thread and 4 wk with surgery). 'Kshaarasootra' offered an effective, ambulatory and safe alternative treatment for patients with fistula-in-ano (ICMR, 1991).

"Kshaarasootra' has also been found to give encouraging results in 5 patients of chronic non healing milk-fistula 'stannadi-vrana' with additional local application of "jatyaditaila and oral administration of 'shigru guggulu' (two tablets t.i.d.) during the course of treatment (Singh et al., 1994b).

Diuretic

A clinical trial was undertaken at the Government Ayurvedic College Hospital, Gwalior, involving 15 cases of general anasarca (shoth) to evaluate the diuretic effect of A. aspera. The decoction of plant, its seed or saponin did not possess a significant diuretic property (immediate or late diuresis) in cases of general anasarea (Shankar et al., 1980)

Bronchial Asthma

A pilot study was carried out at the Central Research Institute for Siddha in Madras on 15 cases of bronchial asthma. The oil obtained from the root soaked in cows' urine was smeared on betel leaf and administered thrice a day to these patients. In most of the cases symptoms like wheezing, gasping, dyspnoea, sneezing and cough disappeared. A fall in the total WBC and cosinophil counts and ESR was observed (Suresh et al., 1985)

TOXICOLOGICAL STUDIES

A. aspera Linn.

The allergenic pollens of the plant are common in the area of Pondicherry. The period of anthesis has been found to be between November and December (Saha and Kalyanasundaram, 1962).

கற்சுண்ணம்.

LIMESTONE

இஃது இயற்கையில் தாராளமாகக் கிடைக்கின்றது. இதனைக் காள வாயிலிட்டு நீற்றி எடுத்து, நீர் விட்டுத் தாளித்துக் கொள்ள வேண்டும். கற்சுண்ணத்திற்குப் புண்ணாக்கி, உடல் தேற்றி, தாது வெப்பகற்றி, துவர்ப்பி, நச்சரி, வயிற்றுப் புளிப்பகற்றி ஆகிய செய்கைகள் உள.[39]

பொதுக்குணம்.

"உண்டவனஞ் சீரணமா நெய்ச்சிக் கேகு
மூறில்விந்து விற்கூக்க முறுங்காண் வாயில்
விண்டவெயி றிதுகுமறற் பேதி வற்றும்
வெந்தளித்த நீர்ச்சுருக்கும் விலகு மெய்யை
யண்டுபல விடமொழியுங் கறையோ நிற்கு
மழலுறுபுண் சிரங்குநமை யழுபுண்ணாறுங்
கண்டவுரு வன்மீகஞ் சிரநோய் சந்நி
களுங்கரக்குங் கற்கண்ணங் காணுங் காலே."

(பொ-ரை) கற்சுண்ணம் அருந்திய உணவைச் செரிக்கவைத்து, குடலில் பற்றிய நெய்ச்சிக்கை நீக்கி, விந்துவிற்கு ஊக்கமும் பல்லுக்கு வலிவும் கொடுத்து, நீர்ப்பேதி, வாத நீர்ச்சுருக்கு, விடங்கள், இரத்தப் பெருக்கு, தீச்சுட்டபுண், சிரங்கு, நமைச்சல், அழுகிய புண், களநோய் யானைக்கால், தலைநோய், சந்நி முதலிய நோய்களையும் நீக்கும் என்ப.

உபயோகங்கள்:

பழஞ் சுண்ணாம்பு காரையைப் பொடித்து ஒட்டிலிட்டு வறுத்துத் துணித்துண்டில் வைத்துச் சுருட்டி, நோயுள்ள விடத்தில் ஒற்றடமிட உடனே அங்குள்ள வேதனை நீங்கும்.

கற்சுண்ணத்தை உத்தாமணிச் சாற்றால் தாளித்து எடுத்துத்தினம் வெற்றிலையில் தடவி, தாம்பூலமாகத் தரிக்க கபசந்நி வாயு நீங்கும். இதனை.

தாளியாத சுற்கண்ணத்தின் மீதிலுத்
தாமணிச்சுதை தன்னை யழுத்தினால்
ஆவிபோல வெதிர்த்துப் புகைந்துநீ
றானசேதி யறியுமுன் சன்னியா
மீளியோசனை தூரமகலும்பொன்
வேந்தனுக்கும் பயஞ்சிறி தாமையா
கேளிதைத்தின முண்ணடை காயொடு
கேதம் போய்விடு மோதுமளவிலே."

என்ற செய்யுளால் உணரலாம்.

சுண்ணாம்புத் தண்ணீர்:

2 பலம் (70 கிராம்) தாளித்த சுண்ணாம்பைக் கல்லடைப்பானுள்ள ஒரு குப்பியிலிட்டு, 6 ½ புட்டி (4.2 லிட்) தண்ணீர் விட்டு, இரண்டு அல்லது மூன்று நிமிடம் குலுக்கி, அசையாமல் வைத்துத் தெளிந்தபின், தெளிவை வடிகட்டிப் பச்சைநிறப் புட்டியிலிட்டுக் காற்றுப் புகாமல் அடைத்துக் கொள்ளவும்.

கற்சுண்ணாம்பு:

அளவு:- 1/8(21 மி.லிட்) முதல் ஆழாக்கு 1/2 (84 மி.லிட்) வரை கொடுக்கலாம். இதனைப் பசுவின் பாலில் கூட்டிக் கொடுக்க, வயிற்றிலுண்டாம் புளிப்பை நீக்கிவிடும். குழந்தைகளுக்கு இந்நீரைக் கொடுக்க, பால் முறிந்து தயிர்போல் வாந்தியெடுத்தல் நிற்கும். சிசுக்களுக்குத் தேக்கரண்டி (4 மி.லிட்) அளவு எடுத்து, சம அளவு பசுவின் பாலில் கலந்து, மூன்று மணி நேரத்திற்கொருமுறை உண்ணுமுன் கொடுக்கவும்.

சுண்ணாம்பு நீர், தேங்காய் எண்ணெய் சமனளவு சேர்த்துக் குலுக்கி, நெருப்பு, வெந்நீர் இவைகளால் உண்டான புண்களுக்குப் போட குணமாம்.

விடகுணமுள்ள திராவகங்களை விடமிக்கும்படி சாப்பிட்டவர்களுக்கு, தேவையான, சுண்ணாம்பு நீரைப் பாலில் கூட்டிக் கொடுப்பதினால் விடம் முறிந்து விடும்.

இதைத் தேன்விட்டுக் குழைத்துத் தொண்டைக் குழியில் தடவிவர புகைந்துவரும் இருமல் சாந்தப்படும். இக்கலவையைக் கட்டிகளின் மீது தடவிவர, அவை பழுத்து உடையும்.

கற்சுண்ணத்தைக் குழைத்துப் புறவளையமாக உந்தியைச் சுற்றிப்போட வயிற்றுவலி, நீர்ச்சுருக்கு, நீர் அடைப்பு நீங்கும்.

சுண்ணாம்புடன் குப்பைமேனி இலைச்சாறுவிட்டு மத்தித்துப் பூரான், வண்டு, குளவி முதலியவைகளின் கொட்டு அல்லது கடிவிடங்களுக்கு, மேலே தடவிவர விரைவில் குணமாம்.

தேள் கொட்டிய இடத்தில் சிறிது சுண்ணாம்பை வைத்து அரிந்த வெங்காயத்தைக் கொண்டு தேய்க்க நற்பலனை அளிக்கும்.

கற்கண்ணத்துடன் சிறிது வெல்லம் கூட்டி, மத்தித்து,அடிபட்ட இடத்தில் வைத்துக்கட்ட இரத்தம் பெருகாமல் ஆறும்.

மூத்திரக் கிரிச்சரம், மூத்திர எரிச்சல் இவைகளுக்குச் சுண்ணாம்புத் தெளிநீரைப் பசுவின் பாலில் கூட்டிக் கொடுக்கக் குணமுண்டாம்.

ஓர் அவுன்ஸ் (28 மி.லிட்) சுண்ணாம்பு நீருடன் இரண்டு அவுன்ஸ் (56 மி.லிட்) முள் இலவம் பட்டைச் சாறு கூட்டிச் சாப்பிட, வெள்ளை, இரத்த வெள்ளை, பெரும்பாடு முதலியவை குணமாம்.

கற்சுண்ணத்தையும் முத்துச் சுண்ணத்தையும் சம அளவு கூட்டி மருவிற்குப் போட்டுவர நற் பலனை அளிக்கும்.

கற்சுண்ணம் எரிப்பிற்கும், தாளகம், சேராங்கொட்டை, ஆமை ஓடு இவைகளைச் சுத்தி செய்வதற்கும், வங்கச் சுண்ணம், சிலாசத்து இவைகளைப் பற்பமாக்குவதற்கும் பயன்படும்.

MODERN ASPECTS

LIMESTONE

Limestone is a sedimentary lock composed largely of the mineral calcite (calcium carbonate $CaCo_3$). Limestone is a key ingredient of quicklime, mortar, cement, and concrete. The solubility of limestone in water & weak acid solution leads to important phenomena.[40]

Description:

Limestone often contains variable amounts of silica in the form of chert And/or flint, as well as varying amounts of clay, slit and some as disseminations, Nodules or layers within the rock**.**

The primary source of the calcite in limestone is most commonly marine Organisms. Limestone makes up to 10% of the volume of all sedimentary rocks.

Calcite can be either dissolved by groundwater a precipitated by ground water, depending on several factors including the water temperature, ptt and dissolved in concentrations.

It is an organic substance

As for the physical properties limestone usually varies between very tiny textured rocks and coarse textured rocks. Further there are many different types of lime stone like chalk, coquina, travertine, tufa, fossiliferous limestone, lithographic limestone, oolitic limestone, they have been categorized based on how each rock is formed. How it looks composition and other some factors.

Lime stone is composed essentially of one carbonate mineral-calcite. Ca(Co,) In pure form it contains 56% CaO and 44% Co_2

Calcite is very common in nature in the form of Lime stone.

By origin, two main varieties of limestone can be distinguished.[41]

1. Biogenic lime stone
2. Limestone of chemical origin.

Lime stones are mainly of the biognic origin. They are formed at the expense of calcareous skeletons and remkants of shalls of orgainsms may either be fully preserved or may appear to have been crushed, disintegrated and recrystallised (E.g) Nandukkal.

Limestones of the chemical origin may be fine-grained frequently olitic and concentric in texture. A group of chemically precipitated limestones are formed by precipitation of calcite as a result of evaporation of springs streams and ground water.

The colour of lime stones is essentially light, white, grey. Their hardness is 3.

Lime stone is water in-soluble except when water is saturated

with carbonic acid or is diluted acids.

Limestone may contain admixture like clay, dolomite, salts and iron hydoxides.

Limestones are greatly widespread. They are found in the rocks of all periods.

Limestones are widely used in many ways.

Uses:

- The manufacture of quick lime (calcium oxide) & slaked lime (calcium hydroxide) cements & mortar.
- As a reagent in desulphurization.
- Glass making, in some circumstances.
- Added to paper, Plastic paints, tiles, and other materials as both white pigment and a cheap filter.
- Tooth paste.
- Added to bread and cereals as a source of calcium.

Lime stone (GEOLOGICAL ASPECTS)

Lime stone is a type of sedimentary rock. That is found naturally in earth's environment. The rock comprises primarily of chemical compound calcium carbonate ($CaCo_3$) which is a type of mineral. It also contains other materials like quarts, clay, minerals pyrite, feldspar and siderite amongst others.[42]

CALCITE:

$CaCO_3$

Origin of Name

From the Latin word calx, meaning **"burnt lime."**

Hand Specimen Identification Calcite is identified by its hardness, rhombohedral cleavage, and effervescence in cold dilute HCl. It may be confused with dolomite or aragonite. Plate 3.8 shows euhedral calcite crystals and Figure 30. shows "sandy" calcite.

PHYSICAL PROPERTIES:

- Hardness - 3
- specific gravity - 2.71
- deavage/fracture - perfect rhombohedral 101/conchoidal
- luster/transparency - vitreous/transparent to translucent
- color - colorless to white; may also be tinted gray, red, blue, yellow, or green; brown to black when impure
- streak - white

OPTICAL PROPERTIES:

Calcite is colorless in thin section and has extremely high birefringence, resulting in pale, washed out, or white interference colors. Polysynthetic twinning is nearly always visible. It shows variable relief upon stage rotation. Calcite may be confused with other. hexagonal carbonates. Orthorhombic carbonates. have parallel extinction and are biaxial. Uniaxial (-), w = 1.658, e = 1.486, 8 0.172. Plates 5.1 and 5.2 show calcite in thin section.

CRYSTALLOGRAPHY:

Hexagonal (rhombohedral), a= 4.99, c= 17.04, Z= 6; space group R32/c, point group 32/m.

HABIT:

Calcite has many habits. The most common are hexag onal prisms with simple to complex terminations; scalenohedra, often with combinations of other forms; rhombohedra, either acute or flattened; and tabs with well-developed basal faces. Polysynthetic twinning is common but usually requires a microscope to detect. Calcite is also found as a massive rock-forming min eral, as nodules or crusts, in speleothems, and as fine to coarse granular aggregates.

Structure and Composition In calcite, Ca^{2+} ions alternate with (CO) groups in a three-dimensional array. The structure is similar to that of cubic salts, such as halite or periclase, but is not cu bic because the structure has been squashed along the equivalent of a main diagonal of the cube. The short ened direction is the c-axis in calcite; planar (CO_2) groups are perpendicular to c, giving the structure a 3 fold axis of symmetry in that direction only. Mg, Fe, Mn, Zn, and a number of others may substitute for some of the Ca; except for Mn, most solid solutions are quite limited.

Occurrence and Associations Calcite is a common and widespread mineral. It is an es sential and major mineral in limestones and marbles, oc curs in cave deposits, and occurs as a vein mineral with other carbonates, sulfides, barite, fluorite, and quartz. Calcite also occurs in some rare carbonate-rich igneous rocks and is a common cement in some sandstones. Calcite is common as a weathering product. Organic cal dite is common in shells and skeletal material.

VARIETIES:

Iceland spar refers to clear calcite, usually in rhombohedral cleavage fragments; dogtooth spar refers to crystals with steep scalenohedral forms; and nail-head spar refers to flat rhombs or stubby prismatic crystals.

RELATED MINERALS:

Calcite has two polymorphs, aragonite and vaterite. It is isostructural with magnesite, MgCO3, siderite, FeCO3; sphaerocobaltite, COCO,; smithsonite, ZnCO; nitratite, Na(NO3); dolomite, CaMg(CO3)2 and gaspeite, (Ni,Mg,Fe) (CO3). Calcite and rhodochrosite form extensive solid solutions at room temperature and a complete solid solution above about 550°C (1,020°F). Calcite forms limited solid solutions with ankerite, CaFe(CO3)2; dolomite, CaMg(CO3)2 and kutnohorite, CaMn(CO3)2, at all temperatures.

Different uses of Limestone:

Interestingly as this rock is so valuable important. Apart from architecture there are several other uses of this rock as well.

Agriculture:

Limestone deposits contains mostly calcium carbonate. Usually, limestones are used in farming sector by crushed in to small particles and various grades are produced.

Finally, these products are sold as “Agricultural lime or algime” which is again used to neutralized soil acidity as well as free up soil minerals like phosphates.

Industries:

There are several industries that make use of limestone. As much powdered limestone is used in textile, paints, paper rubber glass and plastic industries. They are mostly used as fillers. This is also used in steel industry for production process whereas limestone is used to remove impurities.

The minerals that found in limestone are also used in pharmaceuticals, cosmetic products, baking soda and tooth paste etc.

Healthy benefits of limestone:

Good during Pregnancy:

Being rich in calcium it will promote health growth of baby. Ensure normal delivery and will also develop in intelligent and sharpness in the kid.

Treats Anaemia:

Good for developing Kids.

Treats Impotence:

It is helps to treat impotence male.

Treats Jaundice: Helps to treat Jaundice.

4. MATERIALS AND METHODS

The study drug was prepared at NIS laboratory. Then the standardization of drug such as siddha standardization, Organoleptic characters, Physiochemical analysis, Biochemical analysis, and Analytical methods like ICP-MS, SEM-EDS, XRD were done. After completing standardization process, the safety profile of Kothanthi thalaga parpam was done as per OECD Guidelines 423&407.

4.1.KOTHANTHI THALAGA PARPAM(KTP) – DRUG PROFILE:

The study drug KTP has been selected from the classical siddha literature-Anupoga vaithiya navaneetham part-6[10]

Collection of raw drug:

The raw drugs of Kothanthi thalaga parpam were collected from raw drug store in Chennai and salem.

Authentication:

Thalagam (Arsenic trisulphide) authenticated by Department of Gunapadam, NIS, Chennai.

Nayuruvi (Achyranthus aspera) authenticated by Department of Botany, NIS, Chennai.

PURIFICATION:

The pieces of Thalagam (200 gm) was tied in a cotton cloth with loose knot. Of raw limestone is placed above and below the layer of thalagam and hot water is poured over the setup. The procedure is repeated for 11 times for purification.Then it is continued for 21 times in order to eliminate the impurities of thalagam.this forms the best purification method as per the siddha literature & it can be used for medicinal purposes. [43]

-Vaithiya thirupugazh

PREPARATION OF MEDICINE:

Ingredients of Kothanthi thalaga parpam:

Purified Thalagam – Yellow arsenic trisulphide

Nayuruvi sambal – *Achyranthus aspera* ash

Ilanthai viragu - *Ziziphus jujuba* wood for burning purpose

Method of preparation:

¾ coarse ash powder of achyranthus aspera was placed in a mud pot, then the purified drug (thalagam) is placed over the ash and again the ¾ th coarse powder of A.aspera was placed .then the pot was sealed with the cloth of clay,allowed to dired,then the sealed mud pot is placed in fire of flaming wood of ziziphus jujuba for 2 hours and burned.Then the seal is uncovered and the drug is taken ,powdered using pulveriser.The powdered drug is known to be KTP.

Dosage: 32.5 mg to 65 mg, Twice a day (*Arisi edai* to *Kaal kundri*)

Adjuvant: Vetrilai or Paaladai

Medicinal uses:

Swasakaasam (Bronchial asthma)

Irumal (cough)

Suram (All type of fever)[10]

PURIFICATION PROCESS OF THALAGAM

UNPURIFIED THALAGAM

UNPURIFIED THALAGAM POUCH PLACED OVER LIMESTONE POWDER IN A POT

HOTWATER POURED INTO POT

SEPERATION OF OIL FROM THALAGAM POUCH

PURIFIED THALAGAM

PREPARATION OF KOTHANTHI THALAGA PARPAM

PREPARATION OF NAYURUVI SAMBAL (*ACHYRANTHUS ASPERA ASH*) & FILLED OVER THE CLAY PAN WITH PURIFIED THALAGAM

2 hours of burning process

final product of KTP

Grinding of KTP using pulverizer

STANDARDIZATION OF KOTHANTHI THALAGA PARPAM:

SIDDHA STANDARDIZATION OF PARPAM:[44]

A. Floats on the water:

A pinch of the parpam was sprinkled over the water in a vessel. The test drug was float on the water surface. It was noted.

B. Finger lines test:

Parpam should be very fine in manner. While the *Kothanthi thalaga parpam* taken between the thumb and index finger, it can fine enough to enter within the lines of fingers.

C. Tasteless:

Parpam should be completely tasteless. This test was done by placing a pinch of the *Kothanthi thalaga parpam* at the tip of the tongue and then tasted.

D. Lustreless:

If any shining particles present in the Parpam, it indicates the improper medicinal preparation. The Kothanthi thalaga parpam was taken in a petridish and evaluated under the sunlight for any shining particles. There was no shining particles seen in the *Kothanthi thalaga parpam*.

ORGANOLEPTIC CHARACTERS:

A. Colour:

B. Odour:

C. Taste:

D. Touch:

E. Appearance

4.2.QUALITATIVE ANALYSIS:

PHYSICOCHEMICAL ANALYSIS:[45]

The Chemical analysis of drug was done at Cholayil Private Limited,Research and Development Centre, Chennai – 98 as per Indian Phamacopoeia.

A. DETERMINATION OF MOISTURE & VOLATILE MATTER:

Procedure:

Dry an empty Petri dish / weight bottle with stopper in an oven for ½ an hour at 105°C. Cool in a desiccator and weigh. Weigh accurately about5 g of the sample in petri dish and dry to constant mass in hot air oven at a temperature of 105 +/- 2°C. Cool in a desiccator and weigh. Repeat dryingand weighing till difference of not more than 0.1% in the net loss in mass isobtained.

Calculation:

$$\% \text{ Moisture \& Volatile matter} = \frac{W2 - W3}{W2 - W1} \times 100$$

W1 = Weight in gram of empty petri dish/stoppered weighing bottle

W2 = Weight in gram of petri dish/stoppered weighing bottle and samplebefore drying

W3 = Weight in gram of petri dish/stoppered weighing bottle and sampleafter drying

B. DETERMINATION OF pH:

Procedure:

Calibrated pH meter is operated according to the instruction manual provided by the manufacturer. Switch on the pH meter and allow half-an- hour for stabilization before the readings are taken. Standardize the pH meter using buffer 4.0, 7.0 or 9.2 depending on the pH range of the samplebeing tested and then measure the pH of the sample.

C. DETERMINATION OF TOTAL ASH:

Procedure:

Heat a silica crucible to red hot for 10 minutes, allow to cool in a dessicator and weigh (W1) accurately about 2 -3 g of the substance being examined and distribute it evenly in the crucible (W2). Heat the crucible with the substance slowly first till the fumes subside and then to 600 °C +/-25°C in a Muffle furnace. If Carbon-free ash cannot be obtained,

exhaust the charred mass with hot water and collect the residue in a Whatman ashless filter paper. Dry the filter paper in air incinerate the residues and filter paper until the ash is white or nearly so add the filtrate to the crucible,evaporate to dryness and ignite again, cool in a dessicator and weigh (W3).

Calculation:

$$\% \text{ Total ash content} = \frac{(W3 - W1)}{(W2 - W1)} \times 100$$

D. DETERMINATION OF ACID INSOLUBLE ASH:Procedure:

Boil the ash obtained in the determination of total ash for 5 minutes with 25 ml of dilute hydrochloric acid / 4 M hydrochloric acid. Collect the insoluble matter in a Whatman ashless #41 filter paper. Wash with sufficient hot water until the filtrate is free from chloride ion. Air dry the filter paper and ignite it in a previously preheated, cooled and weighed silica crucible (W1). Ignite it to constant weight (W2).

Calculation:

$$\% \text{ Acid insoluble ash} = \frac{(W2 - W1)}{\text{Weight of substance taken}} \times 100$$

E. DETERMINATION OF SULPHATED ASH:

Procedure:

Heat a silica crucible to red hot for 10 minutes, allow to coolin a dessicator and weigh (W1). Weigh accurately about 1.0 g of the substance being examined and distribute it evenly in the crucible (W2). Ignite, gently at first until the substance is thoroughly charred. Cool,moisten the residue with 1 ml sulphuric acid, heat gently until the white fumes are no longer evolved and ignite at 800 °C +/- 25°C in a Muffle furnace until all black particles have disappeared. Allow the crucible to cool, add a few drops of sulphuric acid and heat. Ignite as before, allow to

cool and weigh (W3). Repeat the operation until two successive weighingdo not differ by more than 0.5 mg.

Calculation:

$$\% \text{ Total ash content} = (W3 - W1) \div (W2 - W1) \times 100$$

F. DETERMINATION OF ALCOHOL SOLUBLE EXTRACTIVE:Procedure:

Accurately weigh (W1) about 5 g of the coarsely powdered raw drug/extract and add 100 ml of alcohol (95%). Allow it to stand with occasional shaking for 6 hours at ambient temperature. Then, leave it for another 18 hours stand still. Filter the content using Whatman #1 filter paperas quickly as possible to avoid solvent loss. Take exactly 25 ml of the filtrateand evaporate it in a previously dried cooled and weighed petri dish (W2). Dry it in an oven at 105 °C cool in a dessicator and weigh (W3).

Calculation:

$$\% \text{ Alcohol soluble extractive} = \frac{(W_3 - W_2)}{W_1 \times 25} \times 100$$

G. DETERMINATION OF WATER-SOLUBLE EXTRACTIVE:Procedure:

Accurately weigh (W1) about 5 g of the coarsely powdered raw drug/extract and add 100 ml of chloroform - water. Allow it to stand with occasional shaking for 6 hours at ambient temperature. Then, leave it for another 18 hours stand still. Filter the content using Whatman #1 filter paper as quickly as possible to avoid solvent loss. Take exactly 25 ml of thefiltrate and evaporate it in a previously dried cooled and weighed petri dish(W2). Dry it in an oven at 105 °C cool in a desiccator and weigh (W3).

Calculation:

$$\% \text{ Water soluble extractive} = \frac{(W_3 - W_2) \times 100 \times 10}{W_1 \times 25}$$

4.3.QUANTITATIVE ANALYSIS:

ANALYTICAL METHOD OF KOTHANTHI THALAGAM:[46]

A. INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY (ICP-OES):

ICP-OES analysis was done at SCRI, Arumbakkam to detect the heavy metal content of Kothanthi thalagam.

Procedure:

Take about 20 mg of sample into the Teflon microwave digestion vessel and add 1 mL of ultrapure nitric acid to digest about 45 minutes using Anton Paarmicrowave digestion unit. After that the sample is made up to a 50 mL standard measuring flask. The calibration standard solution is prepared for 0.2 µg/mL to 10 µg/mL by using ultrapure nitric acid and blank also. Agilent ICP-OES 5100 VDV instrument used with the following operation conditions: a RF power 1.2 kW, a plasma gas flow rate 12 L min^{-1}, and a nebulizer gas flow rate 0.70 $Lmin^{-1}$.The samples are introduced into the plasma using nebulizer and spray chamber for the analysis.

B. X-RAY POWDER DIFFRACTION:

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analysed material is finely ground, homogenized, and average bulk composition is determined. Max von Laue, in 1912, discovered that crystalline substances act as three-dimensional diffraction gratings for X-ray wavelengths similar to the spacing of planes in a crystal lattice. X-ray diffraction is now a common technique for the study of crystal structures and atomic spacing. X-ray diffraction is based on constructive interference of monochromatic X- rays and a crystalline sample. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample.

X-ray Powder Diffraction (XRD) Instrumentation

These spectra consist of several components, the most common being Ka and Kp. Ka consists, in part, of Ka1 and Ka2. Ka1 has a slightly shorter wavelength and twice the

intensity as Ka2. The specific wavelengths are characteristic of the target material (Cu, Fe, Cr). Filtering, by foils or crystal monochromates, is required to produce monochromatic X-rays needed for diffraction. Ka1 and Ka2 are sufficiently close in wavelength such that a weighted average of the two is used. Copper is the most common target material for single-crystal diffraction, with Cu Ka radiation = 1.5418A. These X-rays are collimated and directed onto the sample.

As the sample and detector are rotated, the intensity of the reflected X-rays is recorded. When the geometry of the incident X-rays impinging the sample satisfies the Bragg Equation, constructive interference occurs and a peak in intensity occurs. A detector records and processes this X-ray signal and converts the signal to a count rate which is then output to a device such as a printer or computer monitor.

The geometry of an X-ray diffractometer is such that the sample rotates in the path of the collimated X-ray beam at an angle 9 while the X-ray detector is mounted on an arm to collect the diffracted X-rays and rotates at an angle of 29. The instrument used to maintain the angle and rotate the sample is termed a goniometer. For typical powder patterns, data is collected at 29 from ~5° to 70°, angles that are present in the X-ray scan.

Applications

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (eg. minerals, inorganic compounds).

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

C. SEM & EDS:

The particle size of the test drugs was determined using Scanning electron microscopy (SEM) and Energy Dispersive X-ray Analysis (EDAX). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The SEM analysis is carried out by using FEI-Quanta FEG 200-High Resolution Instrument

Resolution:

1.2 nm gold particle separation on a carbon substrate

Magnification:

From a min of 12 X to greater than 1,00,000 X.

Calculation of the particle size:

The horizontal line in the right corner of the micrograph corresponds to micron Bin length would be given. A comparison could be made between the length of the particles visible in the micrograph with this line and the length of the particle was calculated.

Application:

To evaluate grain size, particle size distributions, material homogeneity and intermetallic distributions.

BIOCHEMICAL ANALYSIS:

Experimental procedure:

5gm of kothanthathi thalaga parpam was taken in a 250ml of clean beaker and 50 ml of distilled water was added to it.Then it was boiled well for about 10 min.Then it is allowed to cool and filtered into a 100ml volumetric flask and made up to 100 ml with distelled water.this preparation is used for the qualitative analysis of acidic / basic radicals and biochemical constituents in it. The biochemical analysis of kothanthathi thalaga parpam was done at Biochemistry Lab,National institute of siddha,Chennai-47.

S.No	EXPREMENT	OBSERVATION	INFERENCE
1	Appearance of sample	Honey orange	
2	**Test for Solubility:** a. A little (500mg) of the sample is shaken well with distilled water. b. A little (500mg) of the sample is shaken well with con. HCl.	floating of water Soluble in HCL	Not soluble in water completely soluble in acid
3	**Action of Heat:** A small amount (500mg) of the sample is taken in a dry test tube and heated gently at first and then strong.	No white fumes No brown fumes	Absence of carbonate Absence of nitrate
4	**Flame Test:** A small amount (500mg) of the sample is made into a paste with con. HCL in a watch glass and introduced into non-luminous part of the Bunsen flame.	Bluish green flame not appeared	Absence of copper
5	**Ash Test:** A filter paper is soaked into a mixture of sample and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited	Yellow colour flame appeared	Absence of Sodium

Test for Acid Radicals

S.No	EXPREMENT	OBSERVATION	INFERENCE
1	**Test For Sulphate:** 2ml of the above prepared extract is taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	Cloudy apperence present	Presence of sulphate
2	**Test For Chloride:** 2ml of the above prepared extracts is added with 2ml of dil-HCl is added until the effervescence ceases off...	No coloudy appearance	Absence of chloride
3	**Test For Phosphate:** 2ml of the extract is treated with 2ml of dil.ammoniummolybdate solution and 2ml of con.HNO3	No Cloudy yellow appearance	Absence of phosphate
4	**Test For Carbonate:** 2ml of the extract is treated with 2ml dil. magnesium sulphate solution	No Cloudy appearance	Absence of carbonate
5	**Test For Nitrate:** 1gm of the substance is heated with copper turning and concentrated H2SO4 and viewed the test tube vertically down.	No characteristic changes	Absence Of nitrate
6	**Test For Sulphide:** 1gm of the substance is treated with 2ml of con. HCL	Rotten egg smelling gas evolved	Presence of sulphide
7	**Test For Fluoride & Oxalate:** 2ml of extract is added with 2ml of dil. Acetic acid and 2ml dil.calcium chloride solution and heated.	cloudy appearence	Presence of fluoride and oxalate
8	**Test For Nitrite:** 3drops of the extract is placed on a	No characteristic	Absence

	filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution is placed.	changes	Of nitrite
9	**Test For Borate:** 2 Pinches (50mg) of the substance is made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	Bluish green colour flame not appeared	Absence of borate

II.Test for Basic Radicals

S.No	EXPREMENT	OBSERVATION	INFERENCE
1	**Test For Lead:** 2ml of the extract is added with 2ml of dil.potassium iodine solution.	No yellow precipitate is obtained	Absence of lead
2	**Test For Copper:** One pinch (50mg) of substance is made into paste with con. HCl in a watch glass and introduced into the nonluminous part of the flame.	No blue colour flame	Absence of copper
3	**Test For Aluminium:** To the 2ml of extract dil.sodium hydroxide is added in 5 drops to excess.	No characteristic changes	Absence of alumunium
4	**Test For Iron:** To the 2ml of extract add 2ml of dil.ammonium solution To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added	Mild red colour appear Blood red colour appeared	Presence of iron Presence of iron
5	**Test For Zinc:** To 2ml of the extract dil.sodium	No white	Absence of

	hydroxide solution is added in 5 drops to excess and dil.ammonium chloride is added.	precipitate is formed	zinc
6	**Test For Calcium:** 2ml of the extract is added with 2ml of 4% dil.ammonium oxalate solution	Cloudy appearance and white precipitate present	Presence of calcium
7	**Test For Magnesium:** To 2ml of extract dil.sodium hydroxide solution is added in drops toexcess.	No white precipitate is obtained	Absence of magnesium
8	**Test For Ammonium:** To 2ml of extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution are added.	No brown colour appeared	Absence of ammonium
9	**Test For Potassium:** A pinch (25mg) of substance is treated of with 2ml of dil.sodium nitrite solution and then treated with 2ml of dil.cobalt nitrate in 30% dil.glacial acetic acid.	Yellowish precipitate is obtained	Presence of potassium
10	**Test For Sodium:** 2 Pinches (50mg) of the substance is 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 is treated with 2ml of dil.sodium hydroxide solution.	No yellow precipitate is obtained	Absenceof mercury
12	**Test For Arsenic:** 2ml of the extract is treated with 2ml of dil.sodium hydroxide solution.	brownish red precipitate is obtained	presence of arsenic

Test for other constituents

S.No	EXPREMENT	OBSERVATION	INFERENCE
1	**Test For Starch :** 2ml of extract is treated with weak dil.iodine solution	No blue colour developed	Absence of starch
2	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution is 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 Tannic Acid:** 2ml of extract is treated with 2ml of dil.ferric chloride solution	No black precipitate is obtained	Absence of tannic acid
4	**Test For Unsaturated Compound:** To the 2ml of extract 2ml of dil.Potassium permanganate solution is added.	Potassium permanganate is not decolourised	Absence of unsaturated compound
5	**Test For Amino Acid:** 2 drops of the extract is placed on a filter paper and dried well. 20ml of Biurette reagent is added.	No violet colour developed	Absence of amino acid
6	**Test For Type Of Compound:** 2ml of the extract is treated with 2 ml of dil.ferric chloride solution.	No green colour developed No red colour	Absence of oxyquinole epinrphrine and pyro catechol Anti pyrine,aliphatic

		developed No violet colour developed No blue colour developed	aminoacids and meconic acid are absent Apomorphine salicylate and resorcinol are absent Morphine, phenolcresol and hydroquinone are absent

4.4.TOXICITY STUDIES:

Preclinical toxicity studies of kothanthi thalaga parpam were conducted on wistar albino rats as per OECD guideline 423 and 407 after getting IAEC approval. (NIS\/IAEC-22/R02/16112021/E8). The animal study was carried out in Animal house of National Institute of Siddha, Tambaram sanatorium, Chennai.

4.4.1.ACUTE ORAL TOXICITY[47]

Experimental Details:

Species	: Wistar Albino Rat
Age / Weight	: 6 to 8 weeks / 140-160 g
Gender	: Female
Acclimatization Period: 7 days prior to dosing	
Housing	: Polypropylene cages
Husbandry	:12-h light/12-h dark cycle
Temperature	: Room temperature 22°C (±3°)
Humidity	:30–70
Feed and water libitum	: Rodent Pelleted feed RO purified water ad

Animal selection and identification:

The animals were procured from CPCSEA approved laboratory (Mass Biotech). Animals were kept in individually and marked as head, body, and tail with picric acid solution. The group number, cage number, sex of the animals were mentioned in the front of each cage.

Grouping of acute toxicity animals:

The total of 6 female animals were included in acute toxicity study. These animals were divided into three groups such as Group I & II. Each group consistsof 6 female animals. Group I was administered 300mg/kg b. wt of kothanthi thalaga parpam and Group II animals wasgiven 2000mg/kg b. wt of kothanthi thalaga parpam.

S. No.	Group	Treatment	No. of Animals
1.	I	Kothanthi thalaga parpam (300mg/kg/p.o)	6 Female
2.	II	Kothanthi thalaga parpam (2000 mg/kg/p.o)	6Female
Total No. of Animals: 12 Female			

Route of administration:

Test drug was administered through oral route.

Administration of Dose:

The animals were fasted (only food was withheld) for 12 hours and weighed prior to dosing. A single dose of the test drug was administered by oral gavage. The food was withheld for another 4hrs after dosing and administration of the drug.

Experiment details of acute toxicity study:

The Albino rats of weighing 160-200 g were obtained from authorized animal breeders of the animal laboratory (Mass Biotech), Chennai and stocked in the animal house at National Institute of Siddha, Chennai. Animals were housed in a cage at 25°C and have free access to standard rat pellet diet. The animals were treated with *kothanthi thalaga parpam by* oral route for single dose on the 1st day and monitored for mortality and behavioral parameters for the first 4 hours after drug administration. Body weightof the animal was monitored at weekly intervals. After that, on 14th day, animals of both groups were weighed and sacrificed under the injection of thiopental sodium (intra peritoneal). The toxicological effect was assessed on the basis of mortality and behavioral parameters.

Observations:

Observation was made and recorded systematically and continuouslyobserved after the substance administration as per the guidelines.

- ❖ Mortality, behavioural changes.
- ❖ ½ hour, 1 hour, 2 hours,4 hours and up to 24- hour observation.
- ❖ All rats were observed twice daily for 14 days.

- ❖ Body weight was observed once in a week.
- ❖ Feed intake was calculated daily.
- ❖ Water intake was calculated daily.

a. **Cage-side observation:**

Clinical observation includes Mortality, convulsion, tremor, sedation, excitation, abnormal gait, motor coordination, piloerection, head movements, reactivity to touch, gripping, grooming, exophthalmos, diarrhoea, salivation, lacrimation, posture, dyspnoea, coma.

b. **Gross necropsy:**

At the end of the 14th day (15th day), all the animals were sacrificed by using theinjection of thiopental sodium. Gross necropsy includes an examination of the externalsurface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, lung, heart, spleen, liver, kidney, uterus, testes, ovary of all animals.

4.4.2.REPEATED DOSE 28 DAY ORAL TOXICITY STUDY: [48]

Experimental Details:

Species	: Wistar Albino Rat
Age / Weight	: 6 to 8 weeks / 140-160 g
Gender	: Male and Female
Acclimatization Period	: 7 days prior to dosing
Housing	: Polypropylene cages
Husbandry	:12-h light/12-h dark cycle
Temperature	: Room temperature 22°C (±3°)
Feed and water	: Rodent Pelleted feed, RO purified water ad libitum

Grouping of animals:

The total of 40 animals were included in subacute toxicity study. These animals were divided into 5 groups such as control, Low dose, Mid dose, High dose and Satellite group. Each group consists of 5 Male & 5 female animals. Control group animal was administered betel juice, Low dose was given 15 mg/kg b. wt of kothanthi thalaga parpam,

Mid dose was given 75 mg/kg b. wt of kothanthi thalaga parpam, High dose given 150 mg/kg b. wt of kothanthi thalaga parpam.

Treatment Groups of Repeated Dose 28 days oral toxicity study:

S. No.	Group	Treatment	No. of Animals
1.	I	Control (Betel juice)	5 M + 5 F
2.	II	Low dose KTP (15mg/kg/p.o)	5 M + 5 F
3.	III	Mid dose KTP (75mg/kg/p.o)	5 M + 5 F
4.	IV	High dose KTP (150mg/kg/p.o)	5 M + 5 F

Total No. of Animals: 40 (20 M + 20 F)

DOSE CALCULATION:[49]

Animal dose = Human dose × Conversion factor (Surface factor for 200gm rat = 0.018)

= 130 mg ×0.018 = 2.5mg / 200gm rat

Per kg rat = 2.5 × 5 = 15mg **(15mg)**

- Low dose = 15mg / kg
- Mid dose = 15 × 5 = 75mg / kg
- High dose = 15 × 10 = 150mg / kg

Experimental Procedure:

Wistar albino rats of both Sexes weighing 140-160 g was obtained fromauthorized animal breeders. Animals was housed in Polypropylene cages at 23ºC (±3°),30–70% relative humidity and have free access to standard rat pellet diet and water libitum. Animals were fasted over night before drug administration with free access ofwater. After drug administration all the animals were observed daily for any mortality and morbidity. At the end of the study (28th day) all the animals will be fasted overnight,weighed and sacrificed under excessive Anaesthesia (Thiopental sodium 1mg/100g). Blood was collected from the anesthetized animals through jugular vein. The followinginvestigations

like Haematology, Biochemical analysis study was done. Histopathologywas done in all the vital organs of control and high dose treated groups. Satellite groupswas observed for further 14 days without drug administration. After 14 days ofobservations all the animals were fasted overnight, weighed and sacrificed underexcessive Anaesthesia (Thiopental sodium 1mg/100g). Blood was collected from the anesthetized animals through jugular vein. The following investigations like Haematology, Biochemical analysis study was done. Histopathology was done in all thevital organs of satellite group animals.

Observation

- Mortality, behavioural changes will be observed daily
- All rats will be observed twice daily for 28 days
- Body weight will be monitored at weekly once
- Fee intake and water intake will be observed daily

a. Cage-Side Observation:

Clinical observation includes abnormal Gait (rolling and tiptop), Aggressiveness, Akinesia, analgesia, catalepsy, convulsions, defecation, excitation, exopthalmos, head twitches, lacrimation, lethality, loss of corneal reflex, loss of righting reflex, loss of traction, piloerection, ptosis, reactivity to touch, respiration, salivation, sedation, stereotypies (chewing), sterotypies (head movements), stereotypies (sniffing), straub, tremor and writhes.

b. Gross necropsy

Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Lung, Heart, Spleen, Liver, Stomach, Kidney, Adrenals, Testes, Uterus of all animals.

c. Biochemical analysis:[50]

Serum samples were analysed for High Density Lipoprotein (HDL), Low density Lipoprotein (LDL), Very lowdensity Lipoprotein (VLDL), Triglycerides (TGL), Total Cholesterol, Blood urea nitrogen (BUN), Creatinine, Albumin, Aspartate Transaminase (AST) and Alanine amino Transaminase (ALT) using Mind ray semi-auto biochemical analyser model BA85A.

d. Haematological analysis:[50]

Blood samples were analysed using established procedures and automated Bayer

Haematology analyser. Parameters evaluated include Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Haemoglobin (Hb), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil's, Basophils, Lymphocytes and Monocytes.

e. Histopathological evaluation:[51]

Sample were fixed in 10 per cent buffered neutral formalin and trimmedto a thickness of about 3 mm. The tissues were dehydrated, cleared and paraffinembedded in a routine manual processing. Tissues were cut at 3 to 5 μm thickness, stained with haematoxylin and eosin and mounted with DPX for histopathological examinations Histological slides of organs were made and observed under the microscope. The pathological observations of cross sectionof these organs were performed on gross and microscopic bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological change .

f. Histopathological evaluation:[51]

Sample were fixed in 10 per cent buffered neutral formalin and trimmedto a thickness of about 3 mm. The tissues were dehydrated, cleared and paraffin embedded in a routine manual processing. Tissues were cut at 3 to 5 μm thickness, stained with haematoxylin and eosin and mounted with DPX for histopathological examinations Histological slides of organs were made and observed under the microscope. The pathological observations of cross sectionof these organs were performed on gross and microscopic bases. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological change

5. RESULTS

Siddha specification of parpam preliminary analysis, safety study of kothanthi thalagam was done in animal model as per OECD guidelines. Results of these studies are as follows.

5.1.QUALITATIVE RESULTS OF STANDARDIZATION OF KOTHANTHI THALAGAM:

5.1.1.SIDDHA STANDARDIZATION OF PARPAM:

Result showed that the parpam is honey orange in colour, odorless, Tasteless,lusterless, smokeless on heating, micro fine in nature and weightless.

Siddha standardization of Parpam

S.NO.	PARAMETERS	RESULTS OF KOTHANTHI THALAGAM
1	Irreversibility	Irreversible
2	Luster	Nil
3	Smoke (heating)	Nil
4	Weight (sprinkle test)	Floats in the water
1	Fine enough enter within the lines of finger	Enter into the finger lines

5.1.2.RESULTS OF ORGANOLEPTIC CHARACTERS OF KOTHANTHI THALAGAM:

The test drug is honey orange in colour, smooth, powder inappearance and has no odour and taste.

Organoleptic characters of Kothanthi thalagam

S.NO.	PARAMETERS	RESULTS OF KOTHANTHI THALAGAM
1	Colour	Honey Orange colour
2	Odour	No Odour
3	Taste	No Taste
4	Touch	Smooth
5	Appearance	Powder

5.1.3.RESULTS OF PHYSICOCHEMICAL PROPERTIES OF KOTHANTHI THALAGAM:

pH of test drug is 8.92, Total ash value of is 72.60%, Acid insoluble ash is 64.45% w/w, Water soluble extractive is 3.41% w/w, Alcohol soluble extractive is 0.59% w/w, Moisture and Volatile matter is 1.43% w/w.

Physicochemical analysis of Kothanthi thalaga parpam

S.NO.	PARAMETERS	RESULTS OF KOTHANTHI THALAGAM
1	pH (1% aqua solution)	8.92
2	Total ash (%)	74.60
3	Acid insoluble ash (% w/w)	66.45
4	Water soluble extractive (% w/w)	3.32
5	Alcohol soluble extractive (% w/w)	0.59
6	Moisture & Volatile matter (% w/w)	1.43

5.1.4. BIOCHEMICAL ANALYSIS OF KOTHANTHI THALAGA PARPAM*:*

Test for acid and basic radicals is a very basic study which helps to identify the presence of elements qualitatively and helps in the quantitative estimation of the same. The acid radicals present in the kothanthi thalaga parpam were sulphate,Sulphite,and fluoride & oxalate. The basic radicals present in the drug were Iron , Arsenic , potassium, sodium and calcium.

Test for acid radicals

Sl. No	**Test**	**Results of Kothanthi thalagam**
1.	Test for sulphate	+
2.	Test for phosphate	-
3.	Test for sulphide	+

Test for basic radicals

Sl. No	Test	Results of Kothanthi thalagam
1.	Test for lead	_
2	Test for aluminium	-
3	Test for iron	+
4	Test for zinc	_
5	Test for calcium	+
6	Test for magnesium	_
7	Test for ammonium	_
8	Test for mercury	_
9	Test for arsenic	+
10	Test for potassium	+
11	Test for sodium	+

Test for Miscellaneous

Sl. No	Test	Results of Kothanthi thalagam
1.	Test for starch	_
2	Test for reducing sugar	_
3	Test for alkaloids	-
4	Test for tannic acid	_
5	Test for Type of compound	_

Acid and basic radicals present in KTP:

Acid radicals present	Sulphate, Sulphide, fluoride & oxalate
Basic radicals present	Arsenic, Iron, calcium, potassium, sodium

5.2. RESULTS OF QUANTITATIVE ANALYSIS

5.2.1.ICP-OES ANALYSIS

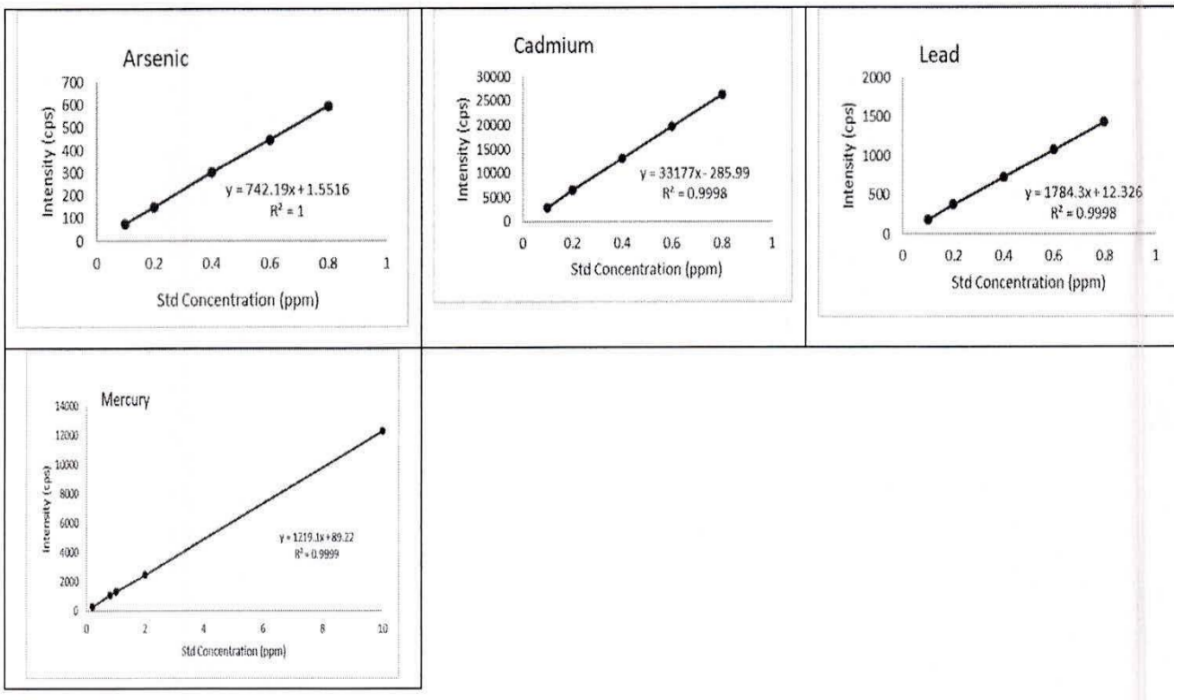
Analytical Report

Sample	Arsenic	Cadmium	Lead	Mercury
Kodhandi Thalaga Parpam	47.30 %	BDL	BDL	BDL

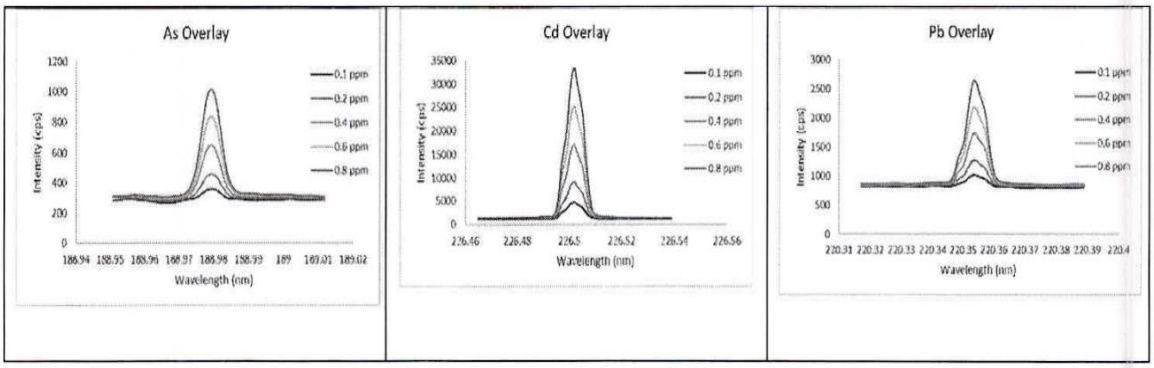
*BDL – Below Detection Limit

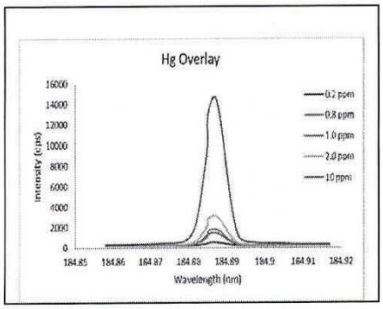
Standard Linearity:-

S.No	Element	Wavelength	R^2 Value
1	Arsenic [As] (µg/g)	188.980 nm	1.0000
2	Cadmium [Cd] (µg/g)	226.502 nm	0.9998
3	Lead [Pb] (µg/g)	220.353 nm	0.9998
4	Mercury [Hg] (µg/g)	184.887 nm	0.9999

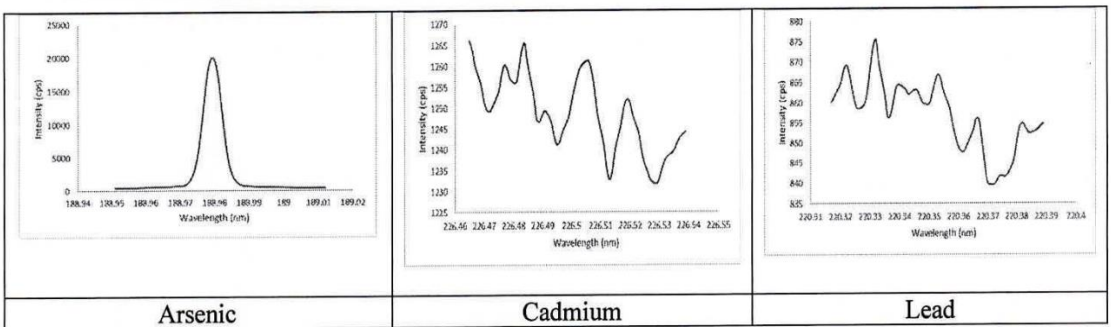


Standard Overlay

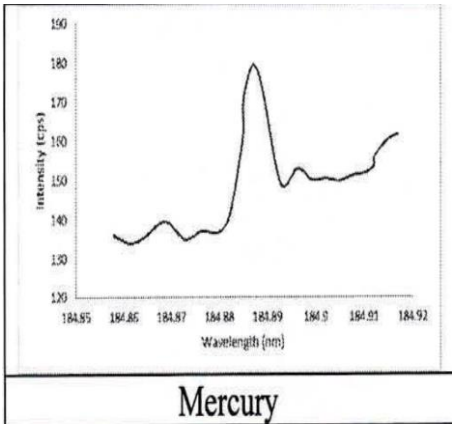




Kodhandi Thalaga Parpam Sample graph

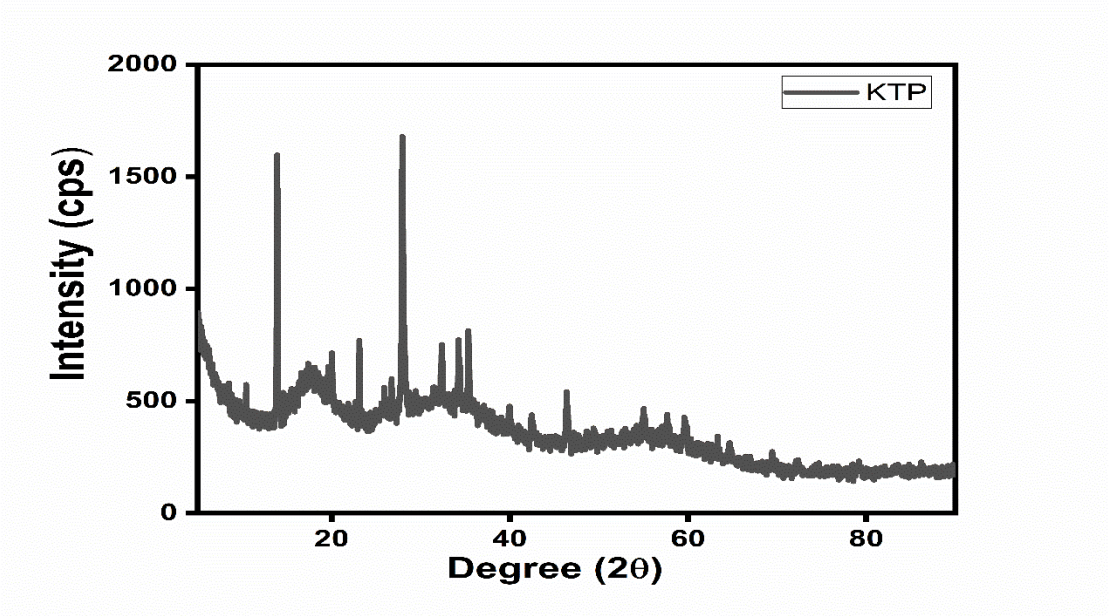


Arsenic | Cadmium | Lead



Mercury

5.2.2.XRD ANALYSIS:



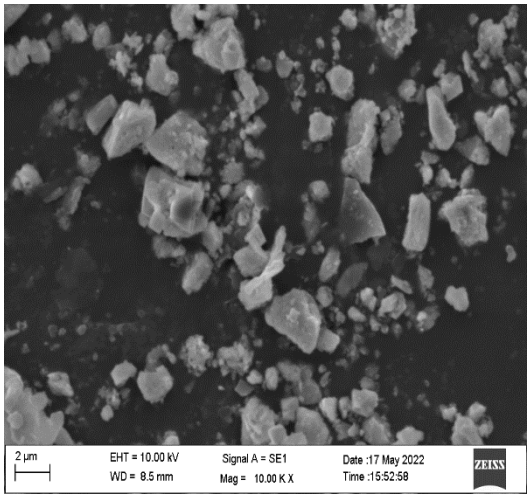
Using a Bruker D8 diffractometer and Cu K (1.5406 °A) radiation as an X-ray source, the XRD pattern was recorded at a scan rate of 1 min^{-1} in the 2-theta range from 5^o to 90^o.

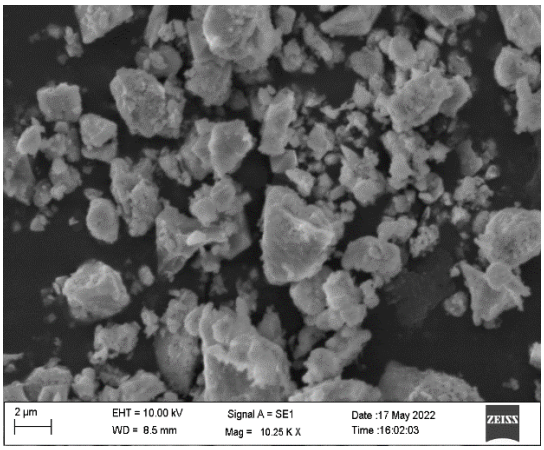
The X-ray diffraction method was used to examine the phase purity and crystallinity of the produced metal drug samples. The characteristic peaks located at 2θ 14.08, 17.30, 19.86, 23.08, 27.9, 32.34, 34.36, 35.29, 46.29, 55.01, 57.69, and 59.57. The crystalline nature and grain size were investigated through XRD.

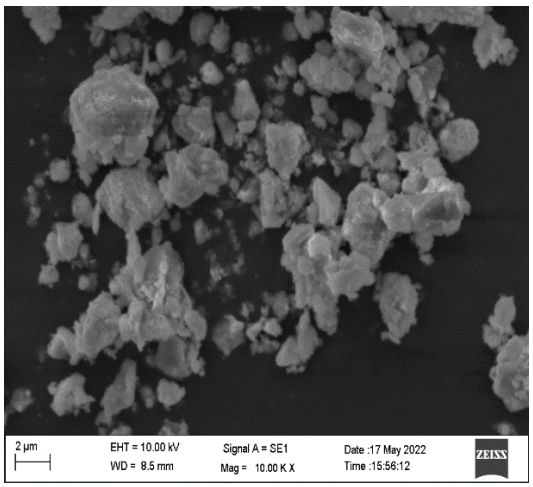
The crystallite size was calculated from the full width half maximum data of the XRD peaks. The peaks observed (2θ) at 17.30, 19.86, 23.08, 27.9, 32.34, 34.36, 35.29 are signatures of As_2S_3 as per JCPDS data (No: 19-0084). The peak which appears at 17.30, corresponds to orpiment (As_2S_3). The peaks (2q) at 13.49, 21.94, 27.54, 28.75, and 32.69 were compared with JCPDS data and found to be As (rhombohedral) with average intensity of 90 (arbitrary unit).

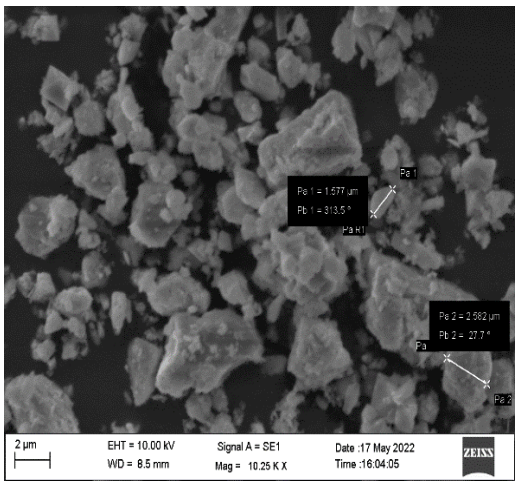
The average crystallite size of metal drug samples of iron based nanocomposites, implying that thalaga parpam played a role of a surfactant to some extent and contributed to the reduction of the nanoparticle size and reduce the unwanted impurities.

5.2.3.SEM – EDS ANALYSIS:





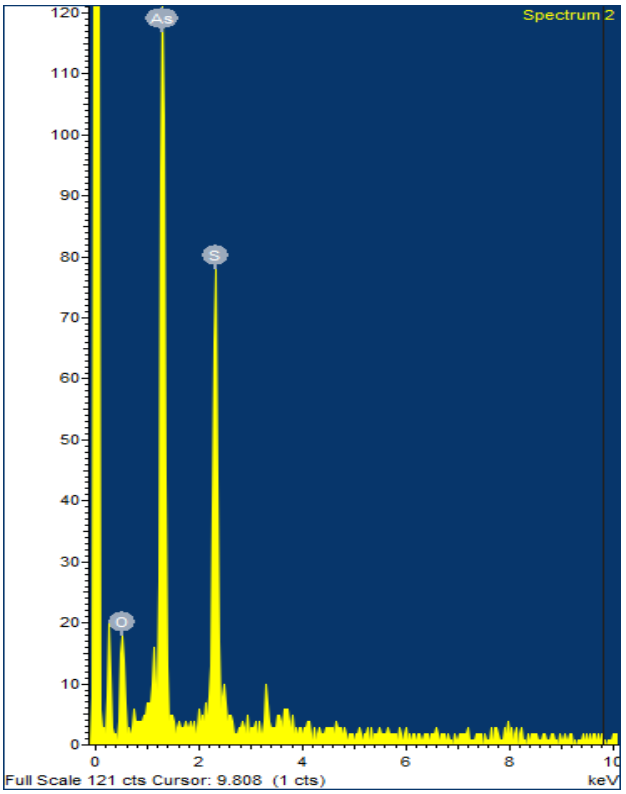


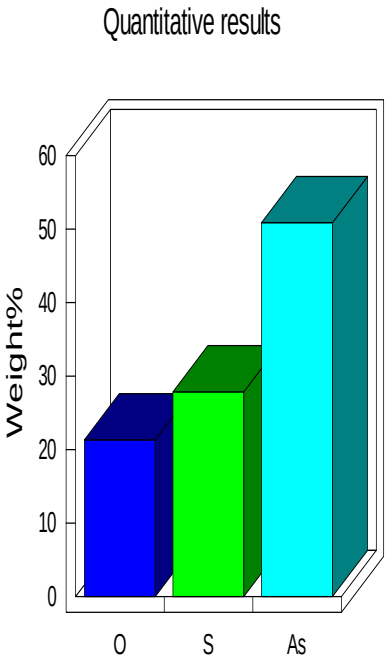


SEM and EDX were used to examine the morphological and elemental composition. Nanoparticles were found to be a heterogeneous collection of sizes ranging from 1.57 to 2.5 µm in diameter. This could improve permeability and effectiveness. They were anisotropic and came in a variety of shapes, from spherical to plate-like. Platelike structures are interesting because they have higher surface-to-volume ratios than spherical structures. Furthermore, shape anisotropy has a significant impact on their chemical reactivity.

The presence of holes within platelets could indicate the aggregation of small particles. A corrugated layered structure of orpiment is likewise supported by the pores. The presence of As (50.86 %), S (27.85 %), and O (21.29 %) may be seen in a section of the EDX spectrum The findings point to the presence of As, S, and O in the formulation. To bond with As, the quantity of sulphur is greater than the proportion of oxygen.

Despite the fact that arsenic may make covalent bonds with both oxygen and sulphur, the atom percent shows that the formulation contains mostly sulphur atoms, either as orpiment (As_2S_3) or realgar (As_2S_4), and less likely As_2O_3. Orpiment and realgar's bonding and atomic configurations may be related to their stability, as they are stable across a wide temperature range.





5.3.TOXICITY STUDIES:

Results of Acute Toxicity Study:

Acute toxicity study of *Kothanthi thalaga parpam* was conducted as per OECD guidelines. No mortality or treatment related toxicity was noted in any animals during the experimental period. The animals were gained bodyweight gradually.

There was no significant difference between the groups in food and water intake throughout the study period. Normal signs were noted in skin, fur, eyes, mucous membranes, salivation, and sleep in control and treated animals. No other signs like tremors, convulsions, lethargy, coma, diarrhea were observed during the study period. No pathological changes were observed in the vital organs of all groups during necropsy.

Table.5.3.1.Effect of *kothanthi thalaga parpam* on behavioural parameters of Wistar albino rats

Group	BEHAVIOURAL OBSERVATIONS																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
GroupI	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Group II	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-

(+) Presence of activity; (-) Absence of activity

Observation:

1.alertness 2. aggressiveness 3. pile erection 4. grooming 5. gripping 6. touch response 7. Decreased Motor Activity 8. Tremors 9. convulsion 10. Muscle spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15. Lacrimation 16. Exophthalmos 17. Diarrhea 18. writhing 19. respiration 20. Mortality

Repeated Dose 28 Day oral toxicity study:

There was no mortality and treatment associated toxicity noted in all groups during the experimental period.

5.3.2.EFFECT OF KOTHANTHI THALAGA PARPAM ON BODY WEIGHT:

Table.5.3.2.1.Body Weight Changes Of Male Wistar Albino Rats In Repeated Dose 28 Day Oral Toxicity Study Of Kothanthi Thalaga Parpam:

GROUP	CONTROL		LOWDOSE		MID DOSE		HIGHDOSE	
	MEAN	S.D	MEAN	S.D	MEAN	S.D	MEAN	S.D
1st Week	168.6	7.47	169	6.45	169	8.05	175.4	6.22
2nd Week	180.8	6.68	180.6	7.55	179	7.16	185.4	2.73
3rd Week	192.6	5.31	188	7.92	190	6.84	198	3.85
4th Week	208	6.29	202.8	8.49	204.4	7.47	215.4	6.71

Values are expressed as Mean ± S.D. Statistically significance (p) calculated by one way ANOVA followed by Dunnett's test (N = 5). The P*<0.05,P**<0.01 calculated by comparing control groups with treated groups.

Figure.5.3.2.1. Body Weight Changes of Male Wistar Albino Rats in Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam

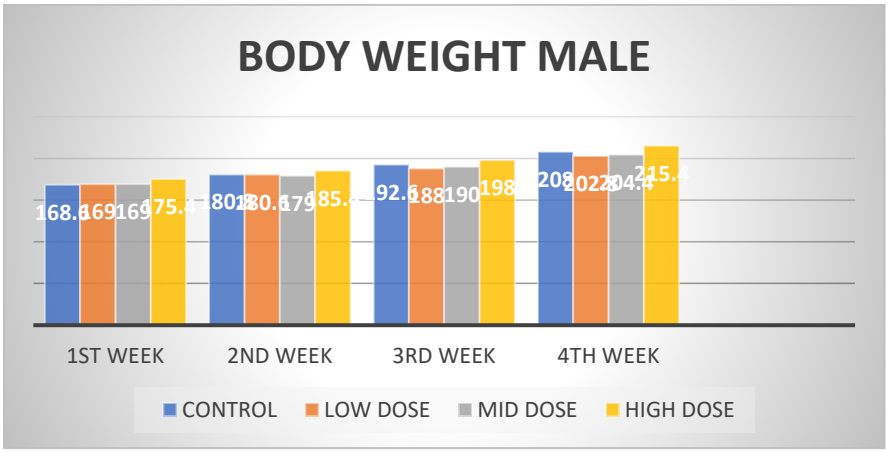
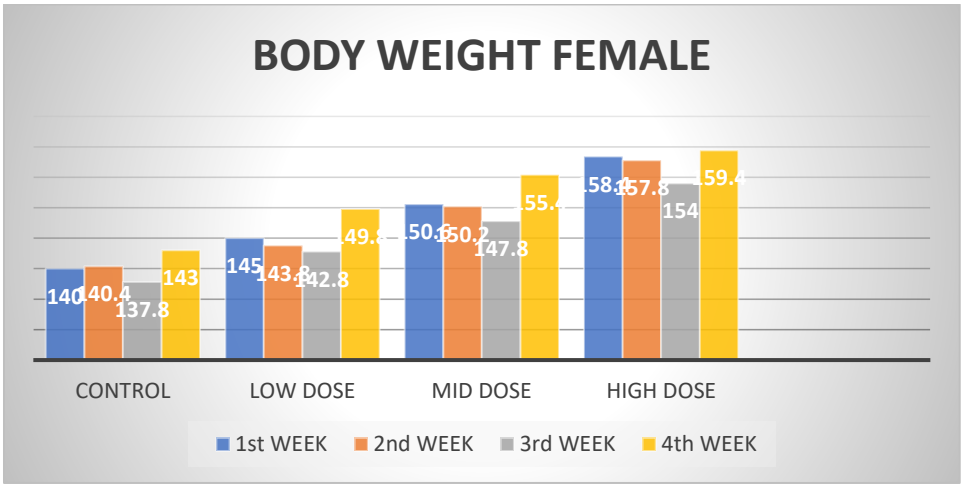


Table.5.3.2.2.Body Weight Changes of Female Wistar Albino Rats in Repeated Dose 28 Day Oral Toxicity Study of kothanthi thalaga parpam

GROUP	CONTROL		LOWDOSE		MID DOSE		HIGHDOSE	
	MEAN	S.D	MEAN	S.D	MEAN	S.D	MEAN	S.D
1st Week	140	3.85	140.4	3.14	137.8	4.96	143	2.09
2nd Week	145	6.98	143.8	6.49	142.8	7.14	149.8	1.6
3rd Week	150.6	6.77	150.2	4.87	147.8	6.91	155.4	1.36
4th Week	158.4	6.22	157.8	4.71	154	6.36	159.4	1.36

Values are expressed as Mean ± S.D. Statistically significance (p) calculated by one way ANOVA followed by Dunnett's test (N = 5). P *<0.05, P**<00.1calculated by comparing control groups with treated groups.

Figure.5.3.2.2.Body Weight Changes of Female Wistar Albino Rats in Repeated Dose28 Day Oral Toxicity Study of *kothanthi thalaga parpam*



The results suggested that body weight of all the kothanthi thalaga parpam treated andcontrol animals were gradually increased during the study period and there were no significant changes observed between control and test drug treated male animals. No Significant changes were observed in all kothanthi thalaga parpam treated female animals (Low Dose, Mid Dose & High Dose) when compared with control group

5.3.3.EFFECT OF KOTHANTHI THALAGA PARPAM ON FEED INTAKE

Table.5.3.3.1.Feed Intake of male wistar albino rats in repeated dose 28 day oral toxicity study of kothanthi thalaga parpam

GROUP	CONTROL		LOWDOSE		MID DOSE		HIGH DOSE	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
1st Week	43.86	5.77	43.29	5.09	48.14	5.84	45	5.50
2nd Week	45.27	5.36	48.86	4.94	46	6.39	46.57	6.28
3rd Week	48.29	5.82	46	7.33	48.57	2.97	47.29	5.50
4th Week	51.71	2.55	48.57	4.69	50	5.15	46.43	5.50

Values are expressed as Mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test (N = 5). P *<0.05,P**<0.01 calculatedby comparing treated groups with control groups.

Figure.5.3.3.1.Feed Intake of male wistar albino rats in repeated dose 28 day oral toxicity study of kothanthi thalaga parpam

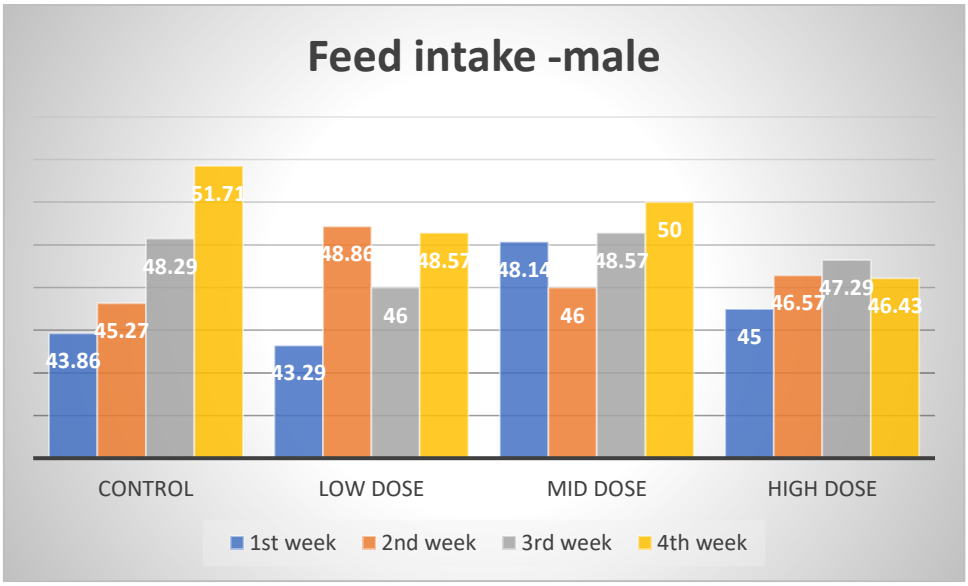
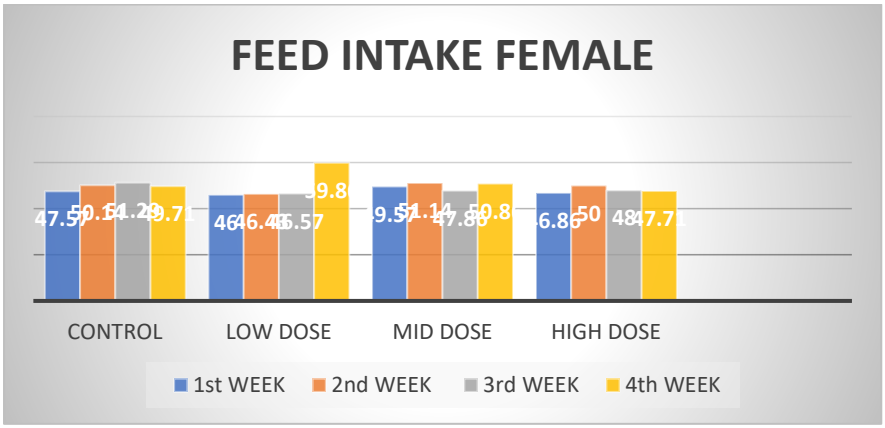


Table.5.3.3.2.Feed Intake of Female Wistar Albino Rats in Repeated Dose 28Day Oral Toxicity Study of kothanthi thalaga parpam

GROUP	CONTROL		LOWDOSE		MID DOSE		HIGHDOSE	
	MEAN	S.D	MEAN	S.D	MEAN	S.D	MEAN	S.D
1st Week	47.57	2.56	46.00	5.55	49.57	3.66	46.86	6.24
2nd Week	50.14	4.76	46.43	6.23	51.14	4.29	50.00	4.87
3rd Week	51.29	2.66	46.57	6.67	47.86	3.44	48.00	7.11
4th Week	49.71	5.36	59.86	5.67	50.86	3.56	47.71	3.92

Values are expressed as Mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test (N = 5). P *<0.05,P**<0.01 calculatedby comparing treated groups with control groups.

Figure.5.3.3.2Feed Intake of Female Wistar Albino Rats in Repeated Dose 28 Day Oral Toxicity Study of kothanthi thalaga parpam



The results suggested that feed intake of control and all the kothanthi thalaga parpam treated animals were gradually increased and there were no significant changes observed between control and test drug treated male and female animals when compared with control group

5.3.4.EFFECT OF KOTHANTHI THALAGA PARPAM ON WATER INTAKE:

Table.5.3.4.1.Water Intake of Male Wistar Albino Rats in Repeated Dose 28 DayOral Toxicity Study of kothanthi thalaga parpam.

GROUP	CONTROL		LOWDOSE		MID DOSE		HIGHDOSE	
	MEAN	S.D	MEAN	S.D	MEAN	S.D	MEAN	S.D
1st Week	45.43	4.27	43.71	2.49	51.43	3.50	49.29	4.16
2nd Week	45.71	7.76	42.14	2.10	48.57	4.40	46.43	4.40
3rd Week	45.00	3.78	42.57	2.82	47.14	5.25	48.57	5.15
4th Week	54.00	2.00	44.00	3.34	50.00	3.78	44.29	3.19

Values are expressed as Mean ± S.D. Statistically significance (p) calculated by one way ANOVA followed by Dunnett's test (N = 5). P* <0.05, P**<0.01 calculated by comparing control groups with treated groups

Figure. 5.3.4.1.Water Intake of Male Wistar Albino Rats in Repeated Dose 28 Day Oral Toxicity Study of kothanthi thalaga parpam

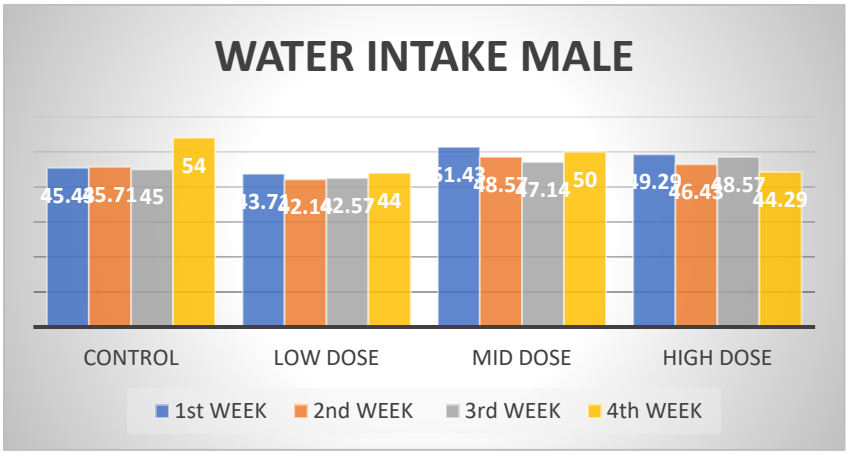
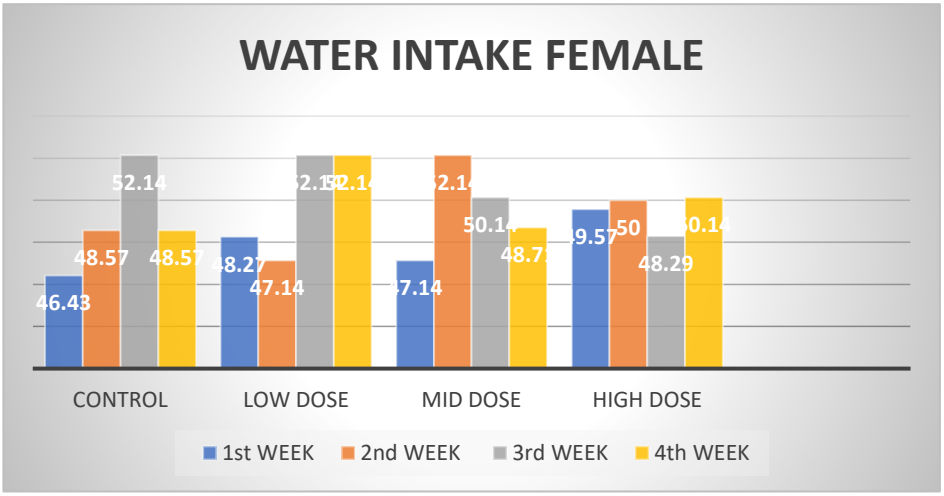


Table . 5.3.4.2.Water Intake of Female Wistar Albino Rats in Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam

GROUP	CONTROL		LOWDOSE		MID DOSE		HIGHDOSE	
	MEAN	S.D	MEAN	S.D	MEAN	S.D	MEAN	S.D
1st Week	46.43	5.15	48.57	5.15	47.14	4.52	49.57	2.56
2nd Week	48.57	3.50	47.14	4.52	52.14	7.00	50.00	4.11
3rd Week	52.14	3.64	52.14	3.64	50.14	6.01	48.29	2.19
4th Week	48.57	3.50	52.14	4.52	48.71	3.19	50.14	3.00

Values are expressed as Mean ± S.D. Statistically significance (p) calculated by one way ANOVA followed by Bartlett's test (N = 5). P* <0.05, P**<0.01calculated by comparing control groups with treated groups

Figure.5.3.4.2.Water Intake of Female Wistar Albino Rats in Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam.



Water intake results suggested that the water intake of all the test animals were gradually increased. No Significant changes were observed in all the test group femaanimals when compared with control group

Table.5.3.5. EFFECT OF KOTHANTHI THALAGA PARPAM ON HAEMATOLOGICAL PARAMETERS.

BLOOD PARAMETER	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
RBC	7.33±0.99	7.21±0.96	7.31±0.95	7.58±0.81
WBC	8.78±2.90	10±2.26	9.92±2.15	8.42±1.72
PLT	642.7±109.80	672.3±53.31	629.2±82.9	671.2±145.3
HGB	12.7±1.51	12.92±1.55	13.25±1.18	13.12±0.93
MCH	17.50±1.66	18.89±2.13	18.09±2.43	17.61±1.77
MCV	58.05±6.08	66.1±7.18	58.37±9.4	64.04±10.04
NEUTROPHIL	2.89±0.93	3.12±1.05	2.75±0.7	2.45±0.84
EOSINOPHIL	1.48±0.22	1.64±0.28	1.47±0.18	1.48±0.27
BASOPHIL	0.44±0.42	0.24±0.33	0.42±0.41	0.47±0.43
LYMPHOCYTES	69.43±6.75	69.33±5.55	75.78±10.81	73.46±4.71
MONOCYTES	3.27±0.60	3.03±0.63	2.99±0.6	3.24±1.11

Values are expressed as Mean ± S.D. Statistically Non significance (p) calculated by one way ANOVA followed by Dunnett's Test (N = 5). The P value considered not Significant. P <0.05, calculated by comparing control groups with treated groups

Figure.5.3.5.1Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam on Haematological Parameters – RBC

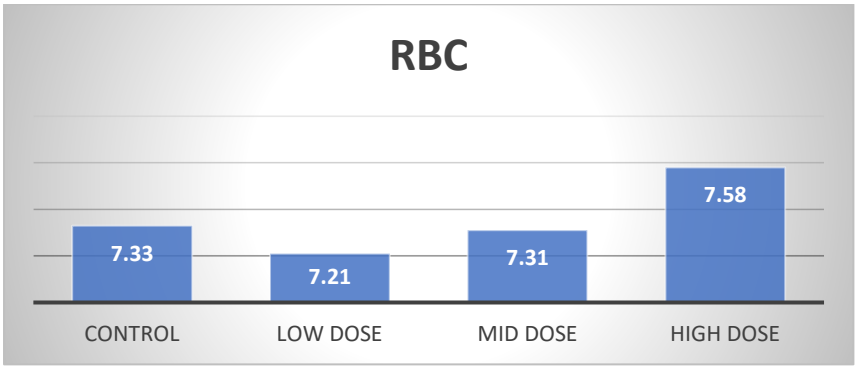


Figure.5.3.5.2.Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam on Haematological Parameters – WBC

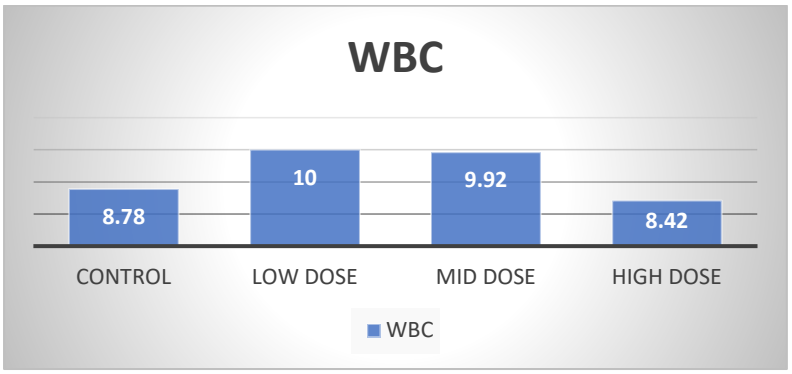


Figure.5.3.5.3.Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam on Haematological Parameters – Platelet count

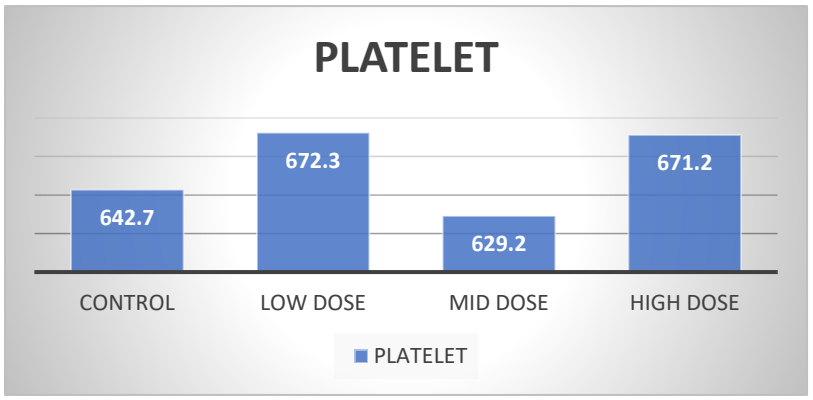


Figure.5.3.5.4.Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam on Haematological Parameters – Haemoglobin

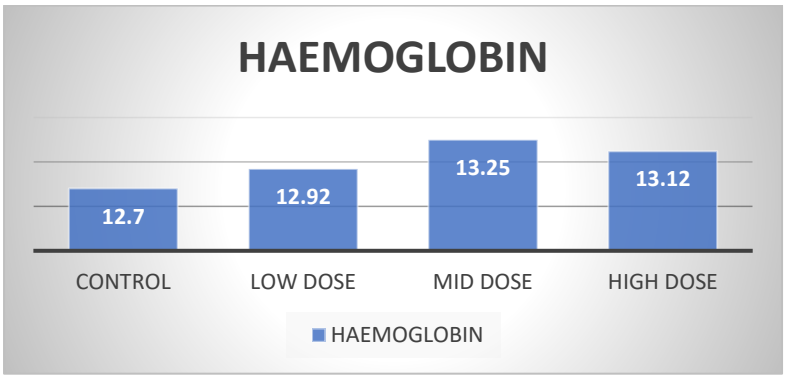


Figure.5.3.5.5.Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam on Haematological Parameters – MCH

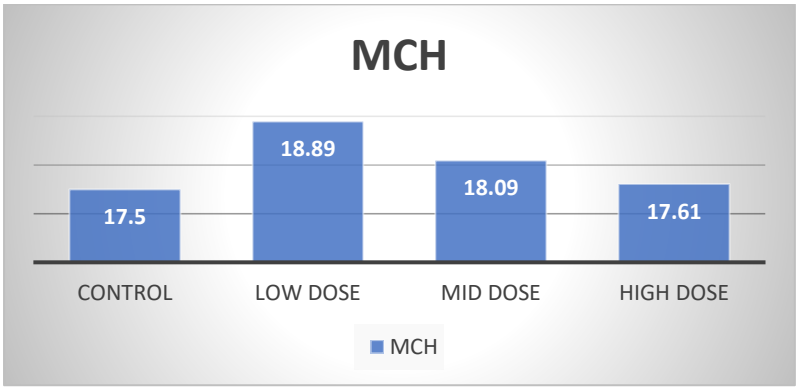


Figure.5.3.5.6.Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam on Haematological Parameters – MCV

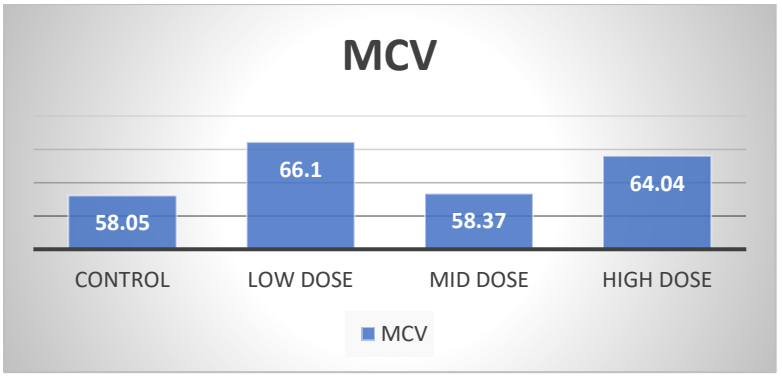


Figure.5.3.5.7.Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam on Haematological Parameters – Differential count

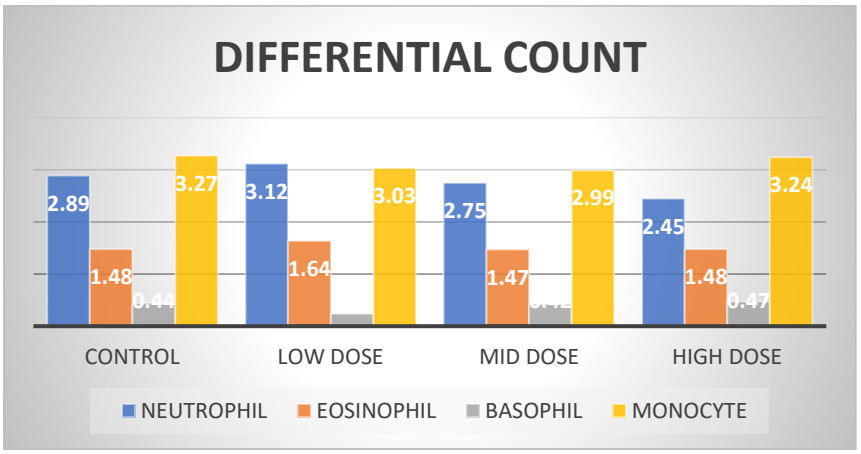
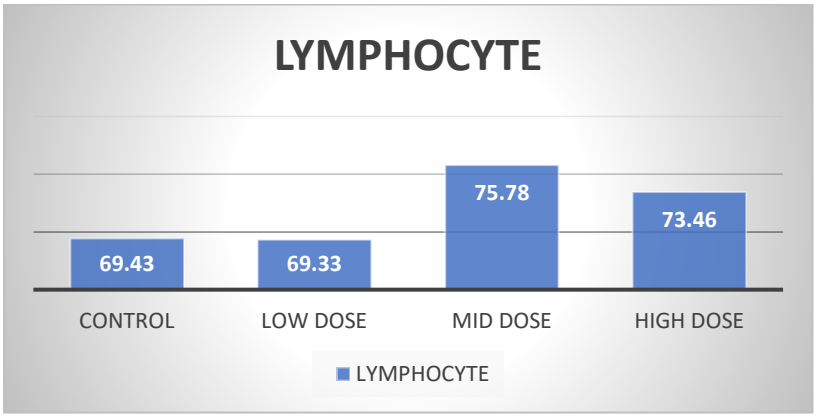


Figure.5.3.5.8.Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam on Haematological Parameters – Lymphocyte



Haematological parameters were assessed in **Kothanthi thalaga parpam** treated animals at end of the study and the results were recorded. It indicates that all the parameters of test animals were within normal level when compared with control group

5.3.6.EFFECT ON KOTHANTHI THALAGA PARPAM ON SEROLOGICAL PARAMETERS – RENALFUNCTION TEST

Table.5.3.6.Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpam on Serological Parameters - Renal Function Test

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BILIRUBIN	15.4±2.32	15.4±2.41	15.8±1.55	14.8±2.04
Serum Creatinine	0.67±0.18	0.63±0.07	0.69±0.14	0.59±0.09

Values are expressed as Mean ± S.D. Statistically Non significance (p) calculated by one way ANOVA followed by Dunnett's test (N = 5). The P value is considered not Significant . P <0.05, calculated by comparing control groups with treated groups

Figure.5.3.6.1.Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpamon Serological Parameters – Renal Function Test – BUN

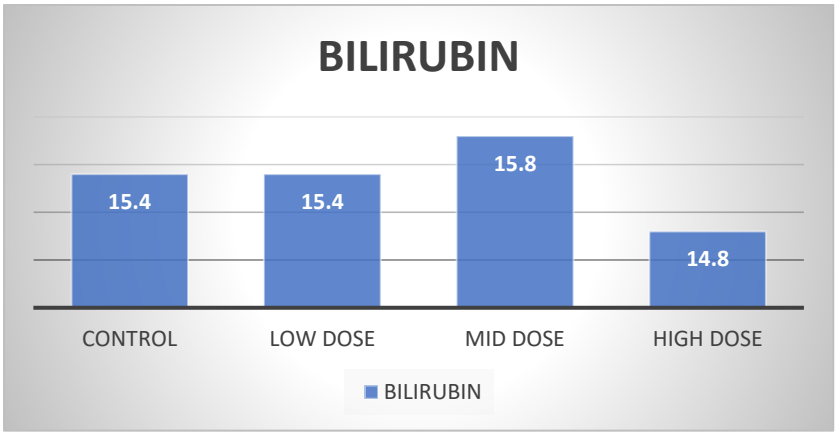
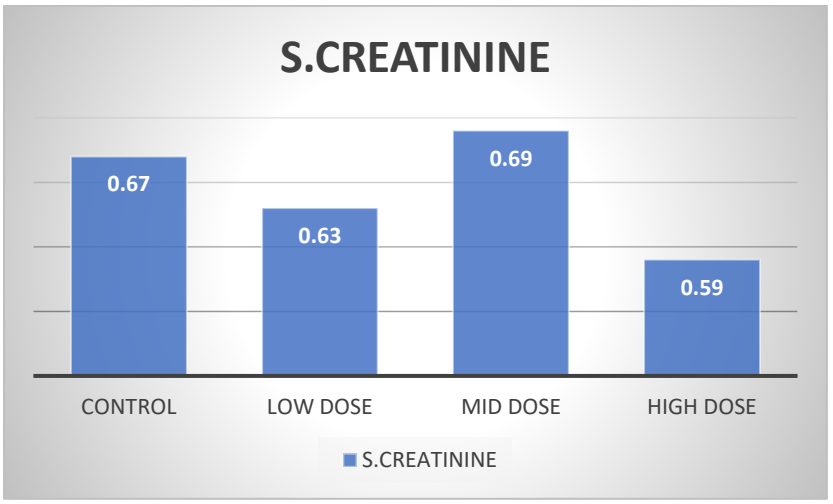


Figure.5.3.6.1.Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpamon Serological Parameters – Renal Function Test – S.Creatinine



Renal parameters were assessed in kothanthi thalaga parpam treated animals at endof the study and the results were recorded. It indicates that all the parameters of test animals were within normal level when compared with control group

5.3.7.EFFECT ON KOTHANTHI THALAGA PARPAM ON SEROLOGICAL PARAMETERS – LIVER FUNCTION TEST

Table.5.3.7.Effect of Repeated Dose 28 Day Oral Toxicity Study of kothanthi thalaga parpam on Serological Parameters - Liver Function Test

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
T. BILIRUBIN	0.35±0.08	0.36±0.1	0.41±0.09	0.4±0.12
SGOT	88.5±9.24	89.2±11.85	79±5.85	85.2±10.2
SGPT	25.4±2.59	25.1±8.92	23.7±5.4	28.1±4.93

Values are expressed as Mean ± S.D. Statistically Non significance (p) calculated by one way ANOVA followed by Dunnett's test (N = 5). The P value is considered not Significant. P <0.05, calculated by comparing control groups with treated groups

Figure.5.3.7.1.Effect of Repeated Dose 28 Day Oral Toxicity Study of kothanthi thalaga parpam on Serological Parameters – Liver Function Test – SGOT

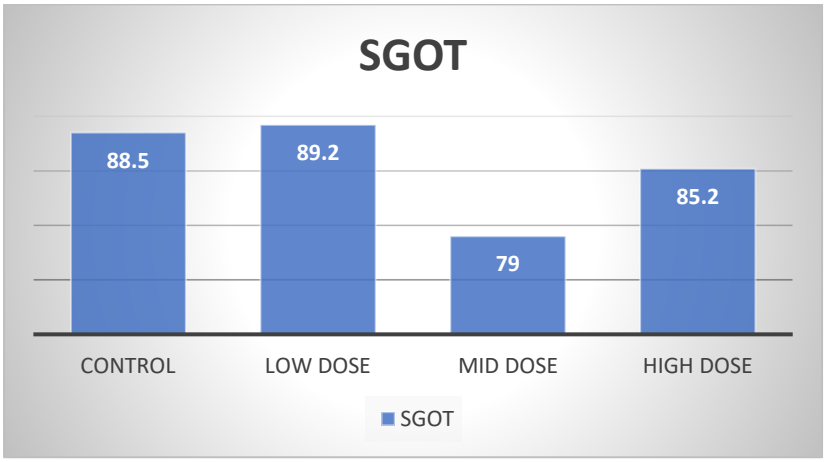


Figure.5.3.7.2. Effect Repeated Dose 28 Day Oral Toxicity Study of kothanthi thalaga parpam on Serological Parameters – Liver Function Test – SGPT

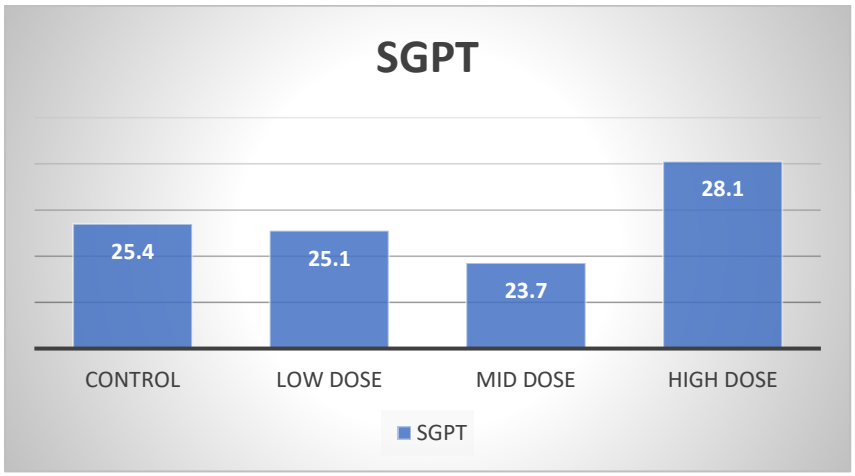
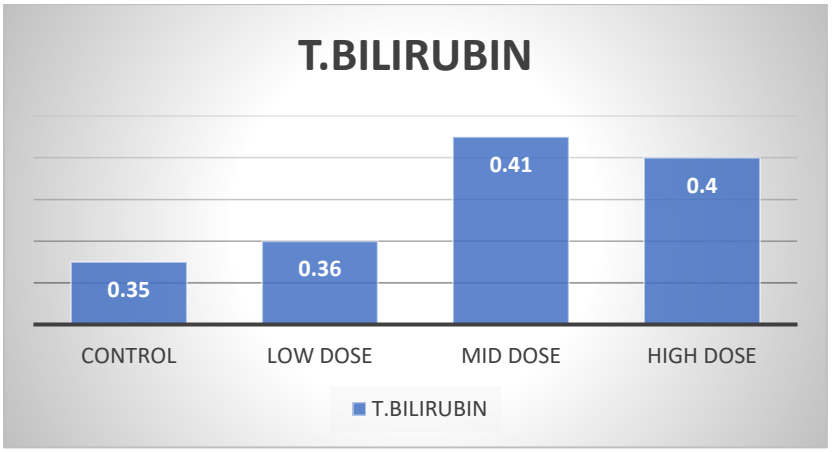


Figure.5.3.7.3.Effect of Repeated Dose 28 Day Oral Toxicity Study of kothanthi thalaga parpam on Serological Parameters – Liver Function Test –



Liver function test were assessed in kothanthi thalaga parpam treated animals at endof the study and the results were recorded. It indicates that all the parameters of test animals were within normal level when compared with control group

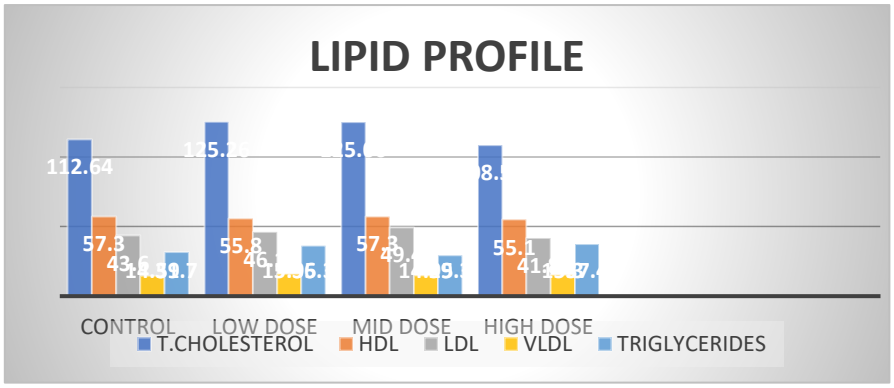
5.3.8.EFFECT OF KOTHANTHI THALAGA PARPAM ON SEROLOGICAL PARAMETERS – LIPID PROFILE

Table.5.3.8.Effect of Repeated Dose 28 Day Oral Toxicity Study of kothanthi thalaga parpam on Serological Parameters – Lipid Profile

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
T. Cholesterol	112.64±13.02	125.26±26.56	125.06±9.59	108.59±7.15
HDL	57.3±5.83	55.8±8.68	57.3±7.54	55.1±3.45
LDL	43.6±7.85	46.1±7.98	49.4±5.87	41.8±6.73
VLDL	14.59±2.5	15.95±1.43	14.95±2.4	15.3±2.63
Triglycerides	31.7±5.48	36.3±9.17	29.3±8.88	37.4±9.52

Values are expressed as Mean ± S.D. Statistically Non significance (p) calculated by one way ANOVA followed by Dunnett's test (N = 5). The P value is considered not Significant. P <0.05, calculated by comparing control groups with treated groups

Figure.5.3.8.Effect of Repeated Dose 28 Day Oral Toxicity Study of Kothanthi thalaga parpamon Serological Parameters – Lipid Profile

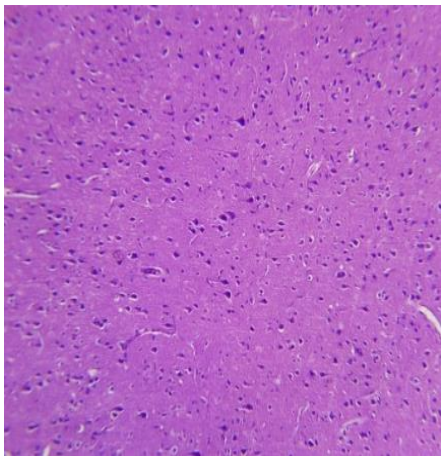


Lipid profile was assessed in test drug treated animals at the end of study and results were recorded. It indicates that all the parameters of test animals were within normal level when compared with control group .

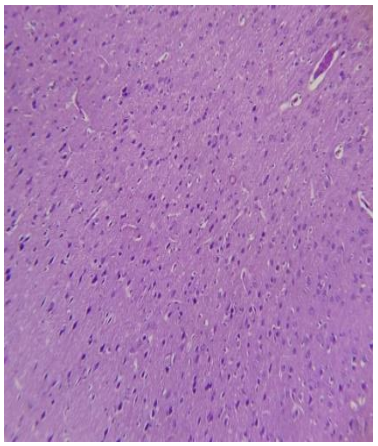
HISTOPATHOLOGICAL FINDINGS AND RESULTS OF 28 DAYS REPEATED ORAL TOXICITY STUDIES OF KOTHANTHI THALAGA PARPAM

HISTOPATHOLOGY OF BRAIN

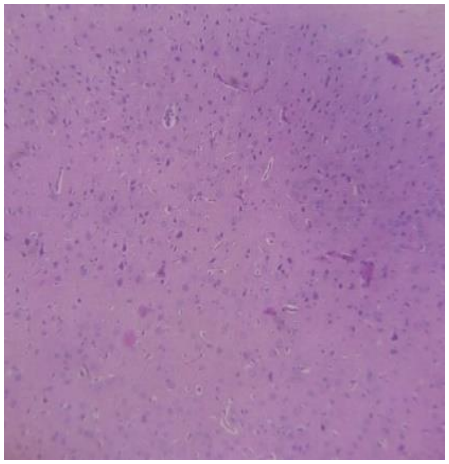
CONTROL MALE



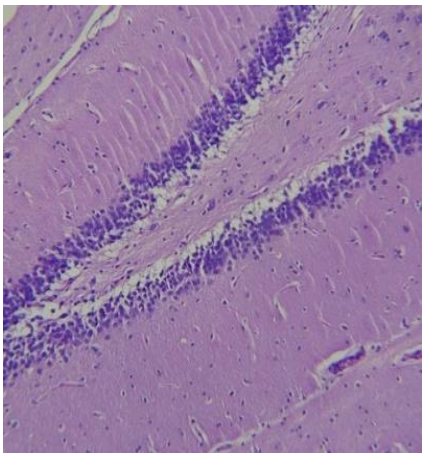
CONTROL FEMALE



HIGH DOSE MALE

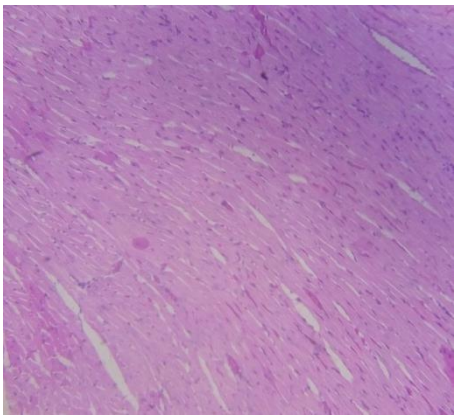


HIGH DOSE FEMALE

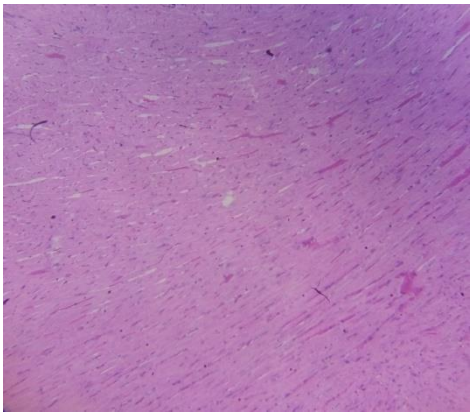


HISTOPATHOLOGY OF HEART

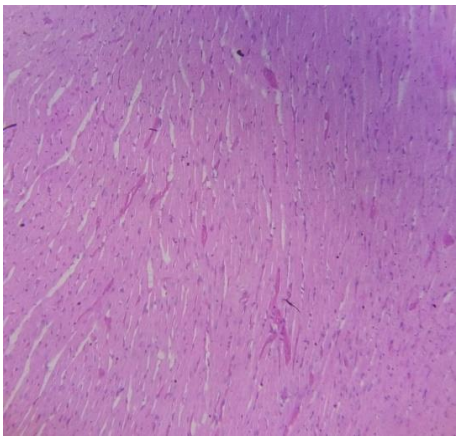
CONTROL MALE



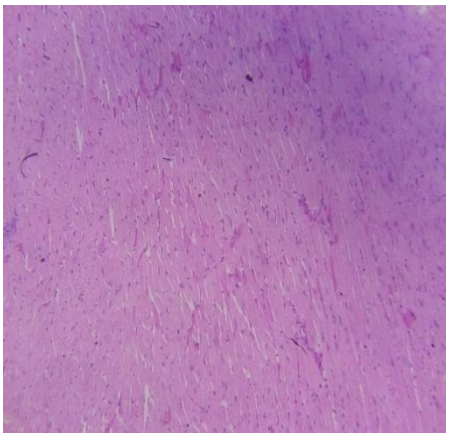
CONTROL FEMALE



HIGH DOSE MALE

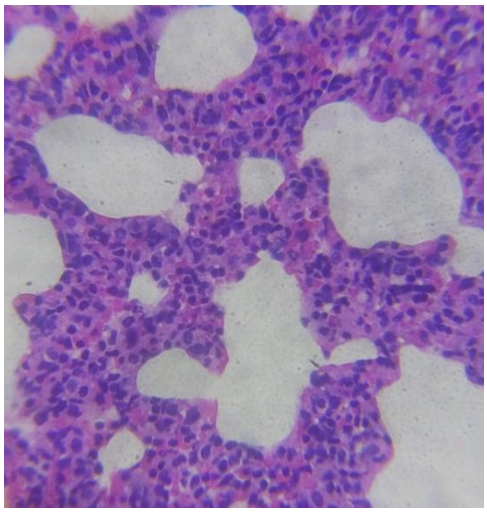


HIGH DOSE FEMALE

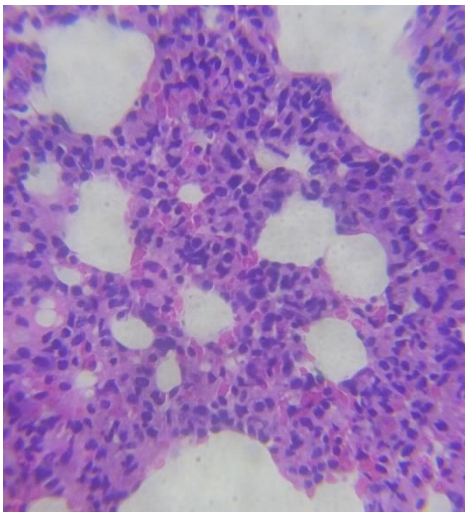


HISTOPATHOLOGY OF LUNGS

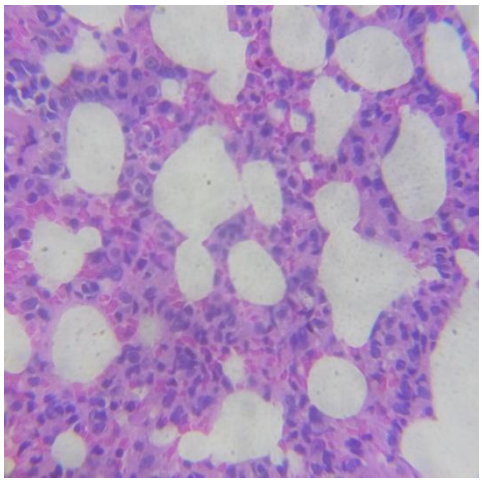
CONTROL MALE



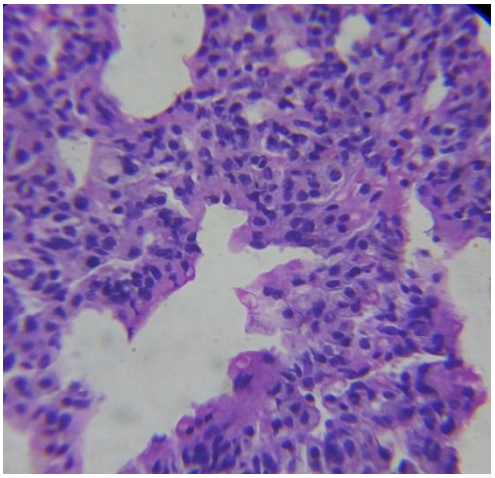
CONTROL FEMALE



HIGH DOSE MALE

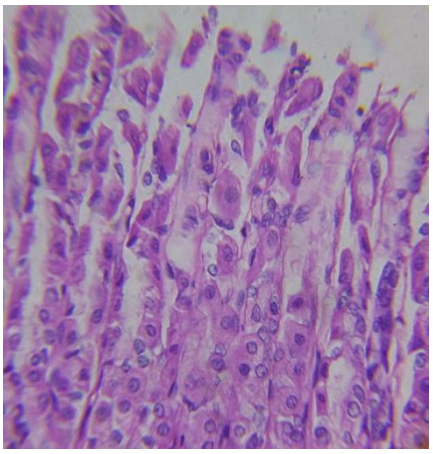


HIGH DOSE FEMALE

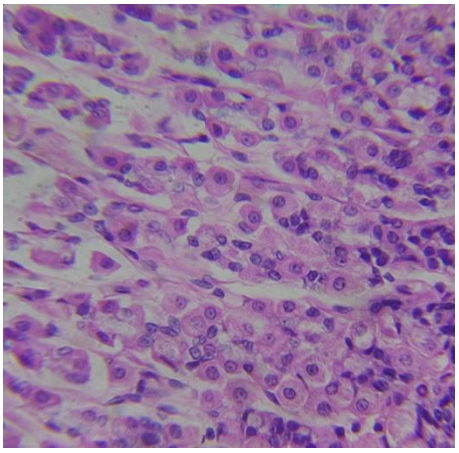


HISTOPATHOLOGY OF STOMACH

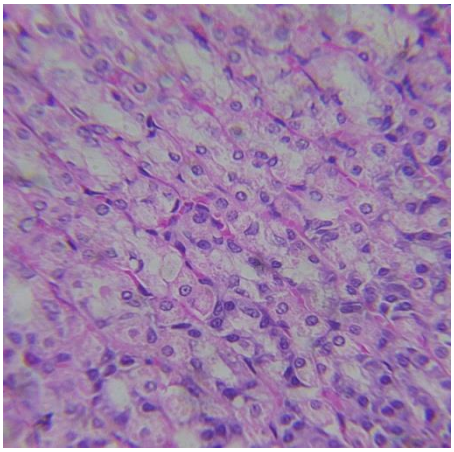
CONTROL MALE



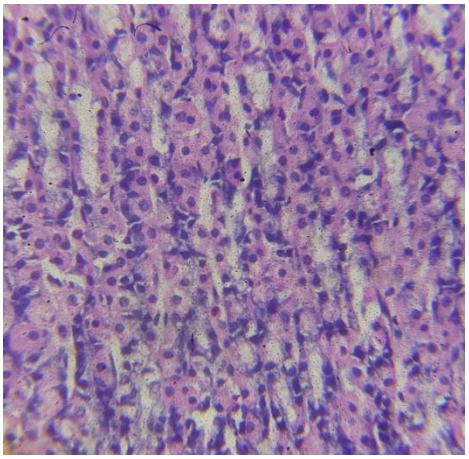
CONTROL FEMALE



HIGH DOSE MALE

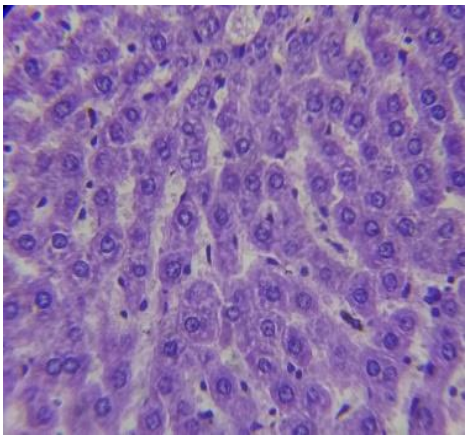


HIGH DOSE FEMALE

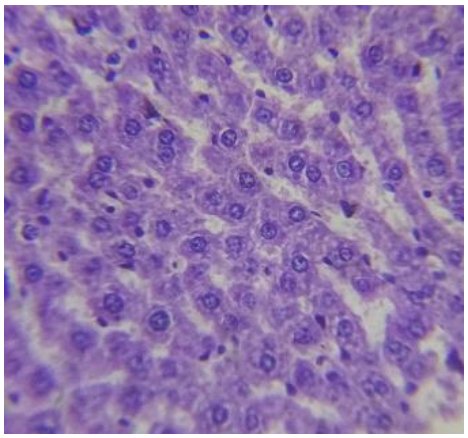


HISTOPATHOLOGY OF LIVER

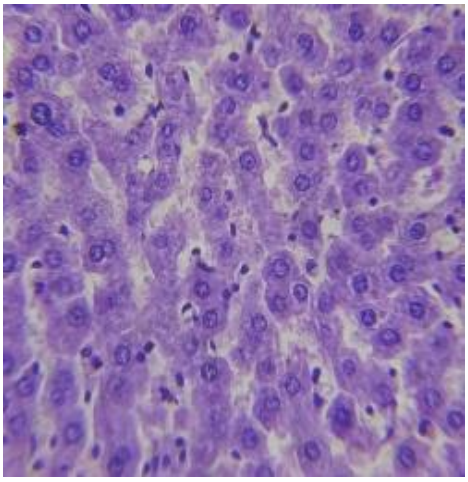
CONTROL MALE



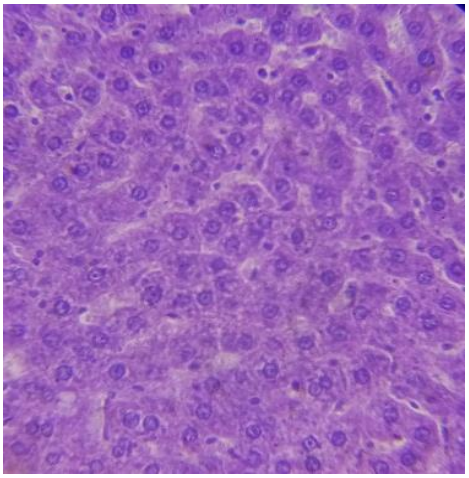
CONTROL FEMALE



HIGH DOSE MALE

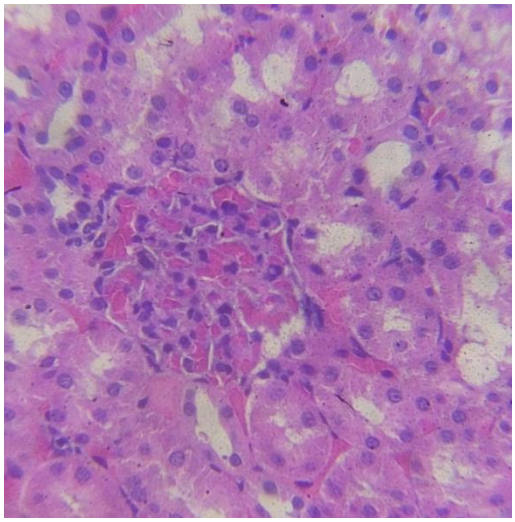


HIGH DOSE FEMALE

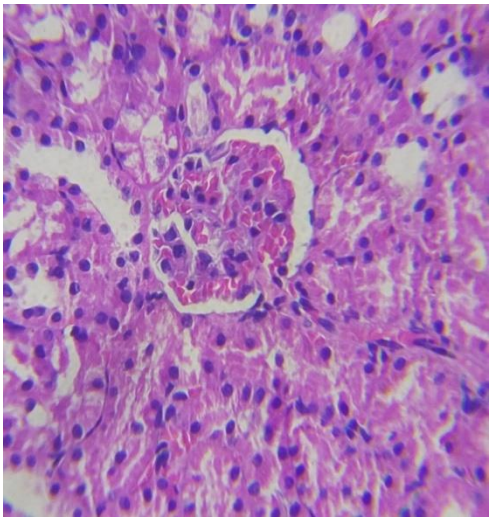


HISTOPATHOLOGY OF KIDNEY

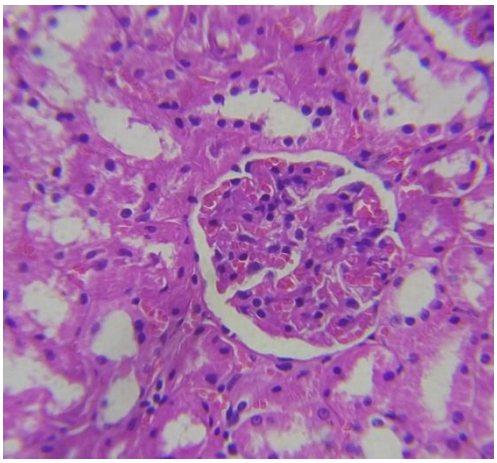
CONTROL MALE



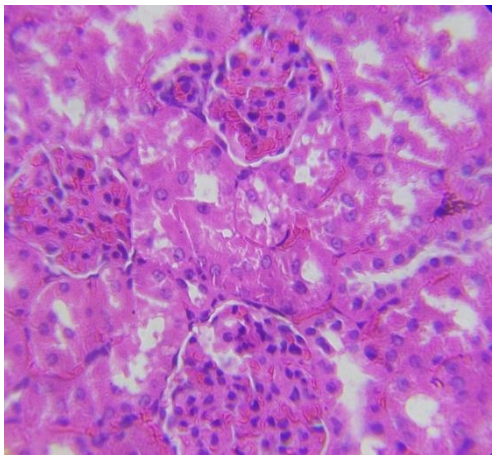
CONTROL FEMALE



HIGH DOSE MALE

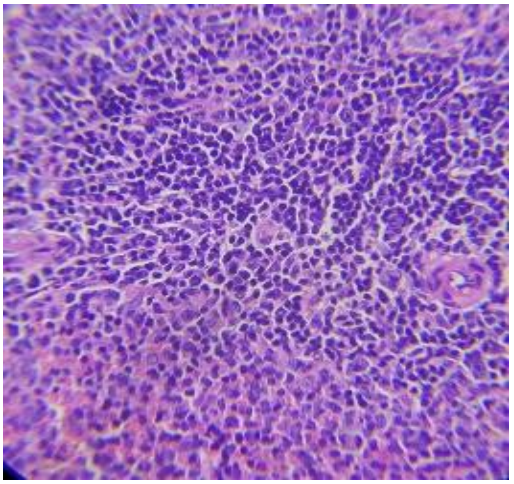


HIGH DOSE FEMALE

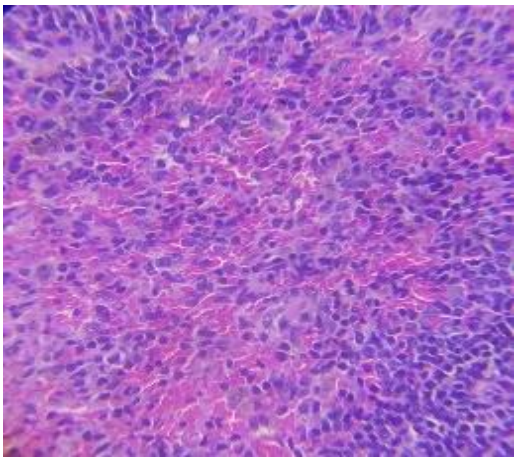


HISTOPATHOLOGY OF SPLEEN

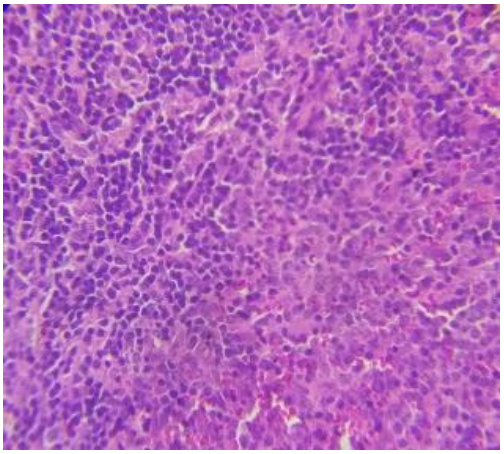
CONTROL MALE



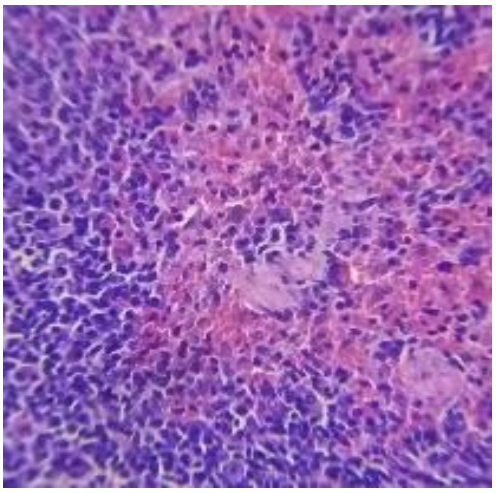
CONTROL FEMALE



HIGH DOSE MALE

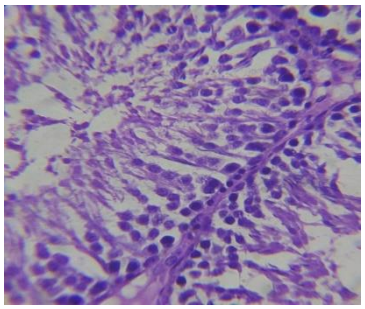


HIGH DOSE FEMALE

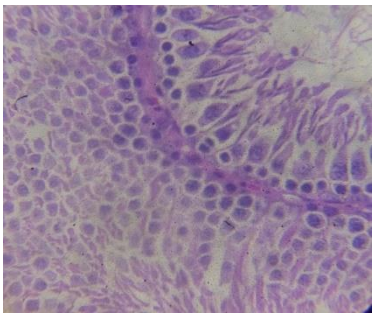


HISTOPATHOLOGY OF TESTES

CONTROL MALE

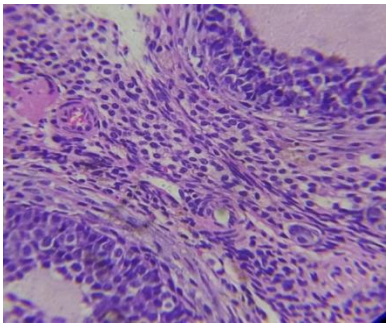


HIGH DOSE MALE

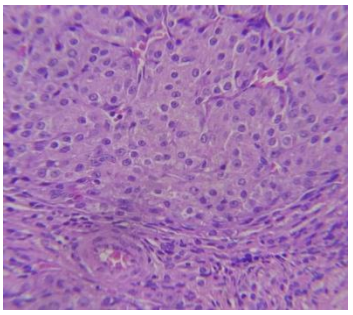


HISTOPATHOLOGY OF OVARY

CONTROL FEMALE

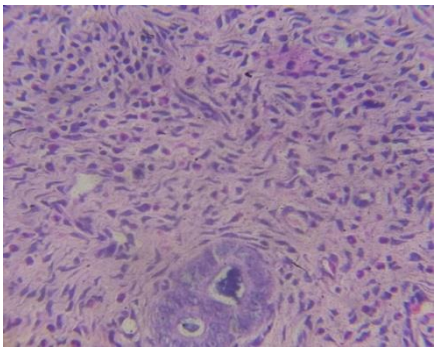


HIGH DOSE FEMALE

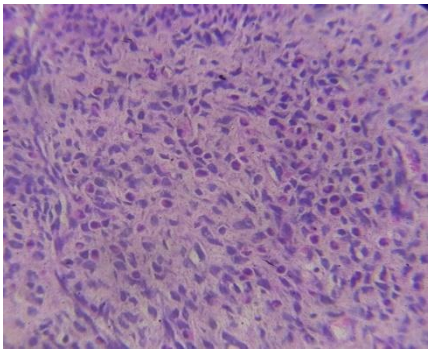


HISTOPATHOLOGY OF UTERUS

CONTROL FEMALE



HIGH DOSE FEMAL



HISTOPATHOLOGICAL FINDINGS OF CONTROL GROUP

TISSUES	FINDINGS		RESULTS
	MALE	FEMALE	
BRAIN	Arrangement of neurons on cerebral cortex appears normal and dense	Showed normal architecture in both cortex and medulla	**Normal**
HEART	Endocardium appears normal with no evidence of necrosis	Appearance of fibrils and cross striations are equidistant	**Normal**
LUNGS	Alveolar epithelium and capillaries appears normal	Opening of lumen of blood vessels appears regular with no invasion of inflammatory cells	**Normal**
STOMACH	Lumina of blood vessels appears normal. Appearance of glandular lumen was normal	Pyloric and fundus zone of stomach appear normal	**Normal**
LIVER	Liver parenchyma appears normal with no evidence of necrosis	Showing normal hexagonal hepatic lobules with normal, regular radiated hepatic cords from the central vein to the peripheral of lobule, central vein	**Normal**
KIDNEY	Normal renal structure with rounded renal corpuscles formed of the Glomerulus and the Bowman's capsule	Appearance of proximal and distal convolutes tubules was normal with no evidence of atrophy	**Normal**

SPLEEN	Erythropoietic cells (EP) are scattered throughout the red pulp. No abnormalities found in lymph node	Regular appearance of red pulp is composed of a three dimensional meshwork of splenic cords and venous sinuses were observed	**Normal**
TESTIS	Appearance of leydig cells, interstitial tissue , seminiferous tubule, Sertoli cells and spermatogonia were normal	------	**Normal**
UTERUS	-----	Normal cyto architecture of uterine layers and glands were observed	**Normal**
OVARY	-----	Follicular cells, cytoplasm and nucleus appears normal	**Normal**

HISTOPATHOLOGICAL FINDINGS OF HIGH DOSE DRUG TREATED GROUP:

TISSUES	FINDINGS		RESULTS
	MALE	FEMALE	
BRAIN	Arrangement of neurons on cerebral cortex appears normal and dense	Brain histology appears normal with no signs of ischemic changes in the cerebral hemisphere	**Normal**
HEART	Myocardial cells appears normal with well-defined mycoplasma and prominent nucleus and nucleolus	Normal appearance of myocytes; myofibres with no evidence of edema	**Normal**
LUNGS	Showing normal alveoli and collagen fibres	Perivascular region appears normal, Alveolar septa and wall appeared widen and normal	**Normal**
STOMACH	Mucosal wall appears normal with regular arrangement of connective tissue	Normal circular muscle and muscolaris mucosa zone	**Normal**
LIVER	Centrilobular zone appears normal with stable network of hepatocytes	Normal hepatocytes with no signs of necrosis	**Normal**
KIDNEY	Appearance of glomerular basement membrane was normal	Lumen of distal convolutes tubule and collecting duct was normal	**Normal**
SPLEEN	Appearance of LF – lymphoid follicle; PALS – periarterial lymphoid sheath was normal with no significant signs of enlargement	Regular histology of Marginal zone along with marginal and germinal centre were observed	**Normal**

TESTIS	Normal sertoli cell aligned properly on the basement membrane with oval dome shaped nucleus shows the normal morphology of the seminiferous tubule were observed	-----	**Normal**
UTERUS	-----	Arrangement of stratum basale, functionale and surface epithelium seems normal	**Normal**
OVARY	-----	Appearance of graafian and antral follicle was normal	**Normal**

RESULTS

The microscopic findings observed in various organs such as Brain, Heart, Lungs, Stomach, Liver, Kidney, Spleen, Testis and Uterus, Ovary of Control group showed no pathological changes. High dose treated group and Satellite group showed no pathological changes in Brain, Heart, Lungs, Stomach, Liver, Kidney, Spleen, Testis and Uterus, Ovary.

These results suggest Kothanthi thalaga parpam did not induce any lesions of toxicological significance in the organs examined under the experimental conditions employed as per the study plan.

DISCUSSION

Kothanthi thalaga parpam, a Herbo -mineral drug consists of heavy metal thalagam and nayuruvi sambal .it is used to cure various ailments like swasakaasam, irumal, suram[7].The present study aimed to reveal the safety of KTP.

Herbo-mineral preparations have been used in Siddha system for centuries to treat various severe illnesses without any toxic effects. The purification process and preparation techniques make the metallic compound to non-toxic form and make it suitable for human consumption[52]. But recently, concerns about the safety of using Herbo-mineral drugs have been raised. So, in this study the safety profiling of kothanthi thalaga parpam is done as per OECD guidelines using Wistar albino rats.

kothanthi thalaga parpam possess anti-convulsant, antimicrobial, anticancer, antiproliferative anticancer, antimalarial, immunostimulant, anthelmintic, spermicidal, antiparasitic, hypoglycemic, hepatoprotective, anti-inflammatory, antiarthiritic, antioxidant, nephroproductive, cardiovascular, bronchoprotective, anti allergic & wound healing activities which correlates well with the indications mentioned for the test drug(KTP).

The standardization procedures such as Siddha standardization procedures, organoleptic characters, physicochemical analysis, biochemical analysis,analytical methods like ICP-OES, XRD,SEM-EDS and safety profiling of the test drug were done as per standard guidelines.

Siddha specification of parpam is mentioned which showed that the parpam is white or grey white colour,odourless, Tasteless, lustreless, smokeless onheating, micro fine in nature and weightless. So, the test drug satisfies the quality of parpam mentioned in Siddha literatures,except colour it may due to sulphite content of drug.

Organoleptic evaluation of kothanthi thalaga parpam is mentioned which showed that test drug is honey orange colour ,no specific odour, taste and has a smooth powdery appearance. Physicochemical analysis of kothanthi thalaga parpam is mentioned which showed that the pH of test drug is 8.92 revealing that the test drug is alkaline in nature. pH of drug plays an important role in drug absorption. kothanthi thalaga parpam being a weak basic drug absorbed more rapidly in intestine, because the basic drugs areunionized when they reach the alkaline medium of intestine where they are rapidly absorbed[53].

Ash values are helpful in determining the quality and purity of crude drugs, especially in powder form[54]. Total ash value of kothanthi thalaga parpam is 74.60% is increased which may be due to presence of metals and mineral compounds in test drug. Acid insoluble ash is 66.45% w/w, Water soluble extractive is 3.32% w/w, Alcohol soluble extractive is 0.62% w/w, Moisture and Volatile matter is 1.53% w/w.

Biochemical analysis revealed the presence of acid radicals such as sulphate and sulphite,fluoride & oxalate. Sulphate is a major contributor to the ionic strength of urine and alters the equilibrium constants governing saturation and precipitation of calcium salts[55].The basic radicals such as Iron, Arsenic, calcium,potassium,sodium were found in the test drug. Calcium lowers risk of diabetes mellitus by normalizing the glucose tolerance[56]. It also controls nerve excitability, maintains the integrity of the skeletal muscles and bones, maintains the tone and contractility of heart, reduces allergic reactions[57]. Potassium and sodium regulate the body's acid base balance ,osmotic pressure,& fluid balance.it necessary for the normal muscle irritability and permeability.Iron helps in transport of oxygen, necessary for electron transport chain andoxidative phosphorylation, involves in phagocytosis and associated with effectiveimmunocompetence of body[5]

ICP-OES result showed that the presence of wavelength is 188.980 nm of Arsenic, 226.502 nm of Cadmium, 220.353 nm of Lead,184.887 nm of Mercury in kothanthi thalaga parpam. ICP-OES result revealed that the heavy metal is present within below detection limit except Arsenic 47.30% in kothanthi thalaga parpam. The variation in results due to composition of major element in test drug.

The X-ray diffraction method was used to examine the phase purity and crystallinity of the produced metal drug samples. The characteristic peaks located at 2θ 14.08, 17.30, 19.86, 23.08, 27.9, 32.34, 34.36, 35.29, 46.29, 55.01, 57.69, and 59.57. XRD result showed that crystalline nature (calculated from the full width half maximum data of the XRD peaks) and grain size were investigated in test drug.

SEM-EDS result showed that Nanoparticles were found to be a heterogeneous collection of sizes ranging from 1.57 to 2.5 μm in diameter. This could improve permeability and effectiveness. They were anisotropic and came in a variety of shapes, from spherical to plate-like. The presence of As (50.86 %), S (27.85 %), and O (21.29 %) may be seen in a section of the EDS spectrum The findings point to the presence of As, S, and O in the formulation. the atom percent shows that the formulation contains mostly

sulphur atoms, either as orpiment (As_2S_3) or realgar (As_2S_4), and less likely As_2O_3. Presence of oxygen indicate calcification process.

So, in order to evaluate the safety of drug, Acute and repeated dose 28 days oral toxicity study of kothanthi thalaga parpam was carried out on animal model as per OECD guideline 423 and 407.

In Acute Toxicity study, there was no abnormal behavioural changes noted atthe dose level of 300 and 2000 mg / Kg b.wt of kothanthi thalaga param in female Wistar albino rats. No mortality and morbidity were observed throughout the study period. And no pathological changes were seen in all the internal organs of sacrificed animals at the end of the study period. These results revealed that the LD_{50} of kothanthi thalaga parpam was greater than 2000 mg / Kg b.wt. So, in reference to the Globally Harmonised System of Classification and labelling of chemicals, the test drug kothanthi thalaga parpam is classified under category 5 or Unclassified[59].

In Repeated dose 28 days oral toxicity study, totally 4 groups of Wistar albino rats of both sexes were used. Each group contains 5 males and 5 females. Group I which was set as control received betel juice and Group II, III, IV received 15, 75, 150 mg/Kg b.wt of kothanthi thalaga parpam respectively by oral administration. All the test animals were observed throughout the study period of 28 days . kothanthi thalaga parpam did not produce any behavioural changes in all group of animals.No mortality had been observed throughout 28 days of observation.

The body weight of all the male and female treatment animals gradually increased during the study period and no statistically significant changes were seen when compared with control group. It revealed that the drug didnot produce any metabolic disturbances in rats. Increase in body weight indicates the positive health status of the animals[60].

Feed intake of all Kothanthi thalaga parpam treated animals and control group animals gradually increased throughout the study period and no statistically significant changes were seen when compared with control group. Water consumption of all the treatment animals gradually increased inthe study period, and no significant increase was seen in high dose male animals when compared with control group.

Haematological and biochemicalparameters were analysed at the end of the study. Blood forms the main medium of transport for many drugs and xenobiotics in the body

and for that matter components ofthe blood such as red blood cells, white blood cells, haemoglobin, and platelets are at least initially exposed to no significant concentrations of toxic compounds. Damage and destruction of the blood cells are inimical to normal functioning of the body[61]. The haemopoietic system is one of the most sensitive targets of toxic compounds and an important index of physiological and pathological status in both humans and animals[62].Results of Haematological parameter analysis showed that there were no significant changes in all test drug treated group animals when compared with control group .

Results of Biochemical parameters showed no significant changes in all test drug treated group animals when compared with control group. Biochemical parametershave no significant roles as markers in toxicological study because of their response to clinical signs and symptoms produced by toxicants[63].

Results of Renal function test showed no abnormal changes in all test drug treated animals compared with controlgroup. The results of renal function test revealed that the test drug doesnot produce any abnormalities in kidney function and the drug is safe.

Liver function test was done to detect the effect of test drug on liver. Results of Liver function test (ALT, AST, T.Bilirubin) showed no abnormal changes in all test group treated animals compared with control group . The results of liverfunction test revealed that the test drug does not produce any abnormalities in liver function. Results of lipid profile showed no abnormal changes in all test group treated animals compared with control group.

At the end of the study, all the animals were sacrificed and all the vital organs were observed. There were no gross pathological changes noted. The histopathology of Brain, Heart, Lungs, Liver, Kidney, Stomach, Sciatic nerve, Testis, Uterus and Ovary were done in control, High dose treatment group, and Satellite group. The evaluation of histopathological changes in organs remains a cornerstone in safety assessment of medicines[64]. The histopathology results revealed no abnormal changes and all organs showed normal architecture.

Study showed that up to 150mg/kg b.w(High dose) no mortality was observed and no changes were observed in Haematological, Biochemical, Histopathological Parameters. So the formulation KTP is safe for human consumption. Further clinical trials ,safety &efficacy studies will be carried out KTP to prove the efficacy of drug.

SUMMARY

The test drug kothanthi thalaga parpam consists of thalagam and nayuruvi sambal which was adopted from the text Anupoga vaithiya navaneetham part-6. The aim of the research work was to study the safety of kothanthi thalaga parpam by acute and Repeated dose 28 days oral toxicity study in the wistar albino rat as per OECD Guidelines

The Standardization procedures such as organoleptic characters, Physicochemical analysis and biochemical analysis were analyzed primarily.

The physicochemical analysis of the test drug showed the increasedbioavailability and purity of the drug.

Qualitative analysis of acid and basic radicals showed the presence of acid radicals such as sulphate, sulphite, fluoride& oxalate and the basic radicals such as Arsenic, iron, calcium, potassium, sodium in kothanthi thalaga parpam.

Heavy metal content of KTP was analysed by using ICP-OES method. It result revealed that the heavy metal is present within below detection limit except Arsenic in Kothanthi thalaga parpam. The variation in results due to composition of major element in test drug.

XRD technique of test drug that result showed that crystalline nature (calculated from the full width half maximum data of the XRD peaks) and grain size were investigated in test drug.

Acute toxicity study of Kothanthi thalaga parpam showed no abnormal signs at the dose level of 2000 mg / Kg b.wt. No mortality, morbidity and pathological changes have been seen in internal organs of treated groups during the study period.

Repeated dose 28 days oral toxicity study results did not reveal any behavioral changes, mortality and morbidity. The body weight, feed intake and water intake of all the animals were gradually increased during the study period.

CONCLUSION

Kothanthi thalaga parpam was taken as test drug, the quality parameters and safety profile of the drug were analyzed as per standard protocol. The qualitative and quantitative analysis of Kothanthi thalaga parpam proved the quality and purity of the drug. The Acute toxicity study results did not show any mortality, behavioural changes and drug related toxicity. From the Acute toxicity study, it is revealed that Kothanthi thalaga parpam is not toxic at a maximum oral dose level of 2000 mg/kg in Wistarrats. So, the median lethal dose (LD_{50}) of Kothanthi thalaga parpam is greater than 2000 mg/Kg b.wt. In Repeated Dose 28 Days oral toxicity study, upto highdose level(150mg/kg b.w) no changes were observed haematological and histopathological parameters showed no significant changes in internal organs compared with control group.No Observed Adverse Effect Level (NOAEL) of Kothanthi thalaga parpam is greater than 150mg/kg.b.wt. Hence, the safety of Kothanthi thalaga parpam is proved through this study. From the study results, it is concluded that the therapeutic dose of Kothanthi thalaga parpam 32.5mg-65mg(Arisi edai -1/4 Kundri) is safe for humanconsumption. Further clinical trials, Pharmacological studies and efficacy studies will be carried out on Kothanthi thalaga parpam to prove the efficacy of drug.

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