SAFETY AND PHARMACOLOGICAL PROFILE OF KALLADAIPPU THOOL

The dissertation Submitted by

Dr. K.VIJAYALAKSHMI

Under the Guidance of

Dr. S.SIVAKKUMAR, M.D(S)

Lecturer, Department of Gunapadam,

National Institute of Siddha, Chennai-47

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1. INTRODUCTION

.Siddha system of medicine is one of the oldest therapeutic systems prevalent predominantly in southern part of India. Siddha is not only a medical a system; it'salso dealing with intense spirituality and immense possibilities for the betterment of human being. Unlike other systems, siddha system aims in both the treatment and prevention of the disease.

The word Siddha comes from the word 'Siddhi' which means 'An object to be attained' or 'Perfection' or 'Heavenly bliss' or 'Eternal bliss.'

To achieve eternal bliss, the physical body alone is the vehicle. But the body is transient and is easily susceptible to disease and suffering hence, is unable to attain salvation. Therefore, in order to get rid of such suffering and to achieve the threefold benefit, long life is essential.

"The real Holy Scripture is the Great Work on Longevity"

For "Salvation" is attainable only by mankind
And as the blessed medicine is extolled
To protect our physique from mortality" - Theraiyar [(1)]

For that, to live the healthiest and longest life, our physical function should be maintained in a balanced state, which means our three humors that are *Vatham*, *Pitham*, *Kapham, viz.*, wind, bile, phlegm should be in the ratio of 4: 2: 1 respectively. If there is any derangement in these three humors, they bring about diseases peculiar to their influence that are classified into 4448 types by Sage Yugimuni. In that, *Kalladaippu noi* is one among them. Based on body humours, it is classified into 4 types likevali kalladaippu, azhal kalladaippu, kapha kalladaippu and mukkutra kalladaippu by Sage Yugimuni. In that, the dietary factors play an important role in the derangement of *pitha kutram*. The raised *Azhal Kutram* dries up the body fluid and urine, resulting in concentration of salts. This further affects the *Keezh Noekku Kaal*. One of the functions of *Keezh Noekku Kaal*is to excrete Urine.So when this *Keezh Noekku Kaal* is affected urine will be obstructed in the urinary tract [(2)].This favours the deposition of urinary salts to develop into calculi anywhere in the kidney or urinary tract.
The features mentioned in *kalladaippu noi* can be compared with urolithiasis mentioned in modern medicine.

Urolithiasisis isone of the commonestdiseases in our country and pains due to kidney stones are known as worse than that of labour pain. In India,approximately 5-7 million patients suffer from stonedisease and at least 1/1000 of Indian population

needshospitalization due to kidney stone disease[3].In India upper and lower urinary tract stones occurfrequently but the incidence shows wide regional variation. The incidence of renal calculi iscomparatively low in the southern part of countrycompared to other parts[4].Even though there are lot of diuretics are available in the allopathic system there are some adverse effects are always reported. There is a need for a drug without causing any adverse effects.

In our Siddha system of medicine besides herbals, metals, minerals and animal products has been used to prepare the medicine[5].Medicine cures diseases through bring back the deranged *kutrams* into balanced state and they are classified into two types that are internal and external medicine. In that, *Kalladaippu Thool* [6]is one type of the herbo-mineral formulated internal medicine to treat urolithiasis.

The ingredients of *Kalladaippu Thool* are *Naayuruvi* (Achyranthes aspera),*Vaazhai charugu*(Musa paradaisiaca), *Panaipoo*(Borasses flabelifer),*Katthari*(Solanum melogena), *Katthakaambu*(Uncaria gambir), *Vengaram*(Sodium Biborate),*Padigaram*(Aluminium PottassiumSulphate), *Induppu*(Sodium Chloride Impurae) and *Savukkaaram*(Sodium Carbonate). In that*Naayuruvi*, *Vaazhaicharugu*, *panaipoo* said to be effective in *Kalladaippu* as mentioned in *Mooligai Marmam.*As per the literature review of *Gunpadam Thathu*,*Vengaram* has lithontriptic and diuretic action and*Padigaram*, *Induppu* has diuretic action.

The above said drug formulation has not undergone any preclinical trial so for. Hence I have selected the siddha formulation *Kalladaippu Thool* for evaluating the safety and therapeuticeffect through the toxicity study and the following pharmacological studies.

- Anti-Uroliathiatic activity
- Diuretic activity
- Saluretic activity

2. AIM AND OBJECTIVES

Aim:

To evaluate the Safety and pharmacological profile of the test drug "*Kalladaippu Thool*" [6] in animal model.

Objectives:

- Collection of variousinformation (Siddha and modern) relevant to the study
- Preparation of the drug as per classical Siddha literature
- Analytical study of the prepared drug
- Physicochemical analysis
- Chemical analysis
- X-ray Diffraction Study
- Toxicity studies
  - ❖ Acute oral toxicity study (OECD – 423 Guideline).
  - ❖ 28 days Repeated oral toxicity study (OECD – 407Guideline).
  - ❖ 90 days Repeated oral toxicity study (OECD – 408 Guideline).
- Pharmacological activities in Wister albino rats
  - ❖ Anti-Uroliathiatic activity -Ethylene Glycol Induced Method
  - ❖ Diuretic activity
  - ❖ Saluretic activity

  } - Lipschitz method

3.1. GUNAPADAM REVIEW

NAAYURUVI - Achyranthus aspera, Linn.

Synonyms:

- *Abamaarki*
- *Krishnabanni*
- *Saramanjari*

Parts used:

Whole plant

Organoleptic Characters:

Taste	:	Bitter, Astringent, Pungent
Character	:	Heat
Division	:	Pungent

Actions:

- Astringent
- Diuretic
- Alterative [10]

General characteristics - நாயுருவி

மலிகாரங்கைப்புள்ளஅபமார்க்கியின்வேரால்வசியமுண்டாம்

இலைமூலஉதிரமந்தம்பேதிகபம்வியர்வுதந்தியிறங்குமேகம்

மலையேறும்படிபுரியுமுள்ளரிசிபசுமாற்றும்வசனமூலம்

பலமாதர்க்குள்ளழுக்கைநீக்குவங்கச்சிந்துரம்பண்ணுமாதோ.

- Theraiyar Gunavagadam

It cures bleeding piles, diarrhoea, *kapha* diseases, excess sweating and leucorrhoea.

Medicinal uses [9]**:**

- Decoction of the whole plant is given for abdominal disorder.
- Whole plant can be used for the obstruction in the post-partum bleeding.
- Its root ash powder is mixed with palm jaggery and given orally to conduct normal delivery without any complication.
- Take 15 ml of its juice with some sugar, and drink for four or five days .This will cure joint pain, menorrhagia, cough and eczema.
- Take equal amount ashes of Achyranthes aspera and flower of Borassus flabellifer and put them in water and collect the clear water from that top and boil it. A salt will be obtained called *Nayuruvi Uppu*. Giving 2or 3 pinches of salt with ghee or butter will cure asthma, ulcer, atrophy of bones and cold.

VAAZHAI – Musa paradisiaca

Synonyms:

- *Kathzhi*
- *Saekili*
- *Ambanam*
- *Arambai*
- *Vosai.*

Parts used:

Leaf, flower, tender vegetable, vegetable, fruit, bark and stem.

Organoleptic Characters:

Taste of banana tuber	:	Astringent
Character	:	Heat
Division	:	Pungent

Actionsof Plantain bark:

- Refrigerant[10]

General characteristics - வாழை

வாழைநீர்தான்குளிர்ச்சிவல்லபலமுண்டாகும்

பேழைவயிறுடைக்கும்பெண்மயிலே! - வீழுவல்லி

ரத்தக்கிரிச்சமெரிநீரிவையுடனே

சிற்றிரணம்போக்குந்தெரி.-Agathiyar Gunavagadam.

It cures all gastro intestinal disorder. It nourishes our body and improves spermatogenesis**.**

Medicinal uses[9]**:**

- Ash of bark is mixed with water and filtered. Then it is boiled until to get salt which has diuretic property.
- Salt which is obtained by using the ash of Plantain bark, Sesame plant, Palm root, Tobacco and Flower plant is used for infantile hepatic problem and cures anemia and ascites.
- 35 ml of banana stem juice is given for kidney stone.
- 15 ml of juice is given for snake bite.

PANAI POO - **Borassus flabellifer, Linn.**

Synonyms:

- *Thaalam*
- *Karumpuram*
- *Aedagam*

Parts used:

Root, flowering stalk,juice,bark and fruit.

Organoleptic Characters:

Taste	:	Astringent
Character	:	Coolant
Division	:	Sweet

Actions:

- Astringent
- Diuretic
- Nutritive
- Refrigerant. [10]

General characteristics- பனைபூ

பனையிலுறுபூலதுதான்கங்கமுறாக்குன்ம

வினையகற்றும்நீர்கட்டைமீட்டும் - முனையான

பன்னோய்ஒழிக்கும்பழஞ்சுரத்தைப்போக்கிவிடும்

முன்னேஇதனைவிளம்பு. - Agathiyar gunavagadam.

It cures peptic ulcer,burning micturition anddental diseases.

Medicinal uses[9]**:**

- Drinking about 100 ml of fresh juice everyday morning before sun rise during summer will be a useful remedy for tuberculosis, chronic dyspepsia, psoriasis and skin diseases.
- A salt is extracted from ash of the burnt male flower is given for urinary disorders, dyspepsia, tooth ache and chronic disorder.
- The seed pulp of palm is remedy for venereal affection and dysentery.

KATTHARI – Solanum melongena

Synonyms:

- *Vazhuthazhai*
- *Vazhithunai*

Parts used:

Whole plant

Organoleptic Characters:

Taste : Bitter and Astringent.

Character : Heat

Division : Pungent

Actions:

- Stimulant
- Hypnotic
- Expectorant(1s0)

General characteristics - கத்தரி

கத்தரிக்காய்பித்தங்கனன்றகபந்தீர்த்துவிடும்

தொத்தசொறிசிரங்கைத்தூண்டிவிடும்மெத்தவுந்தான்

பிஞ்சானகத்தரிக்காய்பேசுமுத்தோடம்போக்கும்

மஞ்சார்குழலே!வழுத்து. - Agathiyar Gunavagadam

It neutralizes *pitham* and reduces *kapham*.Tender brinjal is taken to normalize the *thridosha*.

Medicinal uses:[9]

- Leaves are added in diuretic type decoction. It helps for good sleep.
- Katthari can also be taken in food for three times a day. But it never causes any illness. It comes under in dietary regimen food (*pathiyam* in Siddha).
- Ripped fruits are best for hepatic disease.
- Burnt fruit improves digestion, but it leads to purgation.

KATTHAKAAMBU – Uncaria gambir

Synonyms:

- *Oothalai maram*

Parts used:

Stem

Organoleptic Characters:

Taste : Bitter, Astringent.

Character : Heat

Division : Pungent

Actions:

- Astringent [10]

General characteristics - கத்தக்காம்பு

பேதிசிறுநீர்கட்டும்பேசவொணாவாய்விரணமும்

தீதிலுயருந்திப்புண்தீருமேமேதினியில்

செங்குருதிதோசமெலாம்தீர்ந்துவிடும்விந்திறுகும்

பைன்கூந்தல்மாமேபகார்.

-Siddha Vaithiya Pathaartha Gunavilakkam.

It cures diarrhea, anuria, oral ulcer, chronic ulcer, and throat ulcer, hoarseness of voice and tooth ache.

Medicinal uses:[9]

- 650-1gm of chooranam is prepared from the mixture of 8 gm powder of *katthakaambu,Karuvaapattai*and 2 gm powder of *Saathikai thool* is taken along with honey to cure diarrhoea.

VENGARAM – Borax (Sodium Biborate)

It is composed of boric acid and soda.

Synonyms:

- *Porikaram*
- *Karam*
- *Urukkumithiran*
- *Danganam*
- *Thoomathaiyadakki*

Organoleptic Characters:

Taste	:	Sweet mixed with astringent
Character	:	Heat
Division	:	Coolant

Actions:[8]

- Diuretic
- Astringent
- Lithodialysis
- Emmenogogue

General Characteristics - வெங்காரம்

சொறிபுடையெண்குன்மநமைசோரியாசம்

பறிகிரகணிகல்லானம்பன்னோய்நெறியைத்

தடங்கணங்கபங்கிருமிசர்ப்பவிடஞ்சந்நி

யிடங்கணங்கலக்கிற்போமெண். -SiddhaMateria Medica

It cures urinary calculus, burning micturition, worm's infection, hemorrhoids, hemiplegia, urinary tract infections, abdominal diseases and venereal ulcer with pus.

PADIGARAM- Alum (Aluminium Potassium Sulphate)

It is colorless, transparent crystals with acid. Alum is a general name for a classof double sulphates containing aluminium and such metals as potassium, ammonium, iron etc.

Synonyms:

- *Cheenam*
- *Padigi*

Organoleptic Characters:

Taste	:	Sweetish astringent
Potency	:	Heat
Division	:	Pungent

Actions: [8]

- Astringent
- Diuretic
- Hemostatic
- Antispasmodic

General Characteristics- படிகாரம்

சீனமெனும்காரமதுசீறிவருபல்லரணை

ஆனைக்கால்கண்ணோய்அனிலமொடுமாநிலத்தில்

துன்மாங்கிசம்வாயுதோலாதஉள்ளழலை

குன்மமிவைபோக்குமெனக்கூறு. - Siddha Materia Medica

It cures gingivitis, eye diseases, elephantiasis, *vayu* tumour, sense of heat, gastric ulcer, hypertension, hemorrhage, dysentery, diarrhoea, children's vomiting, whooping cough with expectorant, pharyngitis, menorrhagia and gonorrhoea.

INDUPPU – Rock Salt (Impure Sodium Chloride)

Synonyms:

- *Cynthavam*
- *Chindooram*
- *Chandiranuppu*
- *Mathikoormai*
- *Mathiuppu*
- *Minthachol*

Organoleptic charaters:

Taste : Salt

Potency : Heat

Division : Pungent

Actions: [8]

- Diuretic
- Laxative
- Carminative

General Characteristics - இந்துப்பு

அட்டகுன்மமந்தம்அசிர்க்கரஞ்சூர்சீதபித்தந்

துட்டவையம்நாடிப்புண்டோடங்கள்கெட்டமலக்

கட்டுவிடவிந்தையக்காமியநோய்வன்கரப்பான்

விட்டுவிடவிந்துப்பைவிள். - Siddha Materia Medica

It cures eight types of gastric ulcer, indigestion, blood diseases,*kaphathikkam*,syphilis, derangement of three *humours*, constipation, poison's bite, spermatorrhoea, cataract, hemorrhoids and *vatha* pain

.

SAVUKKARAM-Sulphate of soda(Sodium Carbonate)

General Characteristics–சவுக்காரம்

தீயசவுக்காரஞ்செப்புவேன்கேளு

மாயாதிபூனீர்வார்வெடிச்சாரமுந்

தோய்வெடிநீரிற்சுண்டிடக்காய்ச்சி

வாயூரயெண்ணைவடித்துஉருட்டிடே

உருட்டியகாரம்ஓம்சவுக்காரமும்

மருட்டிடுஞ்சரக்கைவாட்டியெடுத்திடு

திருட்டுகள்பேசிதிரிகிறவாதியை

மெருட்டுமிக்காரம்வேதைகள்கோடியே.

- மச்சமுனி 800 பக்கம் 24

சவுக்காரம்:

கார்மேகத்துர்ப்படையும்கட்டிக்கரப்பனுடன்

கார்மேகவூறற்றழுபலரும்சேர்மேகத்

தொட்டிவரும்நகத்தினூறவகற்றிவிடும்

கட்டிச்சவுக்காரங்காண்.

- சித்தவைத்தியபதார்த்தகுணவிளக்கம்

It is useful in dyspepsia with vomiting, diarrhoea and flatulence.

3.2. BOTANICAL REVIEW

Achyranthes aspera linn,

Common name:

Flower plant

Vernacular names:

Tamil	:	Sirukadaladi
Hindi	:	Latjira, Chirchira
Telugu	:	Uttaraena
Gujarati	:	SafadAghedo
Malayalam	:	Kadaladi
Unani	:	Chirchitaa
Sanskrit	:	Aghata

Taxonomicalclassification :[16]

Kingdom	:	Plantae
Subkingdom	:	Tracheobinota
Super Division	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida

Sub Class	:	Caryophyllidae
Order	:	Caryophyllales
Family	:	Amaranthaceae
Genus	:	*Achyranthes*
Species	:	*aspera*

Parts used:

Whole plant

Phytochemicalconstituents :[11]

- Saponins
- Ecdysterone
- Oleonolicacid
- Dihydroxy ketones
- Long chain compounds
- Alkaloids

Actions:

- Spermicidal activity
- Anti-parasitic activity
- Astringent activity
- Hypoglycemic activity
- Cancer chemo preventive activity
- Hepatoprotective activity
- Antimicrobial activity
- Anti-oxidant activity
- Anti-allergic activity
- Wound healing activity

- ❖ Immunomodulatory activity
- ❖ Cancer Chemo preventive activity
- ❖ Nephroprotective activity.

MedicinalUses :[11)]

- Crushed plant is used in pneumonia.
- Infusion of the root is used as mild astringent in bowel complaints.
- Decoction of powder leaves with honey or sugar candy is useful in early stages of diarrhoea and dysentery.
- The plant is used in the treatment of asthma, bleeding piles, boils, bronchitis, cold, cough, colic, dropsy, ear complications, headache, leucoderma, pneumonia, renal complications, skin diseases, snake bite and scorpion bite.[(8)]
- The flowering spikes or seeds, ground and made into a paste with water, are used as external application for bites of poisonous snakes and reptiles, used in night blindness and cutaneous diseases
- For snake bites the ground root is given with water until the patient vomits and regains consciousness.
- Inhaling the fume of Achyranthes aspera mixed with Smilax ovalifolia roots is suggested to improve appetite and to cure various types of gastric disorders.[12].
- Ash of the plant is applied externally for ulcers and warts.
- The crushed leaves rubbed on aching back to cure strained back.[13].
- Paste of the roots in water is used in ophthalmia and opacities of the cornea.
- Paste of fresh leaves is used for allaying pain from bite of wasps.[(14)]
- The plant is useful in liver complaints, rheumatism, scabies and other skin diseases. It also possesses tranquillizing properties. [15, 16]

Scientific Review:

Achyrathes aspera: Diuretic Activity and Acute toxicity study[(17)]

Muhammad Asif et.al. Studied that the acute toxicity, diuretic activity and saluretic activity of Achyranthes aspera Linn Crude Aqueous Extract in Albino mice and Albino Rats respectively.The findings of this study demonstrated that the crude aqueous extract of the plant showed significant diuretic ($p < 0.001$), natriuretic ($p < 0.001$) and kaliuretic ($p < 0.001$) effects. Lipschitz values showed that, at the dose of 50 mg/kg, the crude extract showed 46 % of diuretic activity as compared with furosemide. No toxic effects were observed among Albino mice even at a higher dose of 3000 mg/kg. The conclusion of this study, the crude extract of Achyranthes aspera increases the urine volume and concentration of urinary electrolytes in a dose-dependent manner. Therefore, this plant has a diuretic potential.

Diuretic Activity:[18]

S.S. Gupta et al. (1972) reported a saponin isolated from the seeds of Achyranthes aspera which shows significant diuretic effect in adult male albino rats. Achyranthine (5 mg/kg, orally) had diuretic activity in rats.

Sub-acute toxicity:[19]

The sub-acute toxicity studies of Methanolic extract of Achyranthus aspera revealed that the mice did not show any external manifestation of toxic symptoms, other behavioural patterns and mortality even after 30 days of drug administration (25mg/kg/day and 50mg/kg/day). It did not produce any statistically significant changes in the body weights food and water intake, haematological,bone marrow studies of differential leucocytes count and chromosomal morphology. But the present study also revealed that the significant changes were found in biochemical, histopathological and weight of the few major organs.

Musa paradisiaca

Common names:

- Banana
- Plantain

Synonyms:

- Musasapientum L.

Vernacular names:

Tamil	:	Vaazhai
Sanskrit	:	Kadali
Telugu	:	Atral, Kadali
Malayalam	:	Vazha

Taxonomical classification:[16]

Kingdom	:	Plantae – Plants
Division	:	Magnoliophyta
Class	:	Liliopsida
Subclass	:	Zingiberidae
Order	:	Zingiberales

Family : Musaceae

Genus : Musa

Species : paradisiaca L

Parts used:

Roots, leaves, fruits and stem.

Phytochemical constituents:[11]

- Nor-epinephrine
- Indole compounds
- Flavonoids
- Vitamin-B
- Vitamin-C
- Albuminoids
- Mineral salts
- Tanins
- Eugenol

Actions:

- Acrid
- Anthelmintic
- Tonic
- Appetizer

Medicinal Uses: [11]

- It is useful in *kapha* disease, pain in the ear, menstrual disorders, diseases of the blood, acid dyspepsia and leprosy.
- The juice of the tender root is used with mucilage for checking hemorrhage from the genital and air passages. Mixed with ghee and sugar it is given for gonorrhoea

- The juice of the stem is cooling, astringent in action, anti-dysenteric, useful in thirst, Urinary discharges and leprosy.
- Unripe fruit in combination with other drug is used in diabetes.
- The ripe fruit is sweet, acrid, cooling, tonic, aphrodisiac, excites appetite, useful in Leprosy, thirst, vaginal and urinary discharges

Borassus Flabellifer Linn.

Common name:

Palmyra palm

Synonyms:

- Borassus flabeliformis L
- Borassus tunicatusLour
- BorassusflabeliformisRoxb

Vernacular names:

Sanskrit	:	Tala
Hind	:	Taltar
Telugu	:	Tatichettu

Toxonomicalclassification:[16]

Kingdom	:	Plantae
Subkingdom	:	Tracheobionata
Division	:	Magnoliophyta
Class	:	Liliopsida
Order	:	Arecales
Family	:	Arecaceae

Genus : Borassus

Species : flabellifer

Parts used:

Root, flowering stalk, juice, bark and fruits.

Phytochemical constituents:

- Gum
- Fat
- Albuminoids [11]

Actions:

Root	:	Cooling and restorative.
Juice	:	Diuretic, cooling and stimulant.
Pulp from unripe fruit	:	Diuretic, demulcent and nutritive.
Terminal buds	:	Nutritive and diuretic.

Medicinal Uses:[11]

- It cures *vayu gunmam*, burning micturition, dental disease and fever.
- Ashes of flowering stalks are diuretics.Salt prepared from ashes of flowering stalk diluted with water then filter it pure ashless diluted water exposure to sunlight. It contains albuminoids, gum and fat.
- Juice is diuretics, cooling andstimulant. It is used for dropsy and inflammation.
- Ashes of flowering stalk are useful in enlarged spleen.

Solanum Melogena, Linn.

Common names:

- Brinjal
- Egg plant

Vernacular names:

Sanskrit	:	Vartaku, Bartaku, Peethaphala
Telugu	:	Vankayi
Hindi	:	Begun

Toxonomical classification:[16]

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Solanales
Family	:	Solanaceae
Genus	:	Solanum
Species	:	melongena

Parts used:

Whole plant

Phytochemical constituents:[8]

Green leaves : Antiscorbutic vitamin C

Dried material :Ether extract

Albuminoids

Nitragen

Actions:

Leaves : Narcotic

Seeds : Stimulant

Fruits : Hypnotic, Phlegmatic.

Tender fruits : Antiphlegmatic, Alleviative of wind.

Riped fruits : Carbonas, Bilious.

Burnt fruits : Light in digestion, Purgative, Beneficial in obesity and phlegm.

Medicinal Uses: [11]

- Fruit is generally used as culinary vegetable, made into pickles.
- Pierced all over with a needle and fried in gingelly oil the fruit is employed as a cure for toothache.
- It is an excellent remedy for liver diseases.
- Seeds are apt to lead to dyspepsia and constipation.

Uncaria Gambir .Roxb,

Common name:

Pale catechu

Synonym:

- Naucleagambir

Vernacular names:

Tamil	:	Ankudu- kurra
Sanskrit	:	Khadir
Hindi	:	Kath
Marathi	:	Kath
Malayalam	:	Gambier, Gambir.

Botanical classification:[16]

Kingdom	:	Plantae
Division	:	Angiospermae
Class	:	Eudicots
Order	:	Gentiales
Family	:	*Rubiacea*
Genus	:	*Uncaria*

Species : *Gambir*

Parts used:

Extract

Phytochemical constituents:[11]

- Catechu-Tannic Acid
- Catechine
- Quercetin,
- Catechu-Red
- Gambir - Fluorescein
- Wax
- Oil

Action:

- Local astringent

Medicinal Uses:[11]

- It is largely used as an ingredient in pan – supari (Betel leaf).
- Externally it is an application to syphilitic sores and ulcers in mouth.
- The official tincture diluted with water can be used as a gargle in sore throat, stomatitis.
- Internally, in combination with chalk, kino and opium. It is a useful preparation in diarrhoea and cholera.

3.3. MINERALOGICAL ASPECT

BORAX

Occurrence:

It occurs as a natural deposit. Crude borax is found in masses by evaporation of water, on shores of dried up lakes in India and Tibet. It is also obtained from the mud of lakes surrounded by hills in Nepal.

Synonyms:

- SodiiBoras
- SodiiBiboras
- Sodium Borate

General Characters:

It is composed of boric acid and soda.

The color is greyish white.On exposure it becomes opaque or dirty white.

It has a faintly balsamic odour.

Physical Characters:

Appearance	:	White solid
Melting point	:	741^0c
Boiling point	:	1575^0c
Molar mass	:	381.37

Density	:	1.73 gm
Related compounds	:	Boric acid, Sodium per borate
Molecular formula	:	$Na_2 B_4 O_7 10H_2O$

Actions:

- Diuretic
- Emmenogogue
- Astringent
- Antacid
- Local sedative
- Antiseptic

Medicinal uses: [20]

- It is given internally at the dose of10-30 grains, for acidity of the stomach, amenorrhea, dysmenorrhea, menorrhagia, puerperalconvulsions and to promote uterine pains during labor.
- Roasted borax is given with tender coconut water for urinary tract infections.
- It is given at a dose of 260-520 mg with betel leaf prevents fever with rigor.
- 65 mg to 325 mg of borax mixed with breast milk is given to children for relief of pain and convulsions.
- 325 mg of borax and 195 mg of pepper powder are taken together with 4 ml of honey thrice a day for controlling asthma and cough.
- *Vengaraparpam* cures *pitha* diseases like burning micturition and urolithiasis.

Supportive articles:

1) The *Vengaram* is one of the ingredients of the drug *Jalamanjari chendooram*in siddha system of medicine which is used for kidney stone diseases . It has significant diuretic activity. [21]

2) The *Vengaram* is one of the ingredients of *Kara Sooda Sathu Parpam* is indicated for diuretic and lithontriptic activity in the management of urolithiasis. [22]
3) The *Vengaram* is one of the ingredients of the drug*Sarva noi Llinga Chendooram* is indicated for diuretic and lithontriptic in the management of urolithiasis. [23]

ALUMEN

Occurrence:

Chiefly fond with peroxide of iron in *Silajit* or in Alum earths of Nepal or prepared from alum shale in the Punjab, Rajputana and Bihar States.Alum is a general name for a class of double sulphates containing aluminium and such metals as potassium, ammonium, iron etc.

Synonyms:

- Alum
- Aluminium Potassium Sulphate

General Characters:

Colourless, transparent crystals. It is with acid and has sweetish astringent taste.

Physical Properties:

Melting point : 660.37^0c

Boiling point : 24670^0c

Specific gravity : 2.6989200^0c

Molecular formula : $KA_1 (SO_4)_2 12H_20$

Actions:

- Astringent

- ❖ Hemostatic
- ❖ Antispasmodic
- ❖ Antiseptic
- ❖ Irritant
- ❖ Purgative in large doses
- ❖ Emetic in repeated doses

Medicinal uses:[20]

- It cures gingivitis, filariasis, heart diseases, *vatham*, fever, peptic ulcer, and tumour.
- *Padikaraparpam* has been used for urinary calculus, urethral stricture, anuria and burning micturition.
- It also controls vomiting when given at a dose of 65 mg.
- 4.7 gm of alum dissolved in butter milk is given for snake bites.
- For severe head injury, 130 mg of alum is administered along with sugar.
- In leucorrhoea with bleeding, alum is given with juice of Adathodavasaica thrice daily.[17]

Supporting article:

The *padigaram* is one of the ingredients of *Kara*S*ooda Sathu Parpam* is indicated for diuretic activity and lithontriptic activity in the management of urolithiasis. [22]

ROCK SALT

Occurrence:

Found in nature in extensive beds mostly associated with clay and calcium sulphate. To obtain it, holes are dug into these rocks which soon become filled up with salt water. The water is evaporated and the salt is left ready for use.

Halitecommonly known as rock salt. It is the mineral form of sodium chloride (NaCl). Halite forms isometric crystals.

Synonym:

- Sodium Chloride impurae

Vernacular names:

Tamil	:	*Indhuppu*
Eng	:	Rock salt
Hindi	:	Sendhalon

General Characters:

It is found in small white crystalline grains or transparent cubes. It is brownish white externally and white internally. It has a pure saline taste and burns with a yellow flame.

Physicochemical properties:

Category	:	Halide mineral

Chemical formula	:	NaCl
Crystal symmetry	:	Isometricalhexoctahedral
Molar mass	:	58.433g/mol
Solubility	:	Water soluble
Crystal system	:	Cubic
Luster	:	Vitreous
Optical properties	:	Isotropic
Streak	:	White
Refractive index	:	1.544
Other characters	:	Salty flavor, Fluorescent

Actions:

- Carminative
- Diuretic
- Laxative
- Purgative
- Stomachic
- Digestive

Medicinal uses :[20]

- It promotes the appetite and assists digestion and assimilation.
- Hot fomentation of the rock salt can be taken for curing the painful swellings.
- It is made into a paste and applied in case of sprain.
- It is given in dyspepsia and other abdominal disorders.

SODIUM CARBONATE

Occurrences and Varieties:

It occurs in porous, granular masses, of a greyish white color or as heavy hard pieces, with a strong alkaline taste of soda.

3 varieties - 1.Sajjikhar, 2.Soda crystals, 3.Very impure carbonate of soda.

All these varieties are found in the ashes of Chenopodiaceous plants, a species of salt worsts growing near the sea.

Crude carbonate or sulphate of soda is an alkaline earth found in large quantities where white forms the sub-soil.

It is found in the hot weather as an efflorescent sand.It is scrapped off from surface with a little quicklime and made into cubes for sale, in cart loads

Synonyms:

- SodiiCarbonasImpura
- Disodium carbonate
- Carbonic acid disodium salt
- Calcined soda.

Vernacular names:

Tamil	:	Choontumunnoo, Sanchhikkaram
Sanskrit	:	Natron, Sarjikakshara

English : Dhobi's earth, Salphate of soda

Telugu : Savitemannuppu

Hindi : Sajjikhar

Constituents:

It contains 25p.c. of Sodium carbonate. Chemically it consists of carbonate of soda with certain impurities such as organic matter, sulphate of soda, potash etc.

Physical properties:

Molecular weight : 105.988g/mol

Color : Greyish – white powder or lumps containing upto 99% Sodium carbonate

Melting point : 856^0c

Solubility : 30.7 gm/100g water at 25^0c

Molecular formula : NA_2CO_3 or NA_2O_3.

Actions:

- Antacid
- Alterative
- Diuretic

Medicinal uses: [20]

- It is useful in dyspepsia, vomiting, diarrhoea and flatulence.
- It is an efficient remedy in urinary diseases, gravel and suppression of urine.
- In Bright' disease of the kidney with abundant sediment in the urine, and in diabetesthe habitual use of this salthas a marked beneficial effect.

4. MATERIALS AND METHODS

The trial drug *Kalladaippu Thool is a* herbomineralpreparationindicated for urolithiasis has been chosen as a trial drug from the Siddha literature *"Anuboga Vaithiya Navaneetham"*,Vol-9,pgno:76,77., *Author: Hakeem P.M.*Abdullah sahib.

Ingredients of *Kalladaippu Thool*:

1. Ash of *Naayuruvi* (Whole plant of Achyranthes aspera) : 2 *palam*(70gm)
2. Ash of *Vaazhai charugu* (Bark of Musa paradaisiaca) : 2 *palam*(70 gm)
3. Ash of *Panai poo* (Flower of Borassus flabelifer) : 2 *palam*(70 gm)
4. Ash of *Katthari* (Whole plant of Solanum melogena) : 2 *palam*(70 gm)
5. Powder of *Katthakaambu* (Stem of Uncaria gambir) : 2 *palam*(70 gm)
6. Purified Borax (*Vengaram*- Sodium Biborate) : 2 *palam*(70 gm)
7. Purified Alum (*Padigaram*- Aluminium PottassiumSulphate) : 2 *palam*(70 gm)
8. Purified Rock Salt (Induppu- Sodium Chloride Impurae) : 2 *palam*(70 gm)
9. Purified Sulphate of soda(*Savukkaaram* -Sodium Carbonate) : 2 *palam*(70 gm)

Collection of the Plant materials:

All plant materials were freshly collected from in and around Trichy, Tamilnadu. Mineral drugs were procured from raw drug shop in Parrys, Chennai**.**

Identification and Authentication of the drug:

All the plant materials were identified and authenticated by the Botanist, Department of *Gunapadam*, National Institute of Siddha.All the mineral materials were identified by the Chemist, Department of Geology, University of Madras, Guindy Campus, Chennai.

Purification of the drugs:

All the drugs mentioned here were purified as per the Siddha literature.

- The whole plant of Achyranthes aspera,Bark of Musa paradisiac,Flower of Borassus flabelifer and Whole plant of Solanum melogena was washed in the running tap water to remove the soil and impurities.
- Extract of Uncaria gambir was cleaned well from dust and impurities.[7]
- Borax was fried till the moisture completely evaporates.[8]
- Alumen was dissolved in water; filtered, boiled when it attained consistency of jelly. It was cooled to get the purified form.[8]
- Rock salt was soaked in goat's urine for three days and insolated to get purified form.[8]
- *Savukkaram* was dissolved in water ,filtered, boiled and then cooled to get purified form.[2]

Preparation of the Drug

The whole plant of Achyrathes aspera barks of Musa paradaisiaca, flowers of Borasses flabelifer and whole plant of Solanum melongena were dried in the shade until complete evaporation of the moisture content.Then these were burnt to get ash. Extract of Uncaria gambir was dried in the shade and then powdered by using pulverize. The purified *Vengaram, Padigaram, Induppu,Savukkaram* were powdered by using stone mortar. Then filtered and mixed all of the ingredients andkept in an air tight container. It was labeled as *Kalladaippu Thool.*

Date of preparation	:	9/8/16 and 14/8/16
Dose	:	4gm
Adjuvant	:	Tender coconut water

Indication : *Kalladaippu* (urolithiasis), Neeradaippu (dysuria)*Sathaiyadaippu*(urethral stricture), *Moothirakiricharam*(burning micturition),*Thurmamisavalarchi*(tumor inrenal tract).

Date of expiry : 1 year.

Ingredients of *Kalladaippu Thool*

Sodium chloride impurae

Before purification **After purification**

Borax

Before purification **After purification**

Alum

Before purification **After purification**

Sodium carbonate

Before purification

After purification

Naayuruvi **- (Achyranthes aspera)**

Katthari **- (Solanum melongena)**

Katthakaambu**- (Uncaria gambir)**

Panai poo **- (Borassus flabellifer)**

***Vaazhai charugu* - (Musa paradisiaca)**

KALLADAIPPU THOOL

4.1. ANALITICAL STUDIES OF *KALLADAIPPU THOOL*

Analytical studyof the prepared drug brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physicochemical properties and to assess the active principles and elements present in the drug. Thus analytical study brings the efficacy and potency of the drug.

As per AYUSH protocol for analytical study, the following parameters were evaluated.

- **Organoleptic characters:**
  - Colour
  - Odour
  - Taste
  - Texture

- **Physicochemical analysis:**
  - Determination of Ash Values
  - Physical characterization

- **Phytochemical analysis:**

- ❖ HPTLC and TLC finger print analysis

- **Chemical analysis:**

  - ❖ Preliminary Basic and Acidic radical studies

- **Elemental analysis:**

  - ❖ X-ray Powder Diffraction Analysis

4.1.1 ORGANOLEPTIC CHARACTERIZATION OF *KALLADAIPPU THOOL*

The organoleptic characters of the sample drug were evaluated. 1gm of the test drug was taken and the following characters were seen.

Colour, Odour, Taste, Texture and other morphology were viewed by naked eye under sunlight. Then the result was noted.

Colour:

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

Odour:

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

Results:

The results of organoleptic character were showed in Table – 1

4.1.2. PHYSICO CHEMICAL ANALYSIS:

Determination of Ash Values:

Total Ash:

3gm is accurately weighed and incinerated in a crucible dish at a temperature not exceed 450^{o}C until free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air-dried powder was calculated.

Water Soluble Ash:

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

Acid insoluble Ash:

The total ash was obtained as the above method for preparation of total ash. The ash was boiled for 5minutes with 25ml 10% HCl. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue were weighed until constant weight is attained.

Loss on Drying:

The powdered drug is dried in the oven at 100- 105°C to constant weight.

Determination of Alcohol Soluble Extractive:

The air dried drug was finely grounded, added with 100 ml of ethanol of specified strength in a closed flask for twenty-four hours, shaken frequently during the course of six hours and allowed to stand for eighteen hours. Then the mixture was filtered rapidly taking precautions against loss of solvent, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, and dried at 105^{o} to constant weight. The percentage of alcohol-soluble extractive with reference to the air-dried drug was estimated.

Determination of Water Soluble Extractive:

The above procedure was repeated but instead of ethanol, chloroform with water was used.

Results:

The results of physicochemical properties were represented in Table - 2.

4.1.3 CHEMICAL ANALYSIS OF *KALLADAIPPU THOOL*

The chemical analysis of *Kalladaippu thool* was carried out in bio chemistry lab, National Institute of Siddha.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Physical Appearance of extract	Dark brown in colour.	
2.	**Test for Slicate :** 500mg of the sample was shaken well with distilled water.	Sparingly soluble.	Presence of Silicate.
3.	**Action of Heat:** 500mg of the sample was taken in a dry test tube and heated gently at first and then strong.	No White fumes evolved. No brown fumes evolved.	Absence of Carbonate Absence of Nitrate.

4.	**Flame Test:** 500mg of the sample was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.	No bluish green flame appeared.	Absence of copper
5.	**Ash Test:** A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	Appearance of yellow colour flame appeared.	Absence of sodium

Preparation of Extract

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

S.No	**EXPERIMENT**	**OBSERVATION**	**INFERENCE**
	I. Test For Acid Radicals		
1.	**Test For Sulphate:** 2ml of the above prepared extract was taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	Cloudy appearance present.	Presence of Sulphate.
2.	**Test For Chloride:** 2ml of the above prepared extracts was added with 2ml of dil-HCl until the effervescence ceases off.	No Cloudy appearance present.	Absence of Chloride.

3.	**Test For Phosphate:** 2ml of the extract were treated with 2ml of dil.ammoniummolybdatesolution.	No Cloudy yellow appearance present.	Absence of Phosphate
4.	**Test For Carbonate:** 2ml of the extract was treated with 2ml dil. magnesium sulphate solution.	No cloudy appearance present.	Absence of carbonate
5	**Test For Nitrate:** 1gm of the extract was heated with copper turning and concentrated H_2So_4 and viewed the test tube vertically down.	No Brown gas was evolved.	Absence of nitrate.
6.	**Test For Sulphide:** 1gm of the extract was treated with 2ml of con. HCL	No rotten egg smelling gas was evolved.	Absence of sulphide.
7.	**Test For Fluoride & Oxalate:** 2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil.calcium chloride solution and heated**.**	No cloudy appearance.	Absence of fluoride and oxalate
8.	**Test For Nitrite:** 3drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution is placed.	No characteristic changes.	Absence of nitrite

9.	**Test For Borate:** 2 Pinches (50mg) of the extract was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	Bluish green colour flame not appeared.	Absence of borate.
II. Test For Basic Radicals			
1.	**Test For Lead:** 2ml of the extract was added with 2ml of dil.potassium iodine solution.	No Yellow colour precipitate was obtained.	Absence of lead.
2.	**Test For Copper:** One pinch (25mg) of extract was made into paste with con. HClin a watch glass and introduced into the non-luminuous part of the flame.	No blue color precipitate appeared.	Absence of copper.
3.	**Test For Aluminium:** To the 2ml of extract dil.sodium hydroxide was added.	No characteristic changes.	Absence of Aluminium.
4.	**Test For Iron:** a. To the 2ml of extract add 2ml of dil.ammonium solution b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 was added.	Red color appeared.	Presence of Iron.
5.	**Test For Zinc:** To 2ml of the extract dil.sodium hydroxide solution was added in 5 drops to excess and dil.ammoniumchloride was added.	No White precipitate was formed.	Absence of Zinc.

6.	**Test For Calcium:** 2ml of the extract was added with 2ml of 4% dil.ammonium oxalate solution.	Presence Cloudy appearance and white precipitate was formed.	Presence of calcium.
7.	**Test For Magnesium:** To 2ml of extract dil.sodium hydroxide solution was added.	No White precipitate was obtained.	Absence of magnesium.
8.	**Test For Ammonium:** To 2ml of extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution were added.	No Brown color appeared.	Absence of ammonium.
9.	**Test For Potassium:** A pinch (25mg) of extract was treated with 2ml of dil.sodium nitrite solution and then treated with 2ml of dil.cobalt nitrate in 30% dil.glacial acetic acid.	No Yellow precipitate was obtained.	Absence of potassium.
10.	**Test For Sodium**: 2 pinches (50mg) of the extract was made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow color flame evolved.	Absence of sodium.
11.	**Test For Mercury**: 2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.	No Yellow precipitate is obtained.	Absence of Mercury.
12.	**Test For Arsenic:** 2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.	No Brownish red precipitate was obtained.	Absence of arsenic.

III Miscellaneous			
1.	**Test For Starch:** 2ml of extract was treated with weak dil.Iodine solution.	No Blue color developed.	Absence of starch.
2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes.	No Brick red color was developed.	Absence of reducing sugar.
3.	**Test For The Alkaloids**: a) 2ml of the extract was treated with 2ml of dil.potassiumlodide solution. b) 2ml of the extract was treated with 2ml of dil.picric acid. c) 2ml of the extract was treated with 2ml of dil.phosphotungstic acid.	No Yellow color developed.	Absenceof Alkaloid.
4.	**Test For Tannic Acid:** 2ml of extract was treated with 2ml of dil.ferric chloride solution	Blue-black precipitate was obtained.	Presence of Tannic acid.
5.	**Test For Unsaturated Compound:** To the 2ml of extract 2ml of dil.Potassium permanganate solution was added.	Potassium permanganate was not decolorized.	Absence of unsaturated compound.

6.	**Test For Amino Acid:** 2 drops of the extract was placed on a filter paper and dried well. 20ml of Burette reagent was added**.**	No violet color appeared.	Absence of amino acid.
7.	**Test For Type of Compound:** 2ml of the extract was treated with 2 ml of dil.ferric chloride solution.	No green and red color developed. No Violet color developed sNo Blue color developed.	Absence of quinolepinephrine pyrocatechoantipyrine, Aliphatic amino acid and meconicacid. Apomorphine salicylate and Resorcinol are absent Morphine, Phenol cresol and hydrouinone are present.

Results:

The results of acid and basic radicals were showed in Table: 3 - 4.

4.1.4 TLC/HPTLC FINGER PRINT ANALYSIS

Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It

may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound.

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

TLC and HPTLC Methodology

4g of fine powdered extracted with 40ml of Ethanol soaked for 24 hours. The extract was filtered, concentrated and made up to 10 ml in a standard flask. 5µl, 10µl, 15µl of the solution were applied on Merck Aluminium plate pre-coated with Silica gel 60 F_{254} of 0.2 mm thickness to a band width of 6 mm using Canag HPTLC system equipped with ATS-Iv applicator. The plate was developed in *Toluene: Ethyl acetate: Formic acid*(4:6:0.1). The plate was dried and visualized in UV 254 and UV 366 nm and photographs were taken. Before derivitization of the plate it was scanned at UV 254 nm and Fingerprint was taken before dipping in Vanillin- Sulphuric acid reagent

Retention Factor:

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

Rf = distance traveled by sample /distance traveled by solvent:

The Rf value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions.

The compound with the larger Rf value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower Rf value.

Results:

The results of Rf were represented in Table - 5.

4.1.5 DETERMINATION OF HEAVY METALS ANALYSIS

The procedure recommended for analysis of Heavy metals as per the guidelines WHO (1998) and AOAC (2005).

Instrument details:

Thermo FisherM Series, 650902 V1.27 model **A**tomic Absorption Spectrometer (AAS) was used for the analysis. The operating parameters:

Lead and Cadmium:

Instrument technique	: sFlame technique
Wavelength (Lead)	: 217 nm
Wavelength (Cadmium)	: 228.8 nm
Slit width	: 0. 5 mm
Lamp current (Pb)	: 4.0 mA
Lamp current (Cd)	: 3.0 mA
Carrier gas and flow rate	: Air and Acetylene, 1.1 L/min
Flow rate	: 2 ml/min

Mercury:

Instrument technique	: Cold vapour technique
Wavelength	: 253.7 nm
Slit width	: 0. 5 mm
Lamp current	: 3.0 mA,
Carrier gas and flow rate	: Argon, 1.1 L/min
Flow rate	: 5ml/min

Arsenic:

Instrument technique	: Flame vapour technique
Wavelength	: 193.7nm
Slit width	: 0. 5 mm
Lamp current	: 6.0 mA,

Carrier gas and flow rate : Acetylene, Argon, 1.1 L/min

Flow rate : 5ml/min

The Hallow cathode lamp for Pb, Cd, Hg and As analysis were used as light source to provide specific wavelength for the elements to be determined.

Results:

The results of heavy metals analysis were represented in Table -6.

4.1.6 DETERMINATION OF MICROBIAL LOAD AND AFLOTOXINS

Test for Microbial load:

The procedures recommended for analysis of microbial load as per the guideline (WHO, 2007).

The test included total bacterial count, total fungal count, and identification of specified organisms such as Enterobacteriaceae, Escherichia coli, Salmonella spp and Staphylococcus aureus.

Test for Aflatoxins:

The procedures recommended for the detection of Aflatoxin as per WHO (2007).

Instrument Details

Name of the Instrument : CAMAG (CAMAG - Automatic TLC sampler, Scanner and Visualiser)

Spray Gas : N2

Lamp used : Deuterium and Tungsten Lamp

Results:

The results of microbial load and aflotoxins were represented in Table -7.

4.1.7 X-RAY POWDER DIFFRACTION (XRD)

The powder method of diffraction was devised independently by Debye and Scherrer. Powder diffraction method involves the diffraction of monochromatic X-rays by a powder specimen. Monochromatic usually means a strong Kα characteristic component of the filtered radiation from an X-ray tube operated above the Kα excitation potential of the target material.

Selection of Kα renders the incident beam to be a highly monochromatised one. The focusing monochromatic geometry results in narrower diffracted peaks and low background at low angles. The sample is mounted vertically to the seemann- Bohlin focusing circle with the scintillation counter tube moving along the circumference of it. It is possible to record the diffracted beam from 2 to160 degrees. The diffract meter is connected to a computer for data collection and analysis. The scintillation counter tube can be moved in step of 0.01 degree by means of a stepper motor and any diffracted beam can be closely scanned to study the peak profile.

Identification of the material:

The powder diffraction of a substance is characteristic of the substance and forms a short of fingerprint of the substance to be identified. The peak of the X-ray diffraction pattern can be compared with the standard available data for the conformation of the structure. For the purpose of comparison, many standards are available, some of which are, Willars hand book, Joint Committee on Powder Diffraction Standards (JCPDS) Pepdfwin and National Bureau of Standards.

Results:

In XRD Study the sample of *Kalladaippu Thool* is matched with standard graph of Natroxlate, Sodium chloride, Sodium boron, Potassium carbonate, potassium sulphide and Boron carbide. So the drug taken for my study indicates the presence of Natroxlate, Sodium chloride, Sodium boron, Potassium carbonate, potassium sulphide and Boron carbide.

5. TOXICOLOGICAL EVALUATION OF *KALLADAIPPU THOOL*

Introduction:

Safety is a fundamental principle in the provision of traditional medicines and herbal products for health care and a critical component of quality control. OECD guidelines provide practical and technical guidance for monitoring the safety of traditional medicines within pharmacovigilance systems. The safety monitoring of traditional medicines is compared and contrasted with that of other medicines, currently undertaken in the context of the WHO International Drug perspective.

Scope of work:

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

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

Hence, the present study was carried out to evaluate the Preclinical toxicity studies of*Kalladaippu Thool* in rodents.

Plan of work:

The following studies were carried out on *Kalladaippu Thool*

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

5.1 ACUTE ORAL TOXICITY STUDY OF *KALLADAIPPU THOOL* (OECD GUIDELINE – 423)

Introduction:

- The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step.
- Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance.
- This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.
- The acute toxic class methodis based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.
- In principle, the method is not intended to allow the calculation of a precise LD50, but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.
- The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

- The use of a selection of pre-defined doses, regardless oftest substance, with classification explicitly tied to number of animals observedin different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

Principle of the Test:

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step; sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.

− No further testing is needed

− dosing of three additional animals, with the same dose

− dosing of three additional animals at the next higher or the next lower dose level.

The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

Methodology:

Selection of Animal Species:

The preferredrodent speciesis the wistar albino rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-200gm) should fall in an interval within±20 % of the mean weight of any previously dosed animals.

Housing and Feeding Conditions:

The temperature in the experimental animal room should be 22ºC $\pm$ 3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventionallaboratory diets may be used with an unlimited supply of drinking water. Animals may be group-cagedby dose, but the number of animals per cage must not interfere with clear observations of each animal.

Selection of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to do sink to allow for acclimatization to the laboratory conditions.

Test Animals and Test Conditions:

Sexually mature Female Wistar albino rats (150-200gm) were obtained fromTANUVAS, Madhavaram, Chennai.All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore).

Preparation for Acute Toxicity Studies:

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the*Kalladaippu thool.* The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design (IAEC NO: IAEC/XLIX/18/CLBMCP/2016).

Test Substance	: *Kalladaippu Thool*
Animal Source	: TANUVAS, Madhavaram, Chennai.
Animals	: Wister Albino Rats (Female-3+3)
Age	:6-8 weeks
Body Weight on Day 0	:150-200gm.
Acclimatization	:Seven days prior to dosing.
Veterinary examination	:Prior and at the end of the acclimatization period.
Identification of animals	:By cage number, animal number and individual marking by using Picric acid.
Numberofanimals	: 3 Female/group,
Routeofadministrate	: Oral

Diet	:Pellet feed supplied by Sai meera foods Pvt Ltd,Bangalore
Water	:Aqua guard portable water in polypropylene bottles.
Housing & Environment	:The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	:between 22ºC $\pm$3ºC.
Relative humidity	:between 30% and 70%,

Air changes :10 to 15 per hour

Dark and light cycle :12**:**12 hours.

Duration of the study :14 Days

Administration of Doses:

Kalladaippu Thool was suspendedin coconut water and administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of5, 50, 300and 2000 mg/kg body weight was administered stepwise. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically andcontinuously as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound andpain, as well as respiratory movements.Finally, the number of survivors was notedafter24 h and these animals were then monitored for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Observations:

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

Observations include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanly killed. When animals are killed for humanreasons or found dead, the time of death was

recorded.

Behaviour:

The animals will be observed closely for behaviour in the first four hours which includes abnormal gait, aggressiveness, exophthalmos, ptosis, akinesia, catalepsy, convolusion, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection reactivity of touch, salivation, scratching, sedation, chewing, head movements, sniffing, straub, tremor and writhes, diarrhea, leathery, sleep and coma.

Body Weight:

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.Table- 8.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups. Table: 9-10.

Mortality:

Animals were observed for mortality throughout the entire period.

Results:

All data were summarized in tabular form Table- 11.

5.2 REPEATED DOSE 28-DAYS ORAL TOXICITY STUDY OF *KALLADAIPPU THOOL*(OECD GUIDELINE – 407)

Sub-acute toxicity study was carried out according to OECD 407 and rats were divided into 4 groups of 10 animals (5 male and 5 female). *Kalladaippu Thool* with tender coconut water was administered to rats at the dose of 140 mg/kg,700mg /kg/day and 1400mg/kg/day continuously for 28 days. The animals were observed daily for gross behavioural changes and other sign of sub-acute toxicity. The weight of the each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per rat was calculated. At the end of 28 days they were fasted overnight ,each animal were anaesthetized with diethyl ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of hematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Test Substance :*Kalladaippu Thool*
Animal Source :TANUVAS,Madhavaram, Chennai.
Animals :Male and Female Wister Albino Rats
Age :6-8 weeks
Body Weight :150-200gm.

Acclimatization	:Seven days prior to dose.
Veterinary examination	:Prior and at the end of the acclimatization period.
Identification of animals	:Bycage number, animal number and individual marking by using Picric acid
Diet	:Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	:Aqua guard portable water in polypropylene bottles.
Housing & Environment	:The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	: between 22ºC $\pm$ 3ºC.
Relative humidity	:between 30% and 70%,
Air changes	:10 to 15 per hour
Dark and light cycle	:12**:**12 hours.
Duration of the study	:28 Days.

Justification for Dose Selection:

The results of acute toxicity study in rats indicated that *Kalladaippu Thool*was nontoxic and no behavioral changes were observed up to the dose level of 2000mg/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and Administration of Dose:

*Kalladaippu Thool*was suspended in with Tender coconut water. It was administered to animals at three doses level 140mg/kg,700 mg/kg and 1400 mg/kg respectively were prepared. The test substance was prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage oncedaily for 28 consecutive days.

Methodology:

Randomization, Numbering and Grouping of Animals:

The rats randomly divided into four groups in each group consist of 10 rats (5male and 5 female) dosing up to 28 days. Animals' acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliparous and non-pregnant.

Observations:

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

i) Body Weight:

Weight of each rat was recorded on day 0 and at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percentage of body weight gain was calculated in Table- 12.

ii) Food and water Consumption:

The quantity of food consumed by groups consisting of ten animals for different doses was recorded at weekly interval. Food and consumed per animal was calculated for control and the treated dose groups. Table: 13-14.

iii) Clinical signs:

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

iv) Mortality:

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

v) Laboratory investigation:

Following laboratory investigations were carried out on 29^{th} day in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

vi) Hematological Investigations:

Haematological parameters were determined by using Haematology analyzer.

vii) Biochemical Investigations:

Biochemical parameters were determined by using auto-analyzer.

ix) Histopathology:

Control and highest dose group animals were subjected to histopathological investigations. If

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

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, and hematology and blood chemistry were subjected to One-way Anova Followed by Dunnet's test using a computer software programme. All data were summarized in tabular form (Table: 15-18).

5.3. REPEATED DOSE 90-DAYS ORAL TOXICITY STUDY OF*KALLADAIPPUTHOOL* (OECD GUIDELINE – 408)

Test Substance : *Kalladaippu Thool*

Animal Source : TANUVAS,Madhavaram, Chennai.

Animals : Wister Albino Rats (Male -3, and Female-3)

Age :6-8 weeks

Body Weight :150-200gm.

Acclimatization :Seven days prior to dosing.

Veterinary examination :Prior and at the end of the acclimatization period.

Identification of animals :Bycage number, animal number and individual marking by using picric acid.

Diet :Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore

Water :Aqua guard portable water in polypropylene bottles.

Housing & Environment : The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature :between 22ºC $\pm$3ºC.

Relative humidity :between 30% and 70%,

Air changes :10 to 15 per hour

Dark and light cycle : 12**:**12 hours.

Duration of the study : 90 Days.

Methodology:

Randomization, Numbering and Grouping of Animals:

24 Wistar Albino Rats (12M + 12F) were selected and divided into 4 groups. Each group consists of 6 animals (Male-3 and Female-3)IAEC approval to IAEC/XLIX/18/CLBMCP/2016.Ist group treated as a control and other three group were treated with test drug (low, mid, high) for 90 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Justification for Dose Selection:

As per OECD guideline three dose levels were selected for the study. They were low dose (X), mid dose dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose (8gm) and the body surface area of the rat (0.018). i.e X dose is 140 mg/kg, 5X dose is 700mg/kg and 10 X dose is 1400 mg/kg of animal.

Preparation and Administration of Dose:

*Kalladaippu Thool*was suspended in tender coconut water to obtain concentrations of 200mg/ml. It was administered to animals at the dose levels of 140 mg/kg, 700 mg/kg and 1400 mg/kg. The test substance suspensions were freshly prepared every two days once for 90 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 90 consecutive days.

Observations:

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

Body Weight:

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study .Table – 21.

Clinical signs:

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

Mortality:

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

Laboratory Investigations:

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

Hematological Investigations:

Hematological parameters were determined using Hematology analyzer.

Biochemical Investigations:

Biochemical parameters were determined using auto-analyzer.

Histopathology:

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

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, hematology and blood chemistry were subjected to One-way ANOVA followed by dunnett'stest using a computer software programme -INSTAT-V3 version.All data were summarized in Table-19

to 24.

6.1 ANTI-UROTHIALITIC ACTIVITY OF *KALLADAIPPU THOOL*

Aim:

Screening of*Kalladaippu Thool*against ethylene glycol inducedUrolithiasis in Wister albino rats.

Experimental animals:

Healthy adult Wister albino male rats of weighing 150-200gm were included in the study. The animals were procured from the animal house of TANUVAS, Madhavaram, Chennai. The animals were maintained in well ventilated room temperature with natural 12 +12hrs light/dark cycle in the polypropylene cages. The animals were fed with balanced diet that is standard rodent pellet diet Sai meera foods Pvt Ltd, Bangalore and water *ad libitum. The animals* were housed for 1week prior to the experiment to acclimate to the laboratory conditions. Approval for the research work and ethical clearance was obtained from Institutional Animal Ethical Committee (IAEC/XLIX/18/CLBMCP/2016).

Animal grouping:

Wistar albino male rats of body weight ranging from 150-200gm were used as experimental animals. They were divided into six groups (6 per group) as follows:

Group I : Normal Control (vehicle)

Group II : Lithiatic Control (ethylene glycol) +vehicle

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

Group IV : Treated with *Kalladaippu Thool* (100mg/kg) + ethylene glycol

Group V : Treated with *Kalladaippu Thool* (200mg/kg) + ethylene glycol

Group VI : Treated with *Kalladaippu Thool* (400 mg/kg) + ethylene glycol

Pharmacological screening for anti- Urolithiatic activity :[24]

Animals were divided into six groups containing six animals in each. Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to Groups II to Group VI for induction of renal calculi till 28 days. Group II received Ethylene glycol alone and served as lithiatic control. Group III received standard anti-urolithiatic drug, Cystone (750mg/kg body weight) from 15th day to end of the study period. Groups IV, Group V and Group VI received *Kalladaippu Thool* (100, 200 and 400 mg/kg body weight respectively) from 15th day to 28th day of the study period. All drugs were administered once daily through oral route by using gastric tube.

On 29th day animals of all the groups were kept in metabolic cages and urine samples were collected for 24h and analyzed for calcium, potassium and oxalate content. The serum analysed for blood urea nitrogen, uric acid and creatinine.

Statistical analysis:

The results were expressed as mean ±SEM (n=6). Statistical analysis was performed by ANOVA test for multiple comparisons followed by Dunnett's test and $P<0.05$ was considered as significant.

6.2. DIURETIC AND 6.3 SALURETIC ACTIVITY OF *KALLADAIPPU THOOL*

Aim:

To evaluate the diuretic and saluretic potential[26] of *Kalladaippu Thool*in Wisteralbino rats.

Experimental animals:

Healthy adult Wister albino male rats of weighing 150-200gm were included in the study. The animals were procured from the animal house of TANUVAS, Madhavaram, Chennai. The animals were maintained in well ventilated room temperature with natural 12 +12hrs light/dark cycle in the polypropylene cages. The animals were fed with balanced diet that is standard rodent pellet diet Sai meera foods Pvt Ltd, Bangalore and water *ad libitum. The animals* were housed for 1week prior to the experiment to acclimate to the laboratory conditions. Approval for the research work and ethical clearance is obtained from Institutional Animal Ethical Committee (IAEC/XLIX/18/CLBMCP/2016).

Study design: [25]

Wistar rats were divided into five groups of six rats in the Group (I) served as normal control (Vehicle). Group II received as furosemide (20mg/kg). Groups (III) to Group (V) received *Kalladaippu Thool* respectively at dose of 100mg/kg, 200 mg/kg and 400 mg/kg orally and immediately after the extract treatment, all the rats are hydrated with saline (15 ml/kg) and placed in metabolic cages. A total volume of urine collected for 24h was

measured at the end. During this period no food and water were made available to animals.The urine volume (ml/day) was measured and then assayed for Na^+, $K^+$and Cl^- concentrations in mMol/l, Cl^- was measured by using routine method.

Procedure:

The method of Lipschitz *et al*[27] employed for the assessment of diuretic activity. All the animals were hydrated with double distilled water. Food and water were withdrawn 8h before the administration of drug. Immediately after dosing, all the animals were placed individually in metabolic cages and urine passed by the animals over a period of 24 h was collected in a conical flask. Total urine, urinary output, urinary electrolyte, Ph was determined.

Estimation of urine output:

Metabolic cage is designed with a stainless steel circular frame. The upper portion is covered with a lid, provided with a wire mesh bottom and a funnel for collecting the urine. Stainless steel sieves were placed in the funnel to retain the faeces, allowing only urine to flow down for collection and measurement. The whole structure is fixed to a metal frame, which keeps the frame in upright position. Conical flask is kept to collect the urine at the bottom exit of the funnel for a period of 24h.Urine volume is expressed as ml/kg. The room temperature is maintained at 27-29°c.

Assessment of lipschitz-value and saluretic effect:

The mean urine volume was determined and it was expressed as Lipschitz-value, i.e., the ratio of T/C in which T was the response of the test compound and C that of control. Indices of 1.0 and more were regarded as positive effect. The saliuretic activity (Na^++Cl^-) and natriuretic activity (Na^+ /K^+) of the test extracts was also calculated. The index values greater than 2.0 indicate favourable natriuretic effect. The diuretic potency and the saluretic effect were assessed by comparison of the urinary excretion and urinary electrolyte excretion respectivelyof the test extracts with respect to the control group.

Statistical analysis:

All values are expressed as mean values ± SEM (Standard error of mean) and data were analyzed by applying an analysis of variance (ANOVA) followed by dunnett's t test. The results were considered statistically significant if $P < 0.05$.

7. RESULTS

ORGANOLEPTIC CHARACTERS

Table 1:Organoleptic characters of *Kalladaippu Thool*

Color	Dark Brown
Odour	Pleasant
Taste	Salty
Texture	Fine powder

PHYSICOCHEMICAL ANALYSIS

Table 2: Physicochemical properties of *Kalladaippu Thool*

S. No.	Parameters	Results
1	Loss on drying	1.338%
2	Ash value a. Total ash (w/w) b. Acid insoluble ash (w/w) c. Water Soluble ash (w/w)	 0.282% 0.028% 0.086%
3	Extractive values a. Alcohol soluble (w/v) b. Alcohol successive soluble (w/v)	3.87% 2.94%
4	pH values (10% solution)	9.8

*****The experimental procedure was analyzed at Regional Research Institute of Unani Medicine (RRIUM), Rayapuram, Chennai- 600 013.

CHEMICAL ANALYSIS

Table 3: Results of acid radicals studies of *Kalladaippu Thool**

S.NO	Parameter	Observation	Result
1	Test for Sulphate	Cloudy appearance present	**Positive**
2	Test for Chloride	-	Negative
3	Test For Phosphate	-	Negative
4	Test For Carbonate	-	Negative
5	Test For Nitrate	-	Negative
6	Test for Sulphide	-	Negative
7	Test For Fluride &oxalate	-	Negative
8	Test For Nitrite	-	Negative
9	Test For Borax	-	Negative

Interpretation:

The acidic radicals test shows the presence of Sulphate**.**

*****The chemical analysis was carried out in Bio chemistry Lab, NIS, Chennai – 47.

Table 4: Results of basic radicals studies of *Kalladaippu Thool

S.NO	Parameter	Observation	Result
1	Test for Lead	-	Negative
2	Test for Copper	-	Negative
3	Test For Aluminium	-	Negative
4	Test For Iron	Blood red color appeared	**Positive**
5	Test For Zinc	-	Negative
6	Test for Calcium	Cloudy appearance and white precipitate was obtained	**Positive**
7	Test For Magnesium	-	Negative
8	Test For Ammonium	-	Negative
9	Test For Potassium	-	Negative
10	Test For Sodium	-	Negative
11	Test For Mercury	-	Negative
12	Test For Arsenic	-	Negative

Interpretation:

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

*The chemical analysis was carried out in Bio chemistry Lab, National Institute of Siddha, Chennai- 47.

TLC AND HPTLC ANALYSIS OF *KALLADAIPPU THOOL*

The procedure recommended for the analysis of TLC and HPTLC analysis as per Wagner H and Bladt S, 1996

Instrument Details:

Name of the Instrument	: CAMAG (CAMAG - Automatic TLC sampler, Scanner and Visualizer)
Spray Gas	: N2
Lamp used	: Deuterium and Tungsten Lamp

The sample was applied for the Thin Layer Chromatography and High Performance Thin Layer Chromatography study with suitable solvent systems. After development the plate was allowed to dry in air and examined under UV – 254nm, 366nm and visible light after derivatised using vanillin – sulphuric acid.

The sample (alcohol extract - 20µl) was applied in TLC aluminium sheet silica gel 60 F 254 (E. MERCK) and plate was developed using the solvent system Toluene: Ethyl acetate: Formic acid (8: 2: 0.2). After development the plate was allowed to dry in air and examined under UV – 254 nm, 366 nm and Visible light (Vanillin –Sulphuric acid).

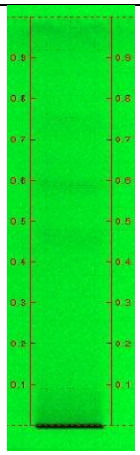
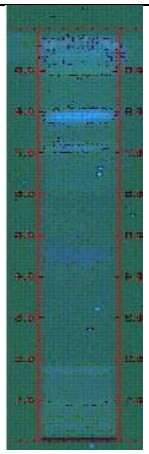
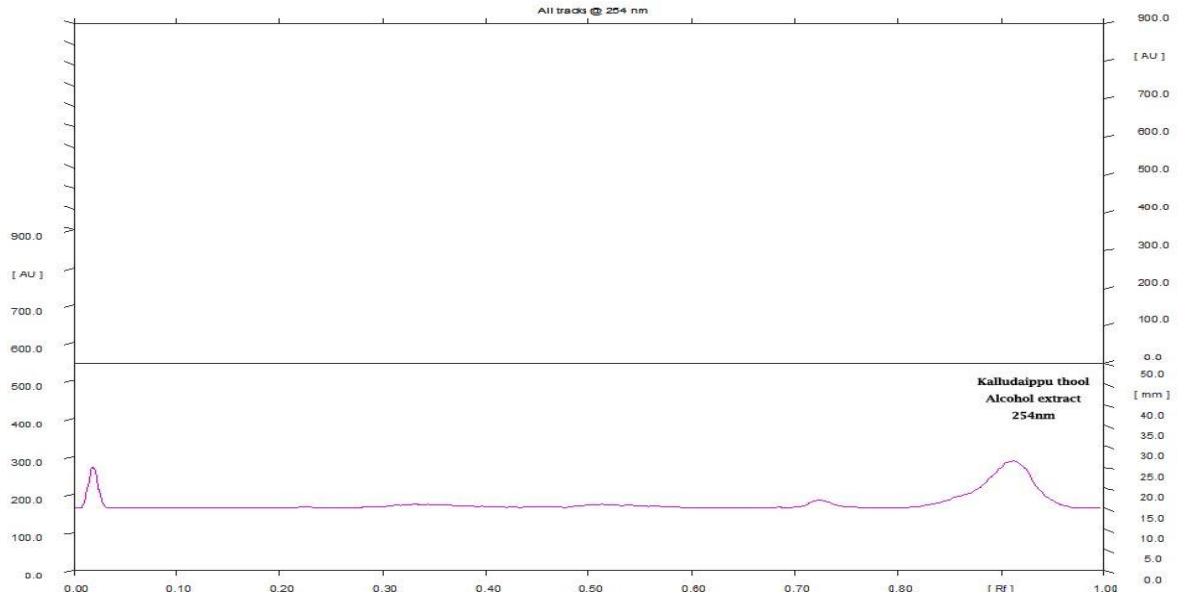
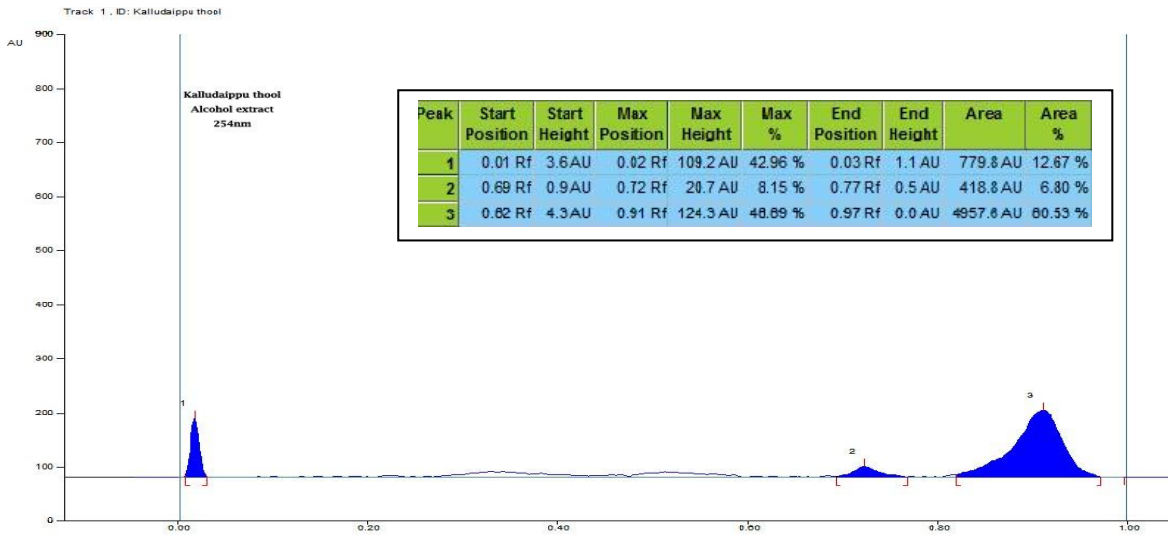
		
UV-254 nm	**UV- 366 nm**	**Visible light (Derivatized with Vanillin-Sulphuric acid)**

Table 5: Rf values

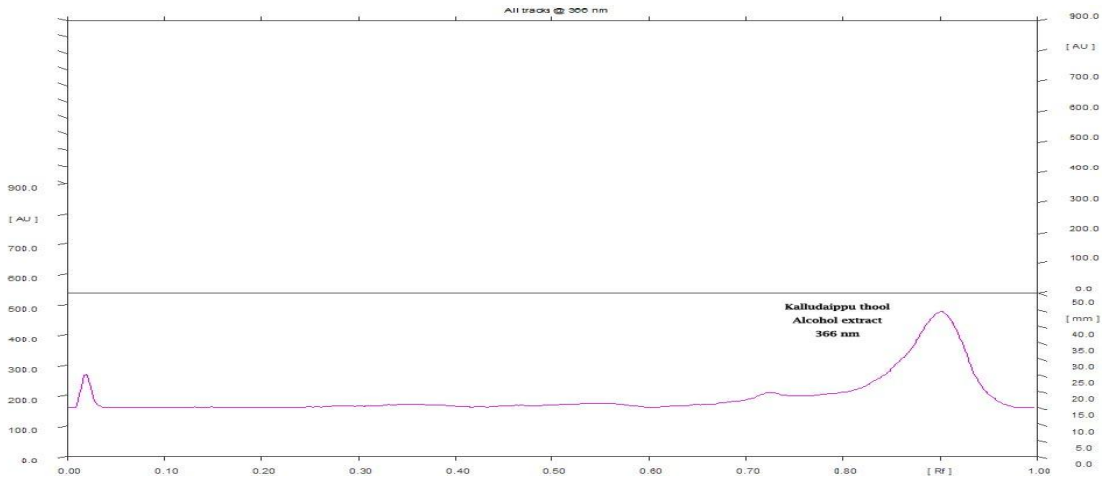
Solvent Systems	Rf values		
	UV- 254 nm	**UV- 366 nm**	**Visible light (Vanillin – Sulphuric acid)**
Toluene : Ethyl acetate : Formic acid (8 : 2 : 0.2)	0.73 Dark green	0.90 Light yellow	0.60 Brown
	0.59 Dark green	0.79 Light blue	0.46 Red
	0.46 Dark green	0.70 Dark blue	
		0.62 Dark blue	
		0.48 Dark blue	
		0.19 Blue	



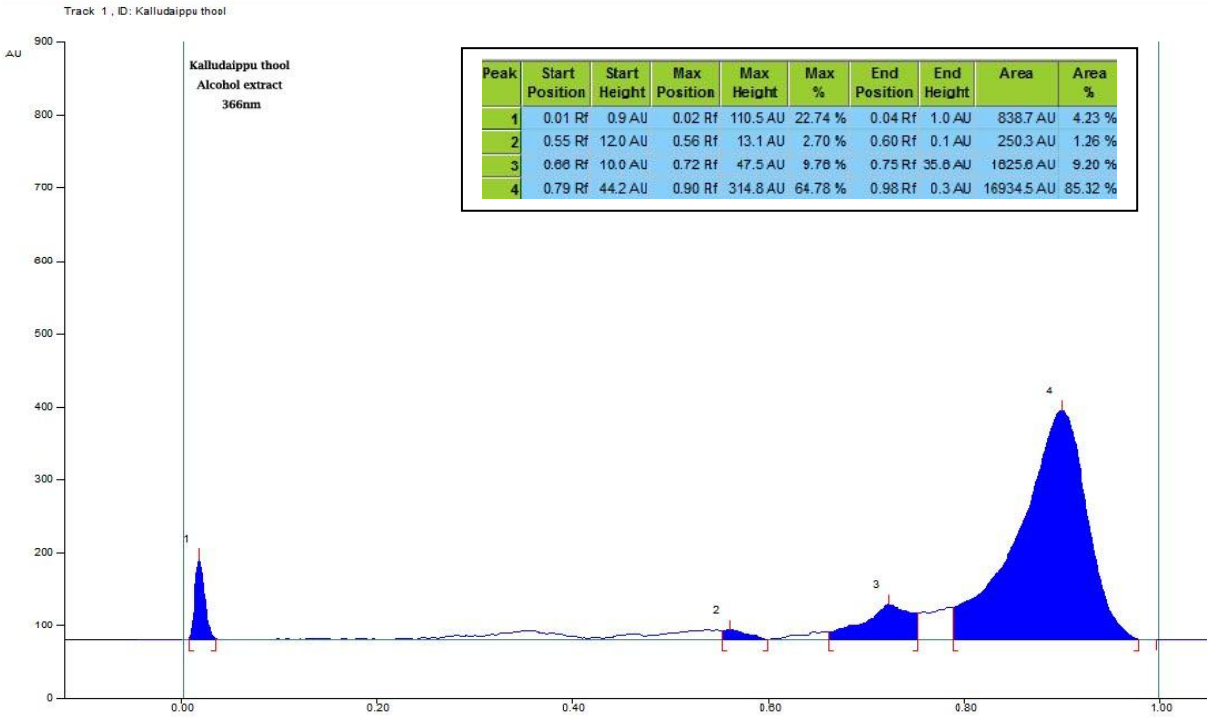
Densitometric chromatogram at UV-254 nm



HPTLC finger print at UV-254 nm



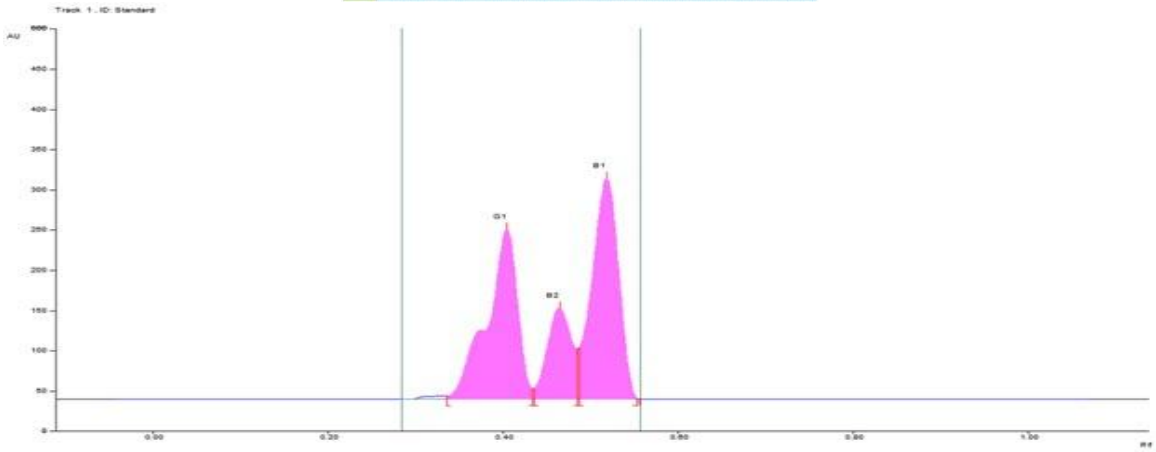
Densitometric chromatogram at UV-366 nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.9 AU	0.02 Rf	110.5 AU	22.74 %	0.04 Rf	1.0 AU	838.7 AU	4.23 %
2	0.55 Rf	12.0 AU	0.56 Rf	13.1 AU	2.70 %	0.60 Rf	0.1 AU	250.3 AU	1.26 %
3	0.66 Rf	10.0 AU	0.72 Rf	47.5 AU	9.76 %	0.75 Rf	35.6 AU	1825.6 AU	9.20 %
4	0.79 Rf	44.2 AU	0.90 Rf	314.8 AU	64.78 %	0.98 Rf	0.3 AU	16934.5 AU	85.32 %

HPTLC finger print at UV-366 nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.34 Rf	4.2 AU	0.41 Rf	211.8 AU	35.25 %	0.43 Rf	13.0 AU	6233.0 AU	39.20 %
2	0.44 Rf	13.3 AU	0.47 Rf	113.7 AU	18.92 %	0.49 Rf	62.9 AU	2680.1 AU	16.85 %
3	0.49 Rf	64.2 AU	0.52 Rf	275.4 AU	45.83 %	0.55 Rf	0.5 AU	6989.0 AU	43.95 %



HPTLC finger print of Aflatoxin standards at UV-366 nm

HEAVY METAL ANALYSIS AND MICROBIAL LOAD ANALYSIS

Table 6: Test for Heavy Metals:

S. No.	Heavy metal	Reference Limits as per API- Vol.-I	Results	Remarks
1	**Lead**	Not more than 10ppm	0.1393ppm	Within permissible limits
2	**Arsenic**	Not more than 3.0ppm	18.1075ppb	Within permissible limits
3	**Cadmium**	Not more than 0.3ppm	0.0043ppm	Within permissible limits
4	**Mercury**	Not more than 1.0ppm	6.9876ppb	Within permissible limits

Table 7: Analysis of Microbial Load:

S. No.	Parameters	Reference Limits as per WHO (2007)	Results	Remarks
1	**Total Bacterial Count (TBC)**	10^5CFU/gm	Less than 10 cfu/gram	Within permissible limits
2	**Total Fungal Count (TFC)**	10^3CFU/gm	Absent	
3	**Enterobacteriaceae**	10^3	Absent	
4	**Escherichia coli**	10	Absent	
5	**Salmonella Spp**	Absent	Absent	
6	**Staphylococcus aureus**	Absent	Absent	

Test For Aflatoxin:

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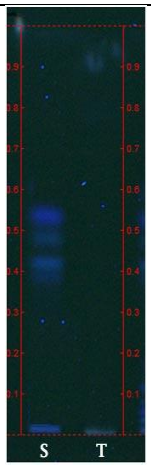
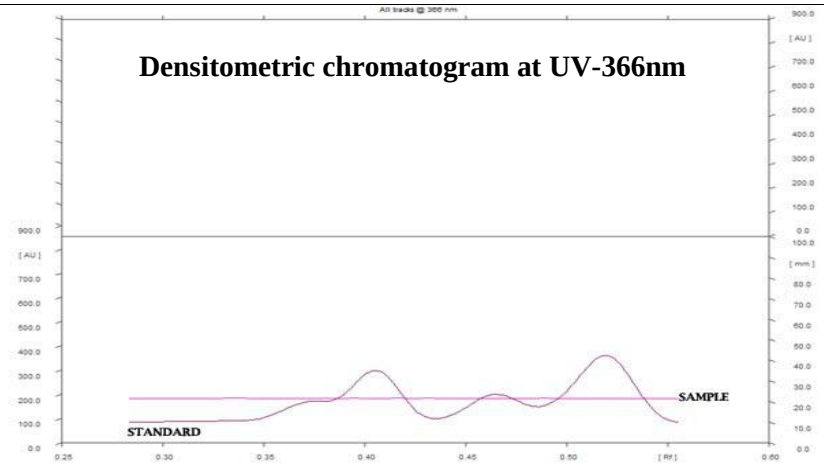
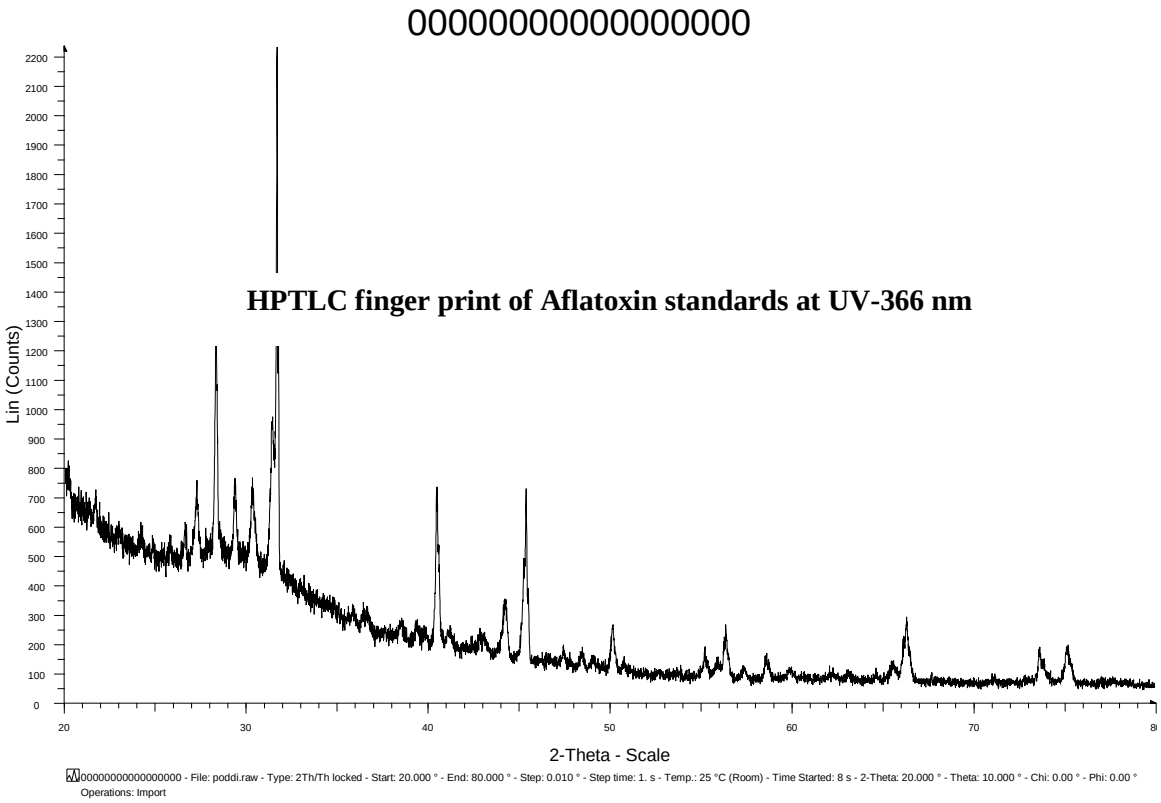
<tr><td> 
</td><td> 
</td></tr>
<tr><td>UV-366nm</td><td>Standard – G2, G1, B2 & B1
The test sample does not show the evidence for the presence of any of the aflatoxins.</td></tr>
</table>

XRD STUDY RESULTS

XRD (image)



Intensity filtered

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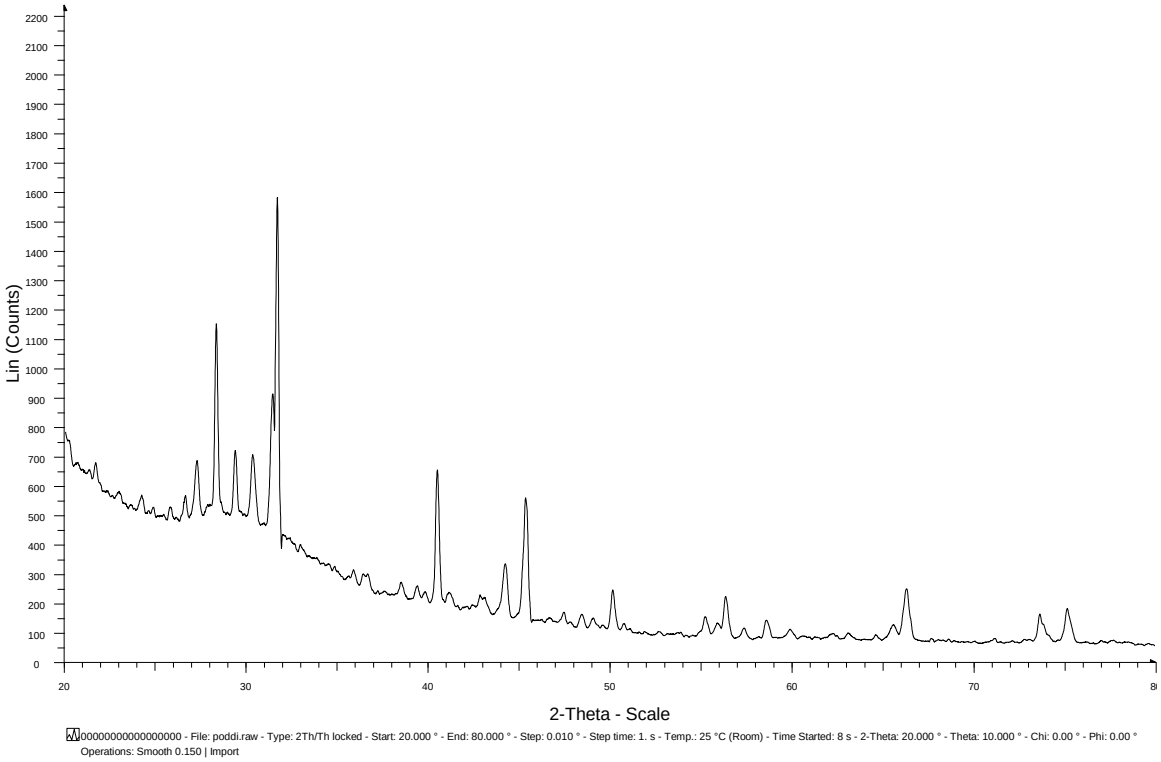
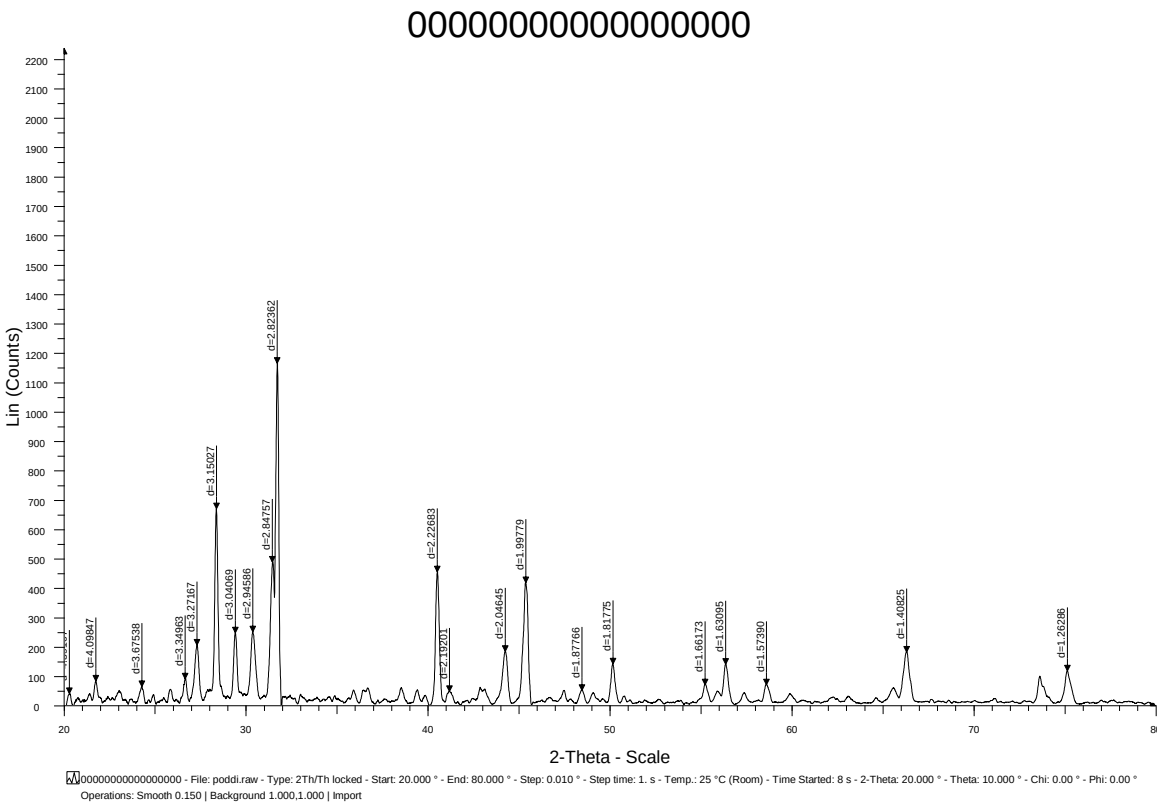


Background and intensity filtered

Peak (detected)

Angle	d value	Intensity	Intensity %
2-Theta °	Angstrom	Count	%
20.207	4.39107	37.5	3.2
21.666	4.09847	79.4	6.8
24.196	3.67538	60.4	5.2
26.590	3.34963	87.1	7.5
27.236	3.27167	203	17.4
28.307	3.15027	669	57.3
29.349	3.04069	244	21.0
30.317	2.94586	248	21.3
31.389	2.84757	486	41.7
31.663	2.82362	1167	100.0
40.476	2.22683	453	38.8
41.148	2.19201	44.6	3.8
44.223	2.04645	181	15.5
45.359	1.99779	416	35.7
48.440	1.87766	47.7	4.1
50.145	1.81775	138	11.8
55.233	1.66173	67.4	5.8
56.367	1.63095	137	11.7
58.605	1.57390	67.1	5.8
66.321	1.40825	179	15.3
75.174	1.26286	115	9.9

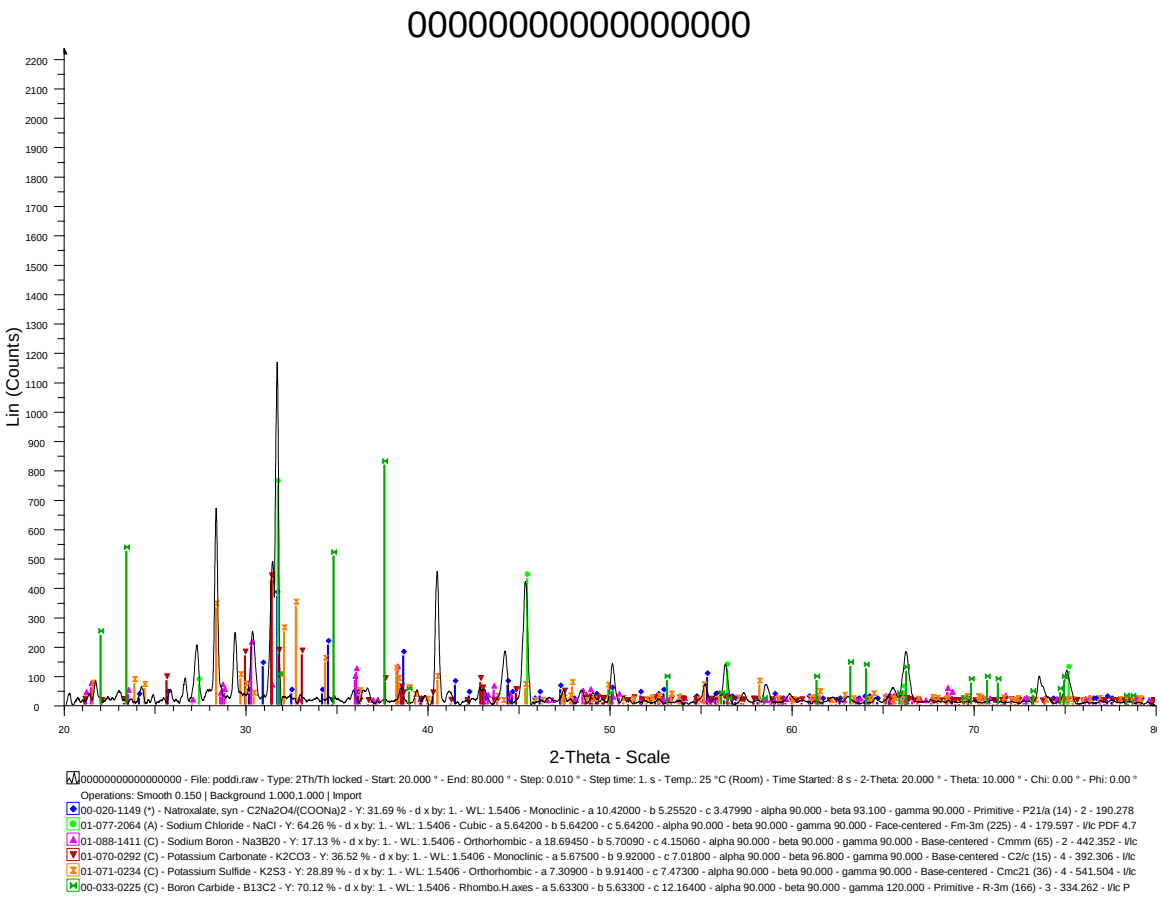
Peak (image)

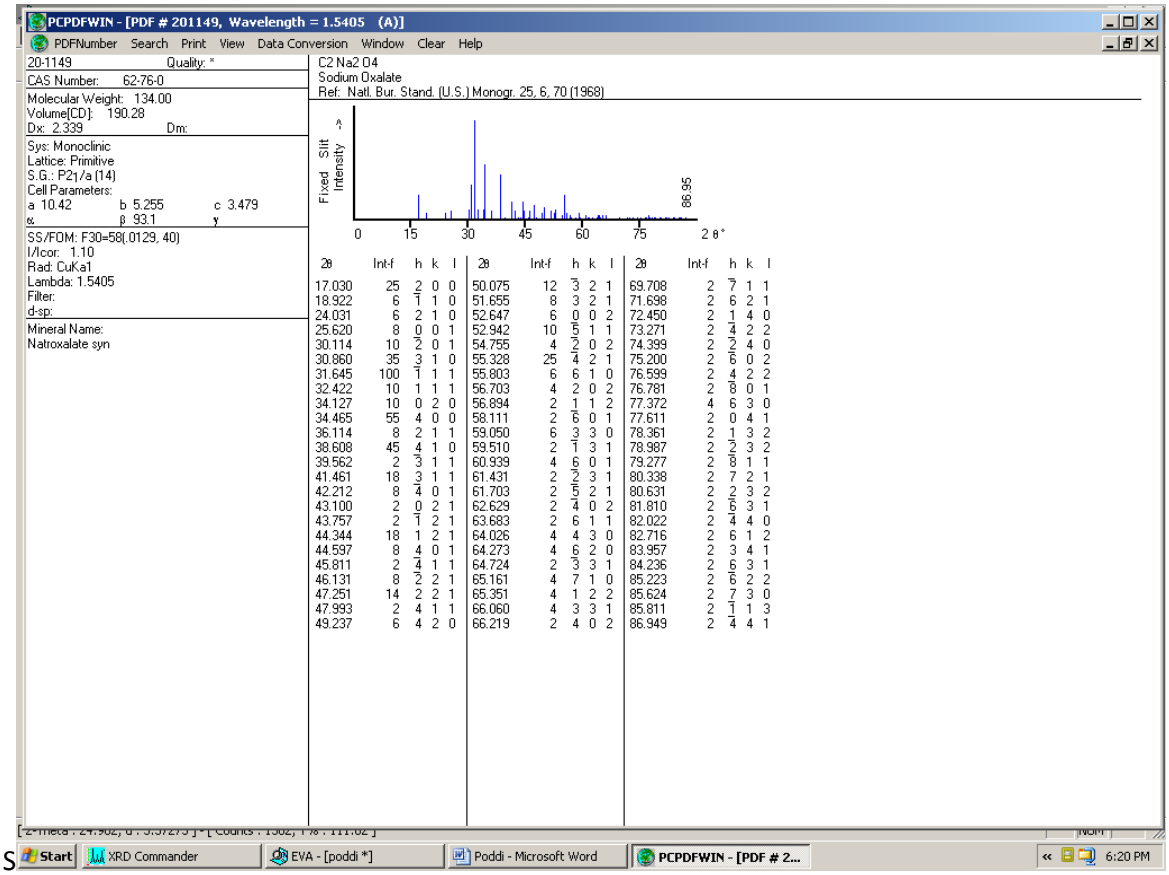


Crystalline

Sample Name	Left Angle	Right Angle	Left Int.	Right Int.	**Obs. Max**	d (Obs. Max)	Max Int.	Net Height	**FWHM**	Chord Mid.	I. Breadth	Gravity C.	d (Gravity C.)	Raw Area	Net Area
	2-Theta °	2-Theta °	Cps	Cps	**2-Theta °**	Angstrom	Cps	Cps	**2-Theta °**	2-Theta °	2-Theta °	2-Theta °	Angstrom	Cps x 2-Theta °	Cps x 2-Theta °
000000000000000000	31.520	31.780	386	386	**31.664**	2.82350	1166	780	**0.159**	31.659	0.153	31.657	2.82414	219.9	119.2

Material (match)

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Lattice: Primitive
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α β 93.1 γ
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Lambda: 1.5405
Filter:
d-sp:
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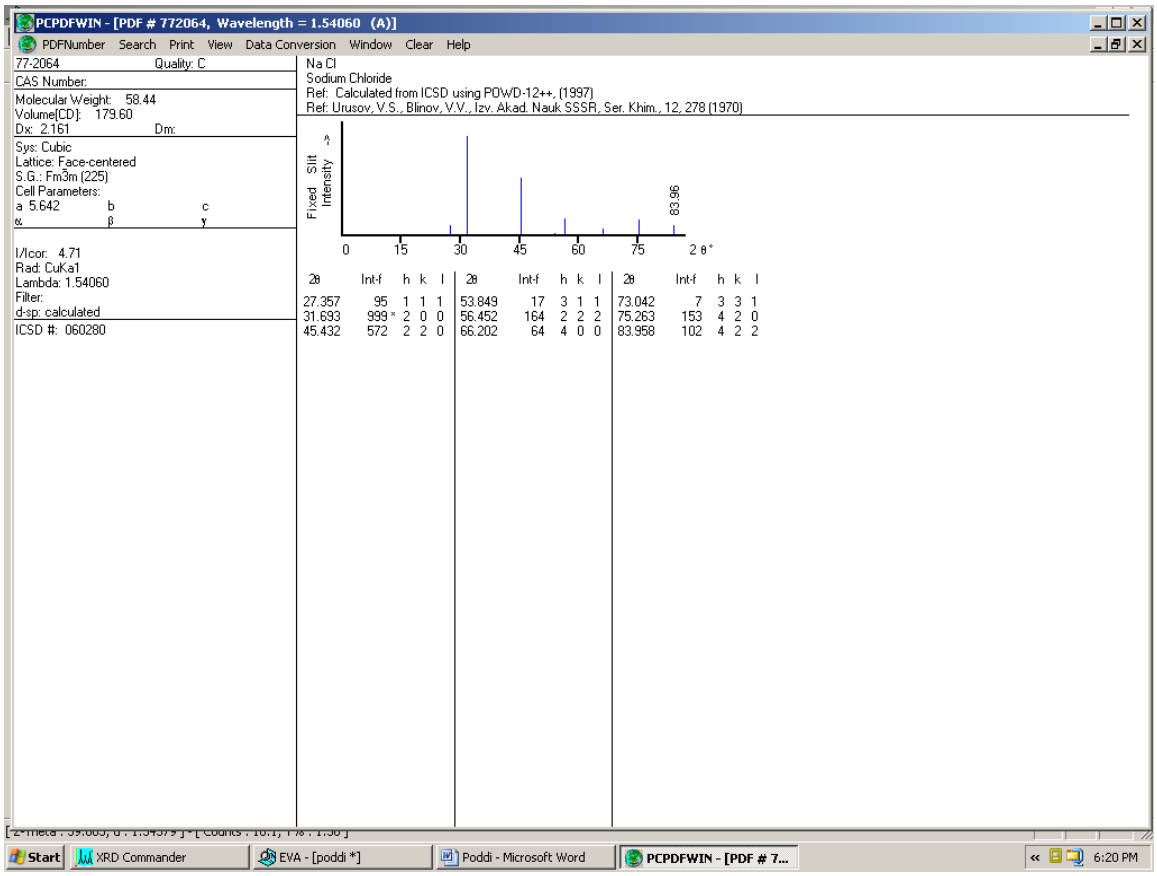
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2θ°
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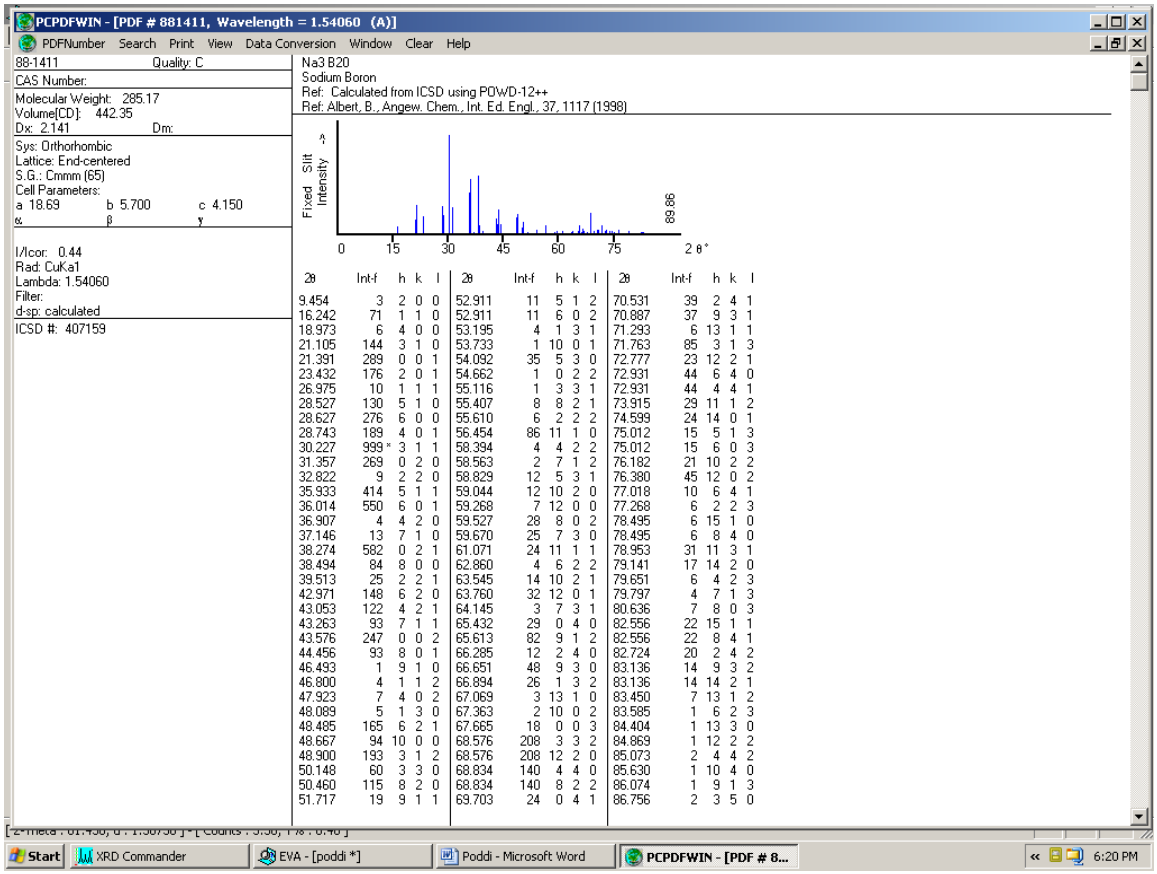
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S.G.: Cmmm (65)
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α β γ
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Lambda: 1.54060
Filter:
d-sp: calculated
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Na3 B20
Sodium Boron
Ref: Calculated from ICSD using POWD-12++
Ref: Albert, B., Angew. Chem., Int. Ed. Engl., 37, 1117 (1998)
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16.242 71 1 1 0 52.911 11 6 0 2 70.887 37 9 3 1
18.973 6 4 0 0 53.195 4 1 3 1 71.293 6 13 1 1
21.105 144 3 1 0 53.733 1 10 0 1 71.763 85 3 1 3
21.391 289 0 0 1 54.092 35 5 3 0 72.777 23 12 2 1
23.432 176 2 0 1 54.662 1 0 2 2 72.931 44 6 4 0
26.975 10 1 1 1 55.116 1 3 3 1 72.931 44 4 4 1
28.527 130 5 1 0 55.407 8 8 2 1 73.915 29 11 1 2
28.627 276 6 0 0 55.610 6 2 2 2 74.599 24 14 0 1
28.743 189 4 0 1 56.454 86 11 1 0 75.012 15 5 1 3
30.227 999 * 3 1 1 58.394 4 4 2 2 75.012 15 6 0 3
31.357 269 0 2 0 58.563 2 7 1 2 76.182 21 10 2 2
32.822 9 2 2 0 58.829 12 5 3 1 76.380 45 12 0 2
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36.014 550 6 0 1 59.268 7 12 0 0 77.268 6 2 2 3
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39.513 25 2 2 1 63.545 14 10 2 1 79.651 6 4 2 3
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44.456 93 8 0 1 66.285 12 2 4 0 82.724 20 2 4 2
46.493 1 9 1 0 66.651 48 9 3 0 83.136 14 9 3 2
46.800 4 1 1 2 66.894 26 1 3 2 83.136 14 14 2 1
47.923 7 4 0 2 67.069 3 13 1 0 83.450 7 13 1 2
48.089 5 1 3 0 67.363 2 10 0 2 83.585 1 6 2 3
48.485 165 6 2 1 67.665 18 0 0 3 84.404 1 13 3 0
48.667 94 10 0 0 68.576 208 3 3 2 84.869 1 12 2 2
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50.148 60 3 3 0 68.834 140 4 4 0 85.630 1 10 4 0
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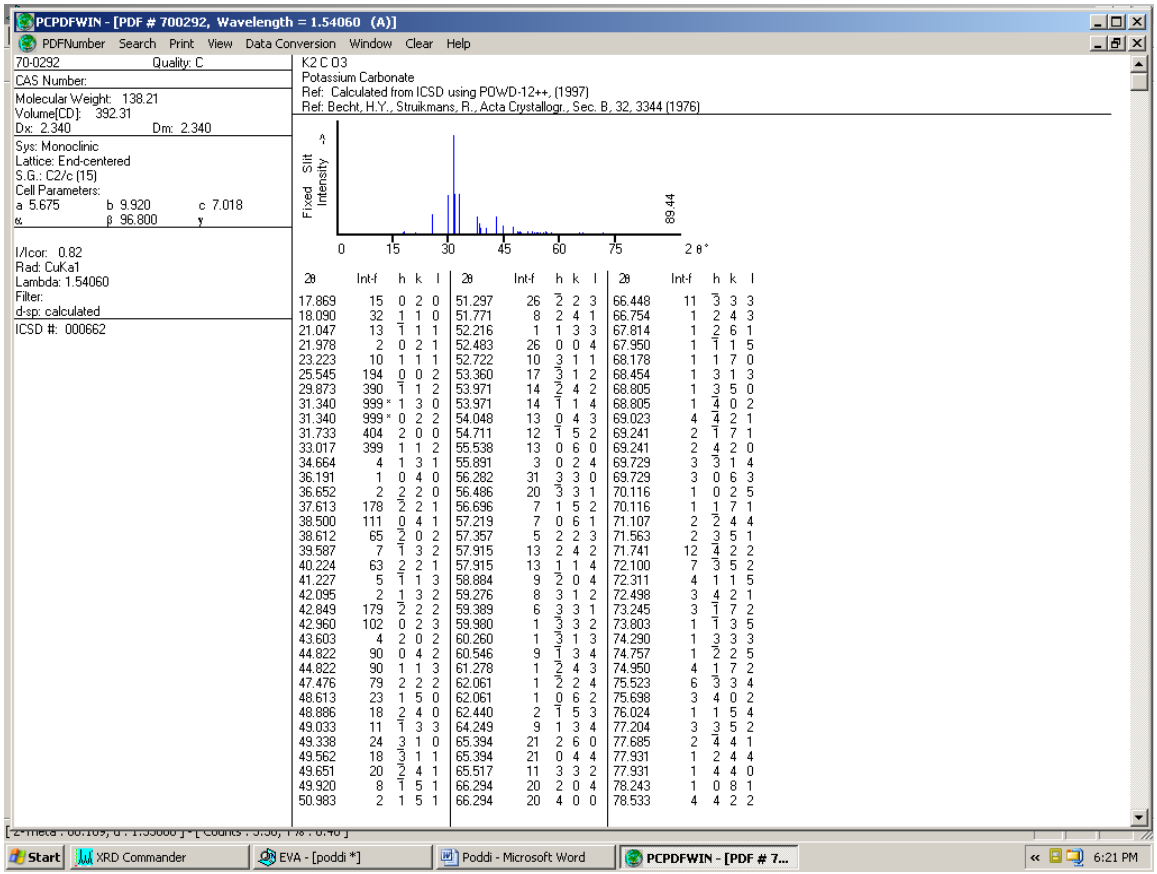
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21.978 2 0 2 1 52.483 26 0 0 4 67.950 1 -1 1 5
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29.873 390 -1 1 2 53.971 14 -2 4 2 68.805 1 -3 5 0
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37.613 178 -2 2 1 56.696 7 1 5 2 70.116 1 -1 7 1
38.500 111 -0 4 1 57.219 7 0 6 1 71.107 2 -2 4 4
38.612 65 -2 0 2 57.357 5 2 2 3 71.563 2 -3 5 1
39.587 7 -1 3 2 57.915 13 2 4 2 71.741 12 -4 2 2
40.224 63 -2 2 1 57.915 13 1 1 4 72.100 7 -3 5 2
41.227 5 -1 1 3 58.884 9 -2 0 4 72.311 4 1 1 5
42.095 2 1 3 2 59.276 8 3 1 2 72.498 3 -4 2 1
42.849 179 -2 2 2 59.389 6 -3 3 1 73.245 3 -1 7 2
42.960 102 0 2 3 59.980 1 -3 3 2 73.803 1 1 3 5
43.603 4 2 0 2 60.260 1 -3 1 3 74.290 1 -3 3 3
44.822 90 0 4 2 60.546 9 -1 3 4 74.757 1 -2 2 5
44.822 90 1 1 3 61.278 1 -2 4 3 74.950 4 -1 7 2
47.476 79 2 2 2 62.061 1 -2 2 4 75.523 6 -3 3 4
48.613 23 1 5 0 62.061 1 -0 6 2 75.698 3 4 0 2
48.886 18 -2 4 0 62.440 2 -1 5 3 76.024 1 1 5 4
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49.338 24 -3 1 0 65.394 21 2 6 0 77.685 2 -4 4 1
49.562 18 -3 1 1 65.394 21 0 4 4 77.931 1 2 4 4
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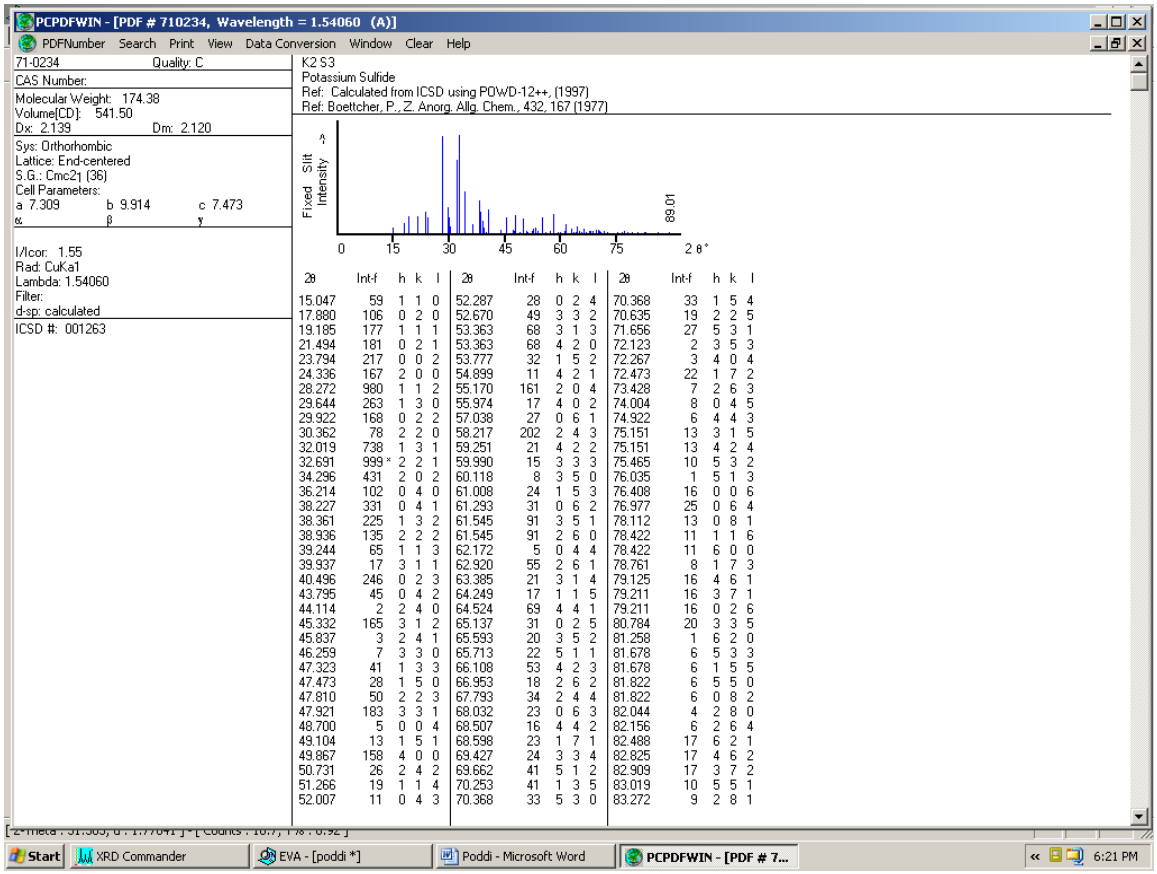
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17.880 106 0 2 0 52.670 49 3 3 2 70.635 19 2 2 5
19.185 177 1 1 1 53.353 68 3 1 3 71.656 27 5 3 1
21.494 181 0 2 1 53.363 68 4 2 0 72.123 2 3 5 3
23.794 217 0 0 2 53.777 32 1 5 2 72.267 3 4 0 4
24.336 167 2 0 0 54.899 11 4 2 1 72.473 22 1 7 2
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29.644 263 1 3 0 55.974 17 4 0 2 74.004 8 0 4 5
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43.795 45 0 4 2 64.249 17 1 1 5 79.211 16 3 7 1
44.114 2 2 4 0 64.524 69 4 4 1 79.211 16 0 2 6
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45.837 3 2 4 1 65.593 20 3 5 2 81.258 1 6 2 0
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47.323 41 1 3 3 66.108 53 4 2 3 81.678 6 1 5 5
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49.104 13 1 5 1 68.598 23 1 7 1 82.488 17 6 2 1
49.867 158 4 0 0 69.427 24 3 3 4 82.825 17 4 6 2
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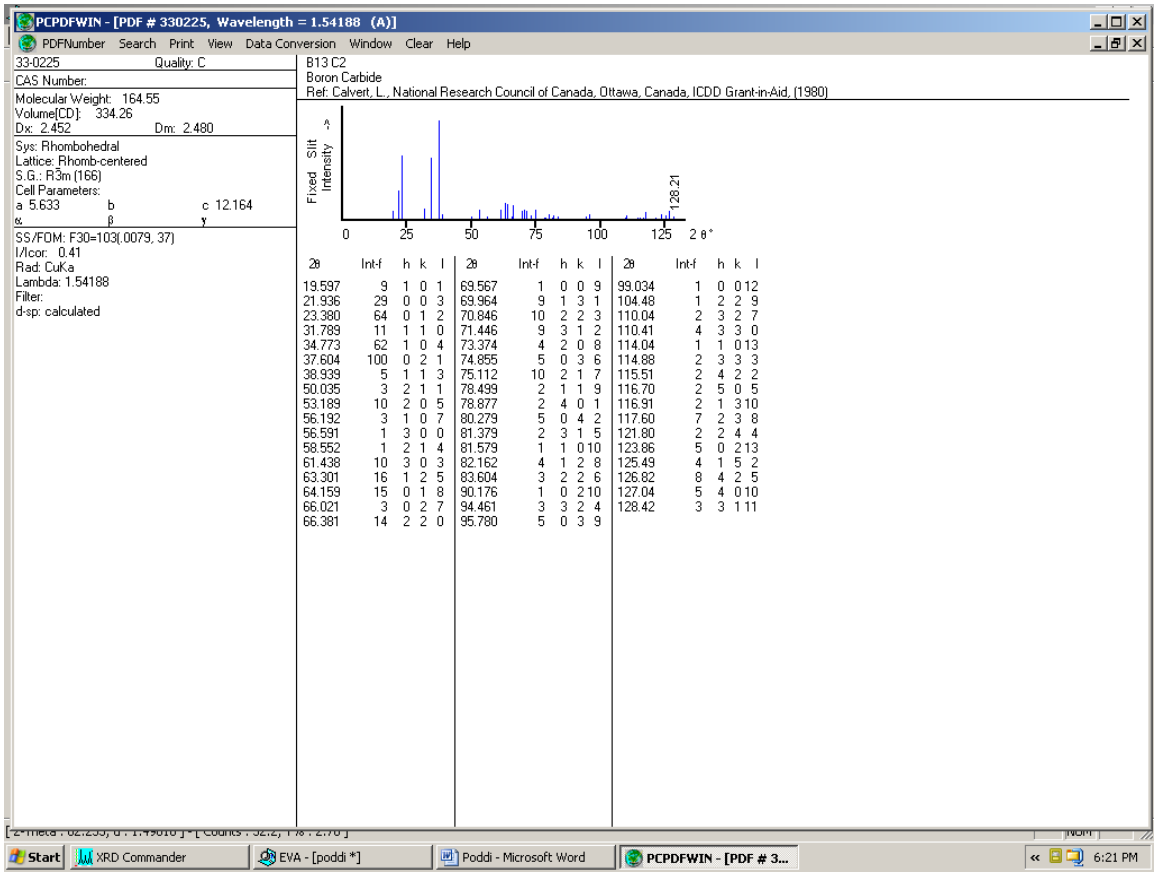
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2θ Int-f h k l 2θ Int-f h k l 2θ Int-f h k l
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21.936 29 0 0 3 69.964 9 1 3 1 104.48 1 2 2 9
23.380 64 0 1 2 70.846 10 2 2 3 110.04 2 3 2 7
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Acute Oral Toxicity Study of *Kalladaippu Thool*

Table: 8Body weights of Wistar albino rats groups exposed to*Kalladaippu thool*

DOSE	**DAYS**		
	1	7	14
Control	158.1±65.70	158.4 ± 41.11	159.7 ±02.12
High dose	157.1± 6.64	157.4 ±7.42	158.8 ± 2.70
P value (p)*	NS	NS	NS

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

Table: 9Water intakes (ml/day) of Wistar albino rats group exposed to*Kalladaippu Thool*

DOSE	**DAYS**		
	1	6	14
Control	54 ± 3.20	54.1±6.10	54.3±5.44
High dose	53.5±1.30	53.8±6.70	54.2±5.64
P value (p)*	NS	NS	NS

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

Table 10: Food intake (gm/day) of Wistar albino rats group exposed to *kalladaippu thool*

DOSE	**DAYS**		
	1	7	14
Control	56.03±2.82	56.2±2.96	57.7±8.86
High ose	58.6±5.44	58.4±5.20	59.8±6.67
P value (p)*	NS	NS	NS

Table 11: Dose finding experiment and its behavioural Signs of Toxicity in wistar albino rats

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

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

+ Presence of Activity

-Absence of Activity

Result

All the data were summarized in the form of Table - 11 revealed no abnormal signs and behavioral changes in rats upto the dose level of 2000 mg/kg body weight administered orally.

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*Acute oral toxicity was done at C.L Baid Metha college of Pharmacy, Thoraipakam, Chennai-97.

28 Days Repeated Dose Oral Toxicity Study of *Kalladaippu Thool*

Table 12: Body weight grams of wistar albino rats exposed to *KalladaippuThool* for 28 days

Dose (mg/kg/day)	Days				
	0	**7**	**14**	**21**	**28**
Control	157±4.37	157.42±1.17	156.92±1.21	157.36±1.41	158.01±1.03
Mid dose	154.13±0.82	154.72±0.55	155.24±0.98	156.1±1.17	157.12±0.52
High dose	155.8±1.03	156.12±0.75	156.69±0.89	157.22±0.75	158.11±1.52

Values are mean of 10 animals ± S.E.M (Dunnett's test) *p<0.05;**p<0.01.N=10

Table 13: Water (ml/day) intakes of wistar albino rats exposed to *Kalladaippu Thool* for 28 days

Dose(mg/kg/day)	Days				
	1	**7**	**14**	**21**	**28**
Control	32.0±0.92	33.36±0.23	33.23±0.23	33.18±0.16	33.64±0.38
Mid dose	35.83±0.49	34.5±1.52	34.33±1.51	35.5±1.05	35.83±1.72
High dose	34.72±1.51	35.19±1.79	36.17±1.51	36.42±1.33	36.59±1.52

Values are mean of 10 animals ± S.E.M (Dunnett's test) *p<0.05;**p<0.01.N=10

Table14: Food (g/day) intakes of wistar albino rats exposed to *Kalladaippu Thool* for 28 days

Dose (mg/kg/day)	Days (gms/rats)				
	1	**7**	**14**	**21**	**28**
Control	33.5±0.84	34.72±1.17	35±0.75	35.32±1.97	35.86±1.37
Mid dose	35.82±0.82	38.16±0.75	38.02±0.98	39.83±1.60	39.33±1.21
High dose	34.75±2.02	37.13±1.33	37.93±1.10	39.33±1.51	39.83±1.17

Values are mean of 10 animals ± S.E.M (Dunnett's test)* p<0.05;**p<0.01.N=10

Table: 15 Hematological parameters after 28days treatment with *Kalladaippu Thool*in wistar albino rats

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin (g/dl)	14.5±0.43	14.60±0.32	14.8±0.23	14.84±0.33	N.S
Total WBC ($\times 10^3$ l)	12.71±0.40	12.82±0.21	12.94±0.60	13.06±1.40	N.S
Neutrophils (%)	26.12±0.40	25.22±0.32	26.31±1.50	26.54±2.20	N.S
Lymphocyte (%)	80.12±1.60	80.74±1.40	81.16±1.44	81.70±1.64	N.S
Monocyte (%)	0.6±0.02	0.56±0.01	0.72±0.04	0.75±0.03	N.S
Eosinophil (%)	0.21±0.02	0.19±0.04	0.23±0.06	0.26±0.06	N.S
Platelets cells10^3/µl	702±2.28	702.32±2.42	702.21±2.60	702.42±3.64	N.S
Total RBC 10^6/µl	7.64±0.32	7.65±0.32	7.75±0.04	7.9±0.06	N.S
PCV%	40.30±0.4	40.32±5.30	40.5±2.70	41.2±1.22	N.S
MCHC g/dL	34.7±1.61	33.8±1.32	34.8±1.35	34.93±1.36	N.S
MCV fL(µm^3)	52.7±3.04	52.2±2.40	51.9±2.20	52.4±1.20	N.S

Values are mean of a 10 animals ± S.E.M (Dunnett's test)* $p<0.05$;**$p<0.01$.N=10

Chart 1: The mean value of T.WBC of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*

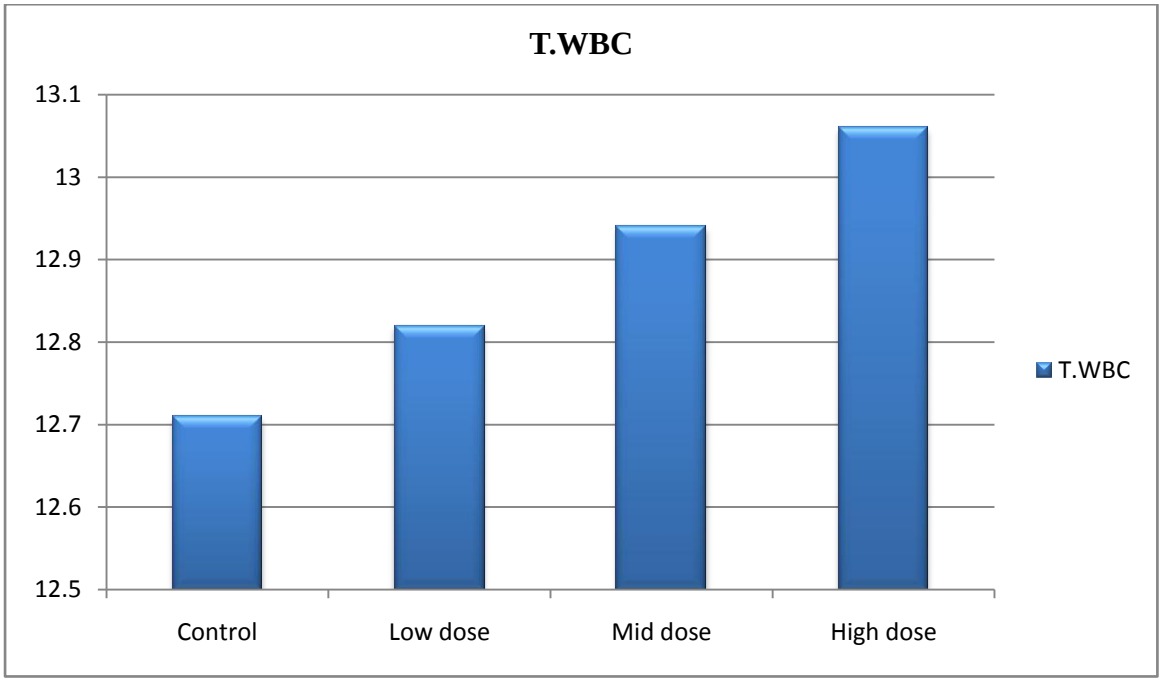


Chart 2: The mean value of Hb and T.RBC of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*

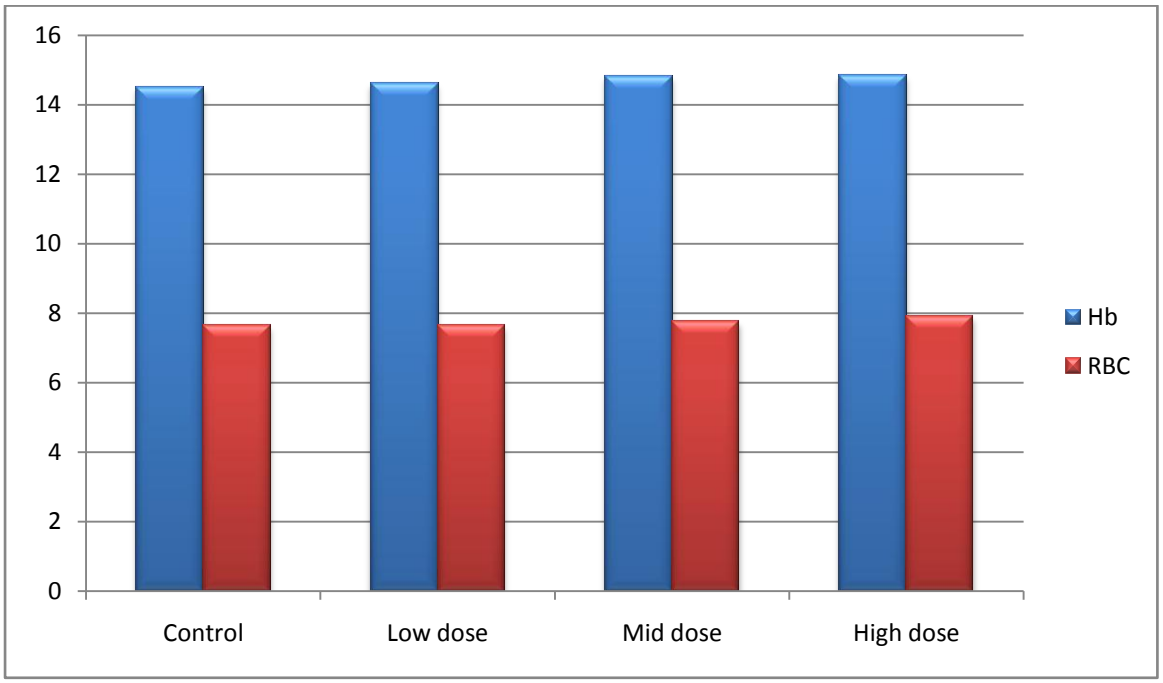


Chart 3:The mean value of Platelet of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool* for 28 days

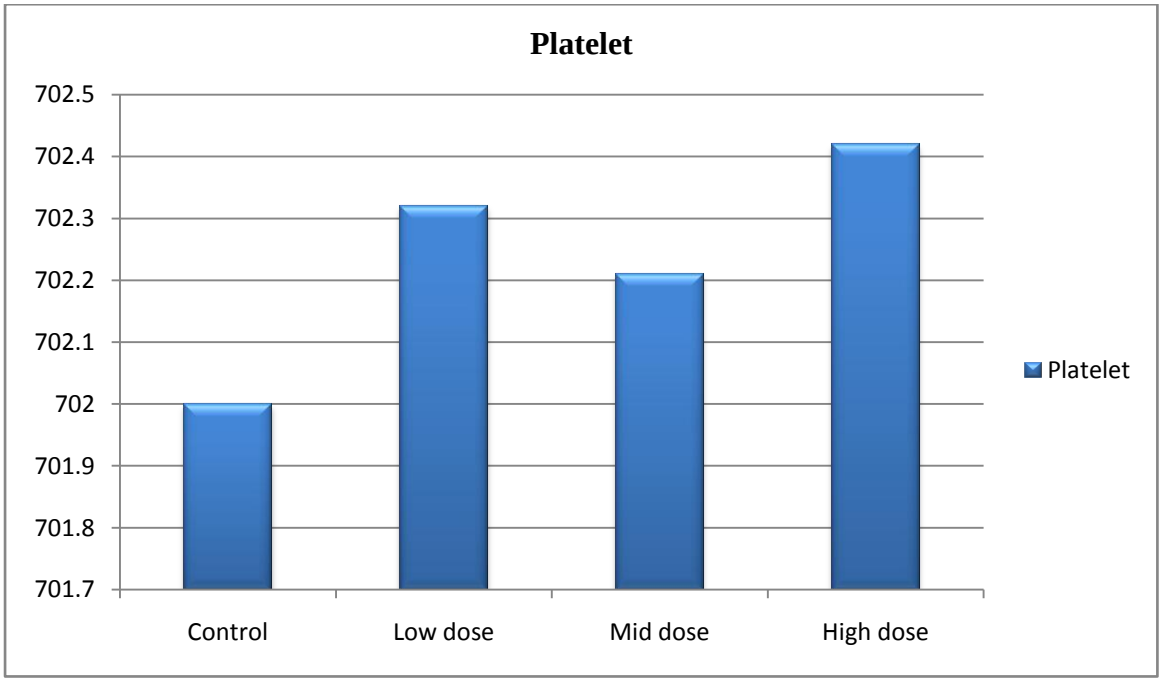


Chart 4: The mean value of Neutrophils and Lymphocytes of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 28 days

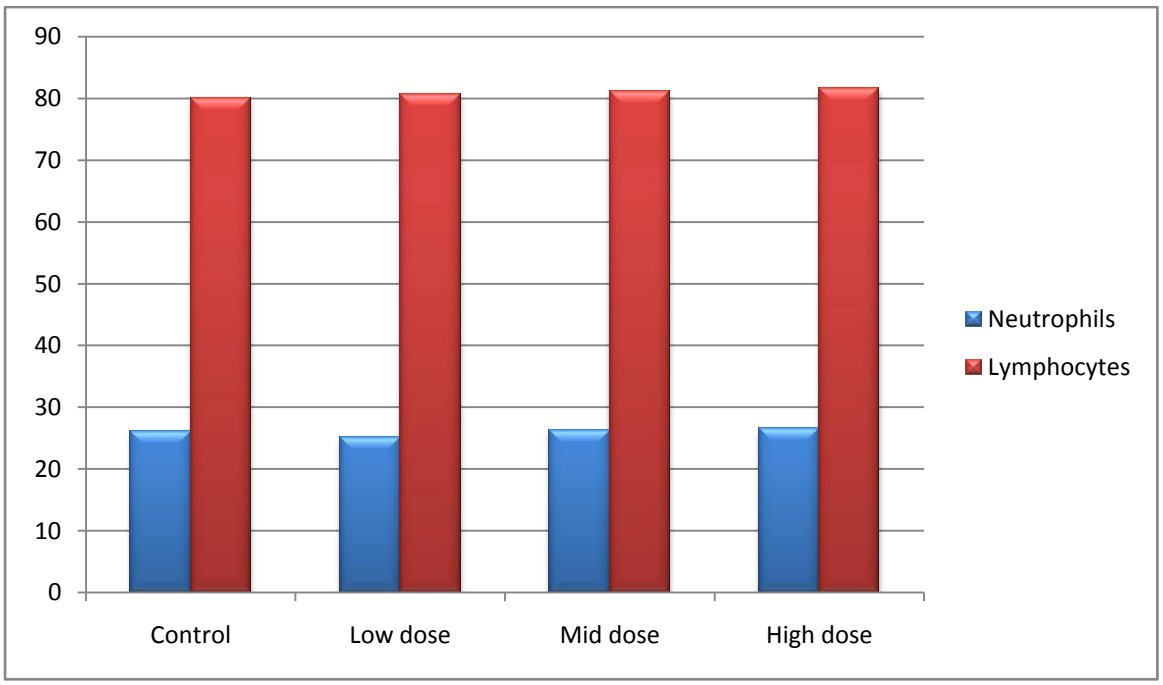


Chart 5: The mean value of Monocytes and Eosinophils of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool* for 28 days

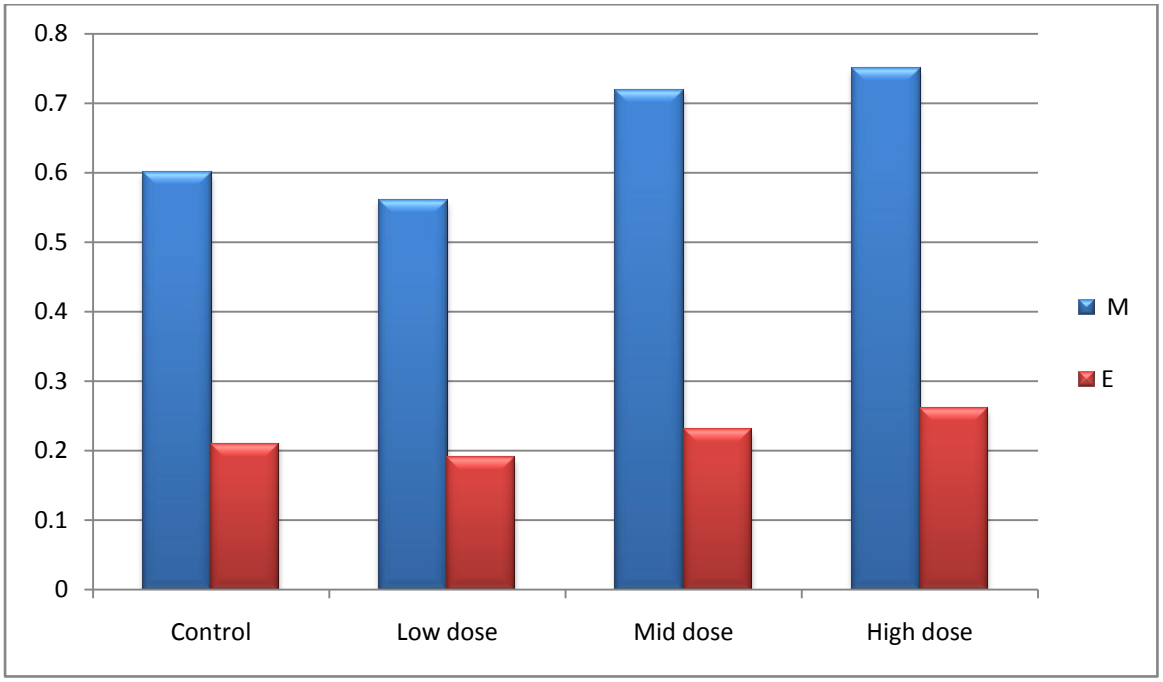


Chart 6: The mean value of PCV, MCHC and MCV of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool* for 28 days

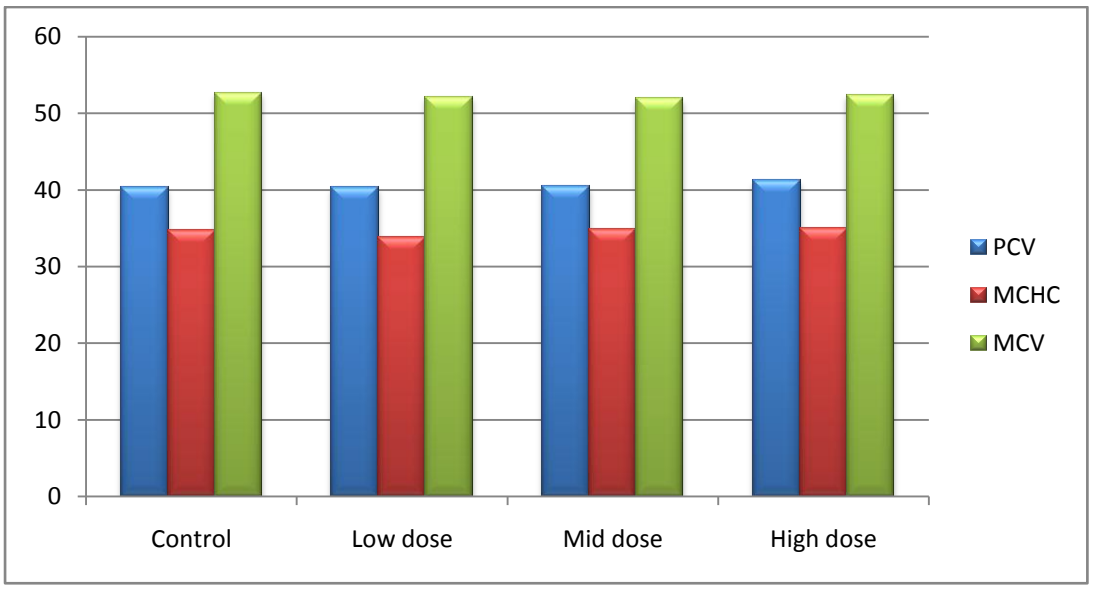


Table: 16 Effect of treatment with *Kalladaippu Thool* on biochemical parameters

Biochemical Parameters	Control	Low dose	Mid dose	High dose	P Value (p)*
Glucose (R) (mg/dl)	98.10±2.40	98.12±1.62	98.56±.08	99±5.25	N.S
Cholesterol(mg/dl)	109.14±3.10	109.25±2.40	109.30±1.58	110.21±1.60	N.S
TGL(mg/dl)	73.05±1.08	73.11±1.02	73.25±1.42	75.26±1.54	N.S
LDL	58.5±4.13	58.1±1.05	57.3±1.03	58.40±2.44	NS
VLDL	15.2±1.30	15.10±1.71	15.22±1.62	15.24±1.55	NS
HDL	32.22±2.30	31.22±2.60	32.46±1.72	33.56±1.43	NS
Ratio 1(T.CHO/HDL)	3.38±1.10	3.49±1.20	3.36±2.32	3.28±2.63	NS
Ratio 2(LDL/HDL)	1.81±2.33	1.86±1.40	1.76±2.10	1.74±04.02	NS
Albumin (g/dL)	4.12±0.50	3.9±0.54	4.25±4.20	4.76±3.24	NS
T. Protein(g/dl)	6.9±0.14	6.9±0.41	7.00±0.60	7.2±0.41	N.S

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

Chart 7:The mean value of Blood sugar of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 28 days

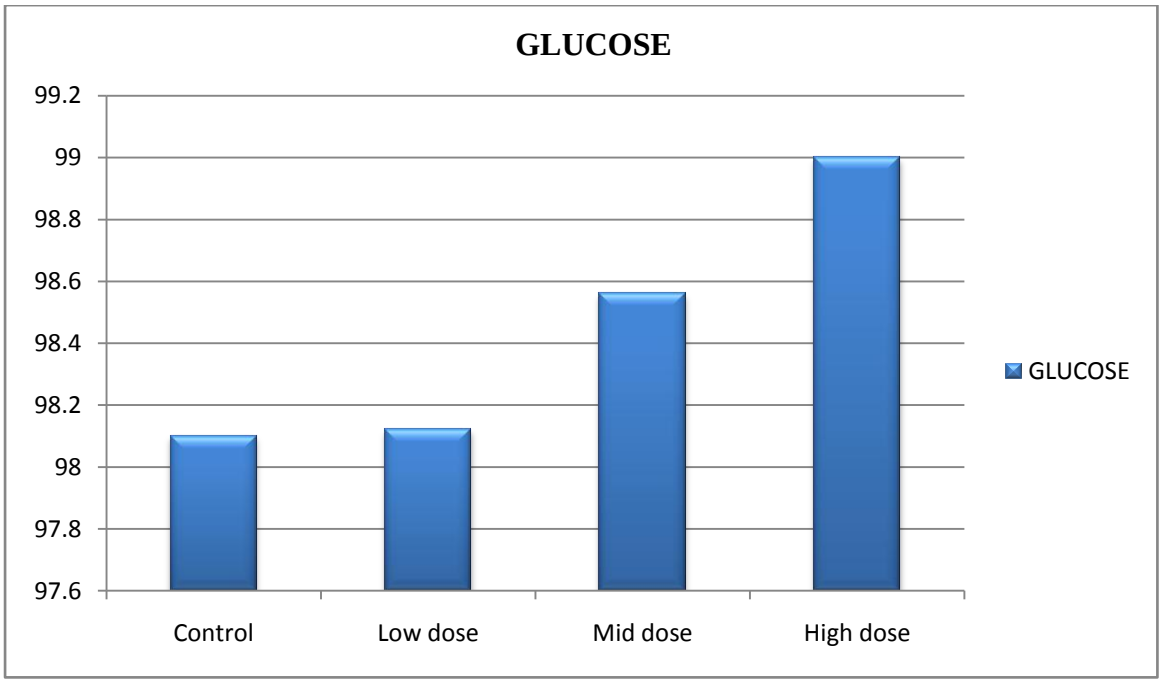


Chart 8: The mean value ofTotal Cholesterol and Triglyceride of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 28 days

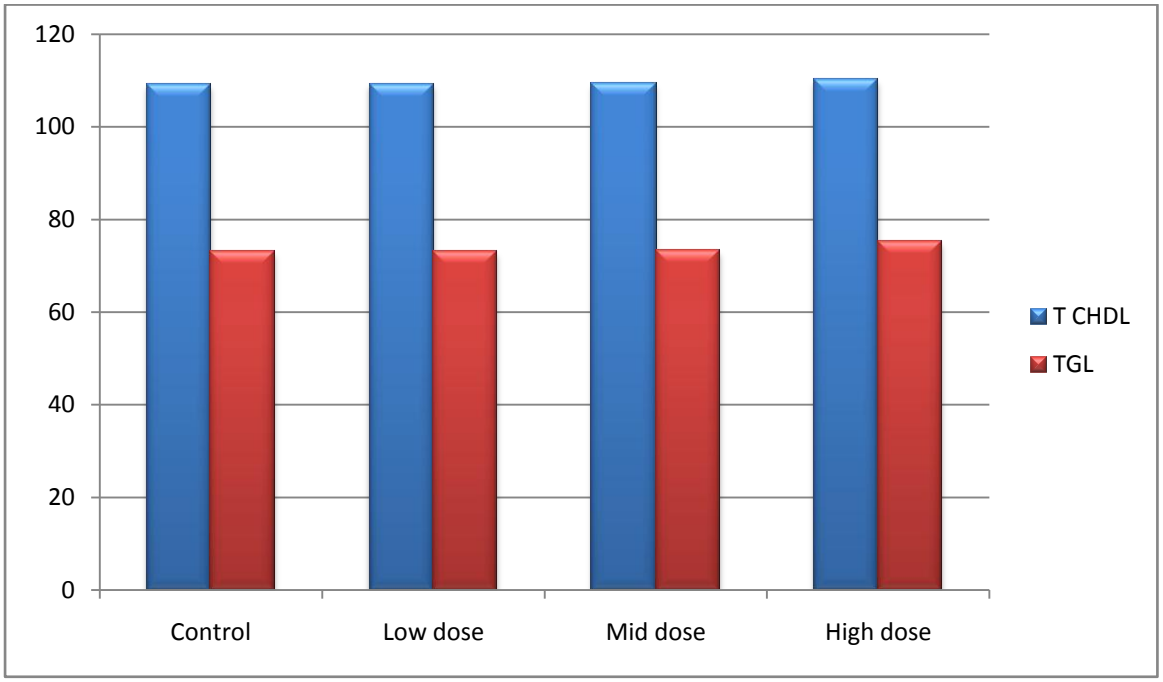


Chart 9: The mean value of HDL,LDL and VLDL of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 28 days

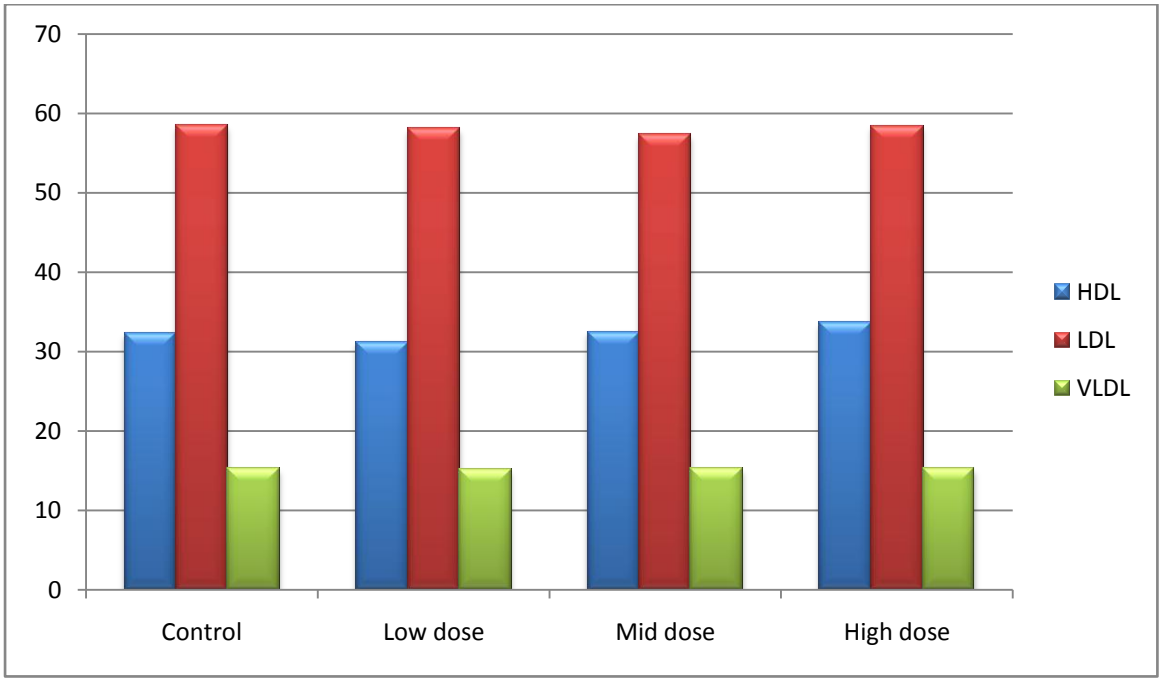


Chart 10: The mean value of Albumin and Total Protein of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 28 days

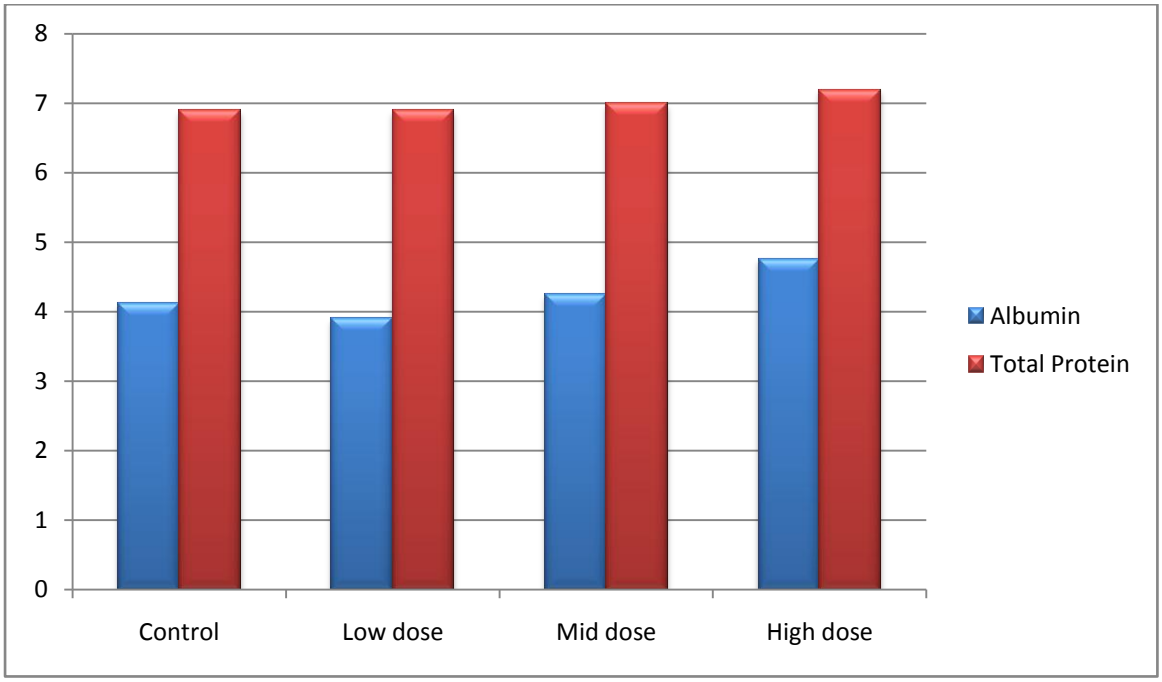


Table 17: Renal function test of Wistar albino rats group exposed to *Kalladaippu Thool*for 28 days

Parameters	**Control**	**Low Dose**	**Mid Dose**	**High Dose**	**PValue (p)***
Urea (mg/dl)	24.31±0.1	24.10±0.19	23.26±1.28	22.34±1.02	N.S
Creatinine(mg/dl)	0.7±0.04	0.71±0.06	0.62±0.04	0.68±0.08	N.S
BUN(mg/dL)	16.8±0.04	16.1±0.24	15.4±0.42	14.9±1.02	NS
Uric acid(mg/dl)	5.04±0.02	5.03±0.20	4.9±0.32	4.2±0.20	N.S

NS- Not Significant, ** (p > 0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 11: The mean value of Urea, Creatinine, blood urea nitrogen and uric acid of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 28 days

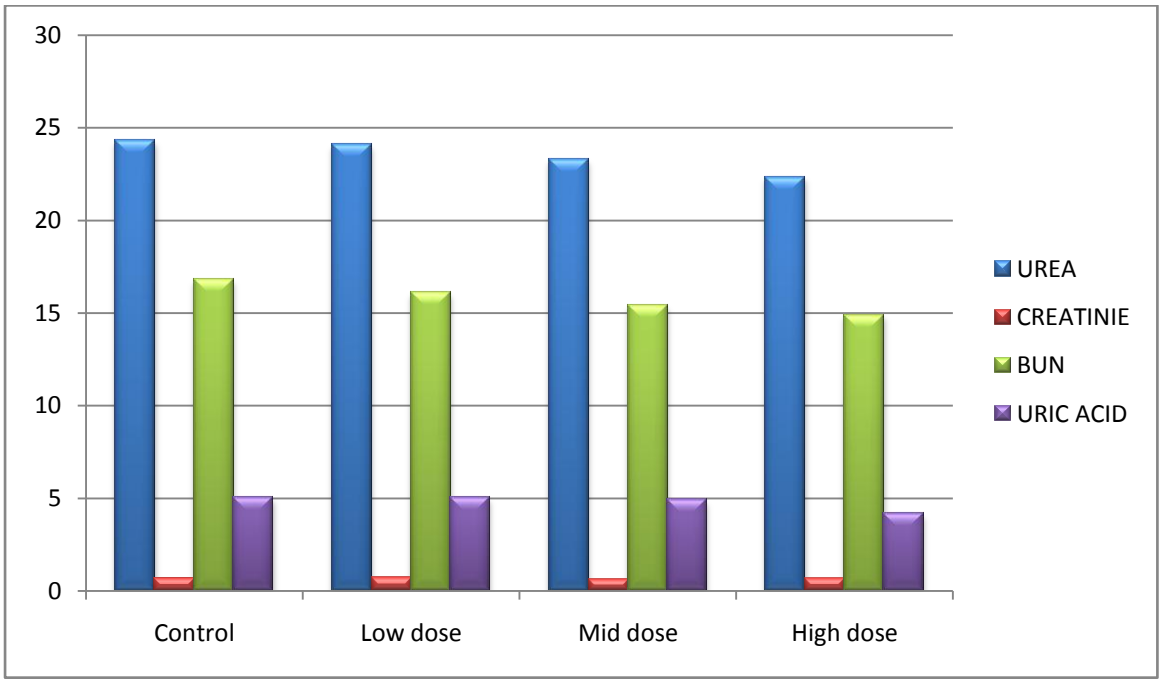


Table 18: Liver Function Test of Wistar albino rats group exposed to *Kalladaippu Thool*for 28 days

Parameters	Control	Low Dose	Mid Dose	High Dose	P Value (p)*
T. Bilirubin (mg/dl).	0.2±0.01	0.24±0.03	0.21±0.03	0.24±0.01	N.S
SGOT/AST(U/L)	15.43.11±1.43	15.12±0.62	14.87±1.34	15.87±1.63	N.S
SGPT/ALT(U/L)	46.11±1.43	46.24±1.14	48.44±1.36	48.23±0.21	N.S
ALP(U/L)	46.30±2.11	46.1±2.10	45.65±1.14	45.98±2.01	N.S

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

Chart 12:The mean value of Total Bilirubin of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 28 days

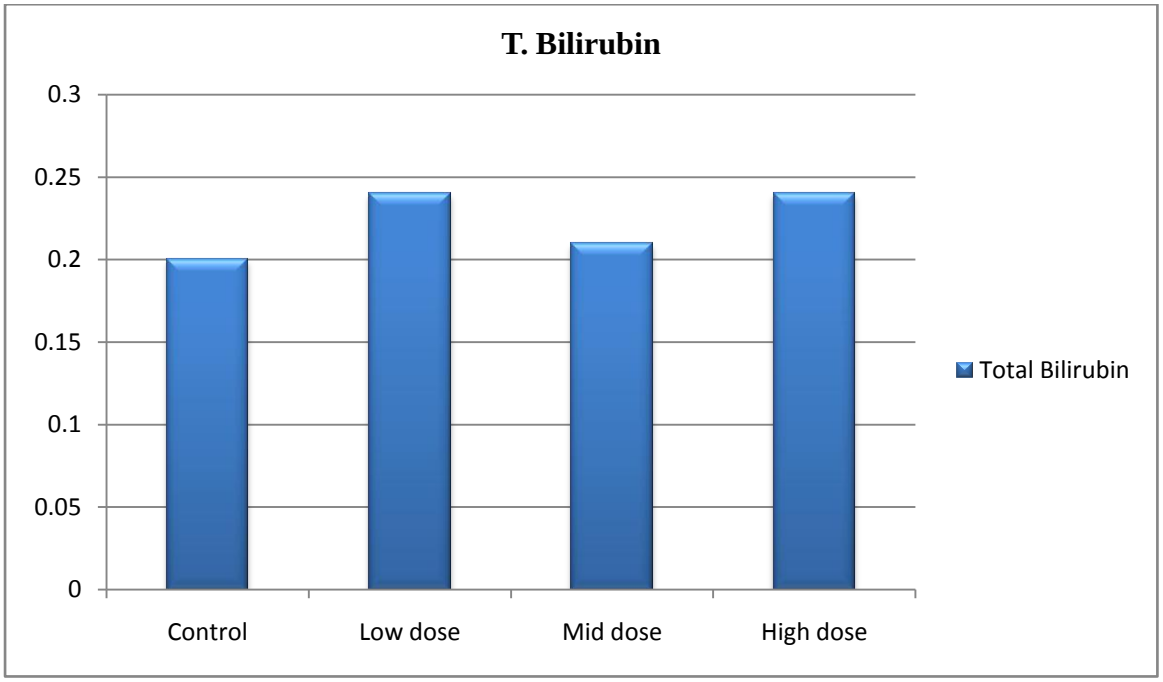
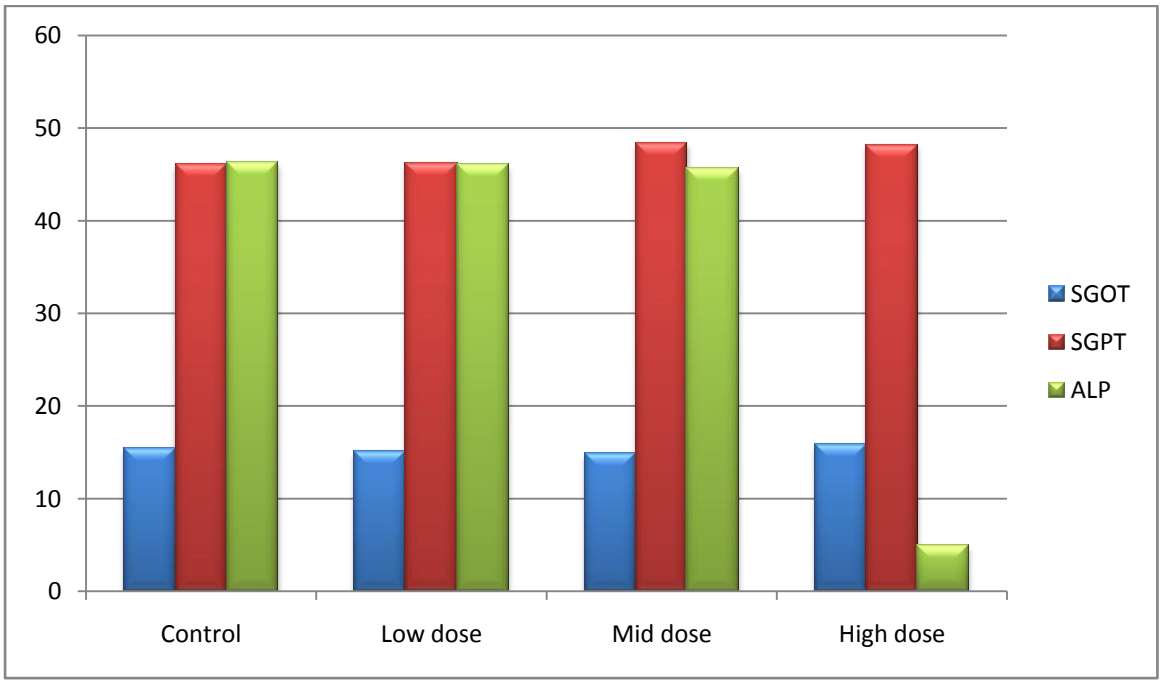


Chart 13:The mean value of ALP,SGOT and SGPT of control and treated groups of wistar albino rats exposed to*Kalladaippu Thool* for 28 days

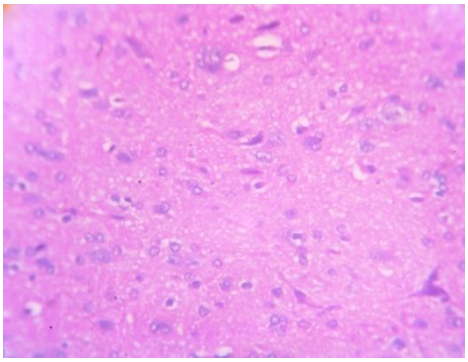


HISTOPATHOLOGICAL EXAMINATION

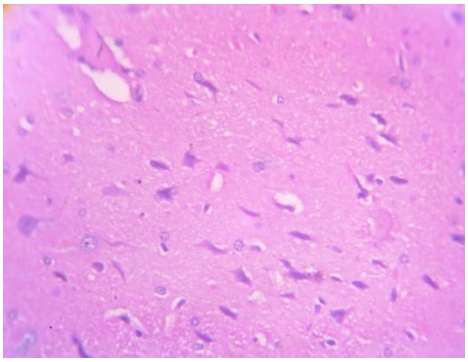
28-Days repeated dose oral toxicity study of *Kalladaippu Thool*

BRAIN

Control

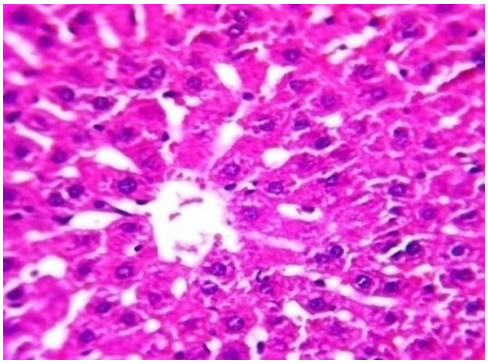


High Dose



HEART

Control



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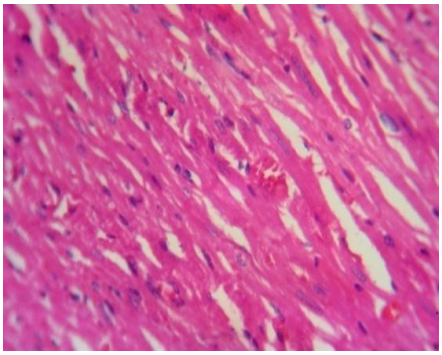

KIDNEY

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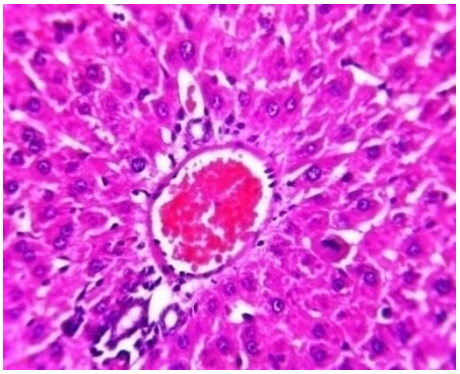

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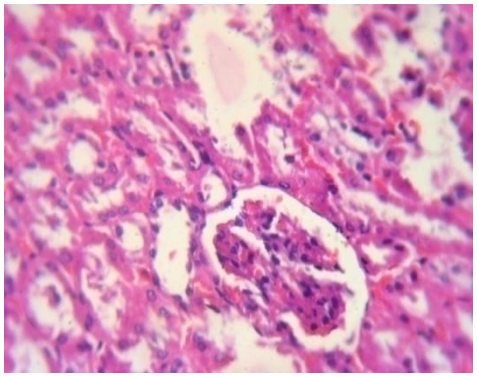

LIVER

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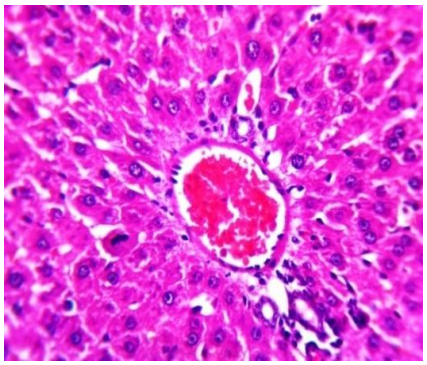

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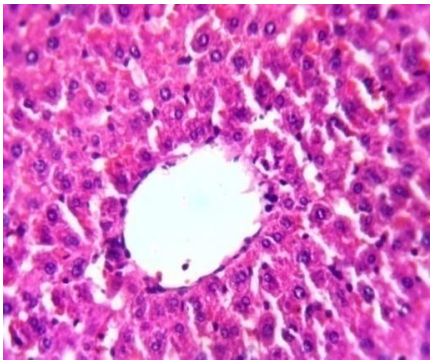

SPLEEN

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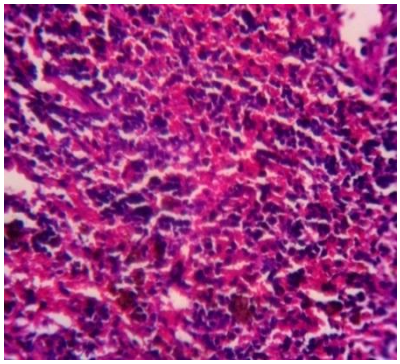
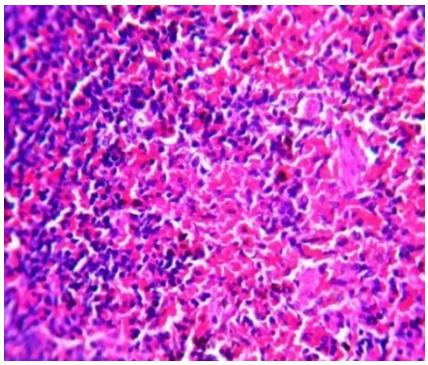


High Dose



Interpretation:

The above slides show the histopathology studies of sub-acute toxicity. There is no toxicological abnormality seen in the vital organs after administration of the test drug *Kalladaippu Thool*. Thus the safety of the drug is revealed so that it can be administered for long time without any side effects.

Results of Repeated Dose 28 Days Oral Toxicity Study:

Repeated dose 28 days oral toxicity of *Kalladaippu Thool*on rats were conducted. All animals from the treated dose survived throughout the dosing period of 28 days. Various parameters were studied and the interpretation of the study result was discussed below.

Clinical signs:

No abnormal behavioural signs were observed during the study period.

Mortality:

The test drug "*Kalladaippu Thool*" did not cause any mortality in mid and high dose levels and were considered as safe dose levels.

Body weight:

The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited over all mild weight gain throughout the dosing period of 28 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

Hematological investigation interpretation:

The hematological investigation results of the rats conducted on 29th day after the repeated dose of the drug revealed the values of different parameters. The increase and decrease in the values obtained were all within the normal biological and laboratory limits.

Biochemical investigation interpretation:

The biochemical investigations were conducted on 29th day and the result was produced. The results revealed there were no significant changes in the values of different parameters with that of the control. All the values were within the normal biological and laboratory limits.

Histopathology interpretation:

Histopathological study of the organ such as brain, heart, kidney, liver and spleen were normal in control and all test groups.

90 Days Repeated Dose Oral Toxicity Study of *Kalladaippu Thool*

Table 19: Body weight of wistar albino rats exposed to *Kalladaippu Thool*for 90 days

DAYS	Weight(gms)/Days				P value (p)*
	Control	Low dose	Mid dose	High dose	
1	161.6±33.68	148.1 ± 21.11	156.6± 13.57	159.5±28.75	NS
15	172.8 ± 28.87	162.5 ± 21.71	166.7 ± 29.01	166.8±32.13	NS
30	181.8 ± 28.31	175.71 ± 14.88	181.8 ± 32.11	181± 28.94	NS
45	204.5± 27.73	194.5± 29.76	204.7± 19.75	208.3± 22.75	NS
60	218.6±33.68	214.6±23.68	226.6±33.68	215.6±23.78	NS
75	236.8 ± 26.85	226.8 ± 28.87	241.8± 28.87	224.8 ±26.87	NS
90	248.5± 27.32	239.5± 27.68	251.4± 27.32	236.3± 36.32	NS

NS- Not Significant, ** ($p < 0.01$),*($p < 0.05$), N = 6; values are mean ± S.D (One way ANOVA followed by Dunnett's test.

Table 20: Hematological parameters of Wistar albino rats group exposed to*Kalladaippu Thool*for 90 days

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Hemoglobin(g/dl)	12.53±0.31	12.7±0.59	12.63±0.45	12.78±0.67	N.S
Total WBC (cells/cu.mm)	11.2±4.02	11±0.54	11.15±5.12	11.4±6.37	N.S
Neutrophils (%)	28.32±0.02	28.23±0.40	28.25±5.24	28.35±2.52	N.S
Lymphocytes (%)	89.12±1.41	89.04±1.42	89.6±1.74	89.7±1.46	N.S
Monocyte (%)	0.1±0.02	0.15±0.04	0.2±0.06	0.22±0.07	N.S
Eosinophils (%)	0.02±0.01	0.02±0.05	0.03±0.01	0.02±0.02	N.S
Platelets lakhs/µl	351±.21	367±25	387.05±26	36±33	N.S
Total RBC (cells/cu.mm)	7.25±0.3	7.08±0.3	7.13±0.2	7.16±0.3	N.S
PCV%	37.59±0.95	38.29±0.18	37.9±1.34	38.36±2.05	N.S
MCHC g/dl	30±3.03	33.66±1.86	31±1.22	30.5±0.07	N.S
MCV%ft	92.16±2.62	91.3±3.75	92.3±1.98	91.5±1.89	N.S
MCH pg.	30± 3.03	32.75±1.86	31±1.22	30.5±0.07	N.S

N.S- Not Significant, ** ($p < 0.01$), *($p < 0.05$), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test).

Chart 14: The mean value of HB, Total RBC, Total WBC of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 90 days

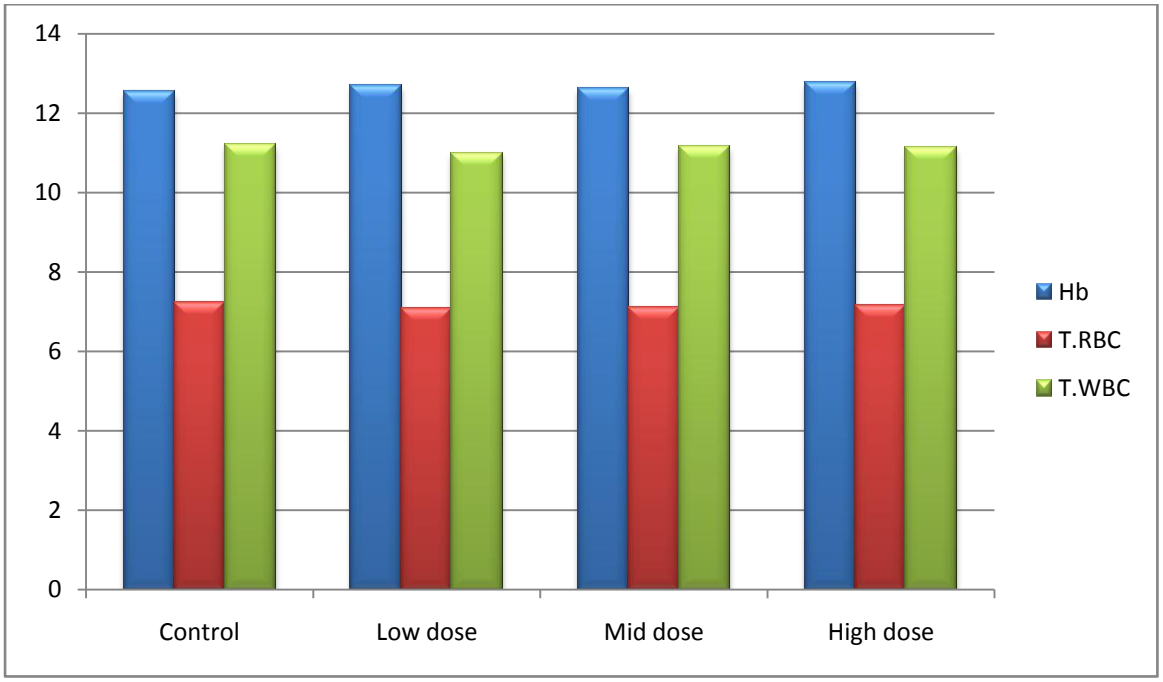


Chart 15: The mean value of Platelets of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool* for 90 days

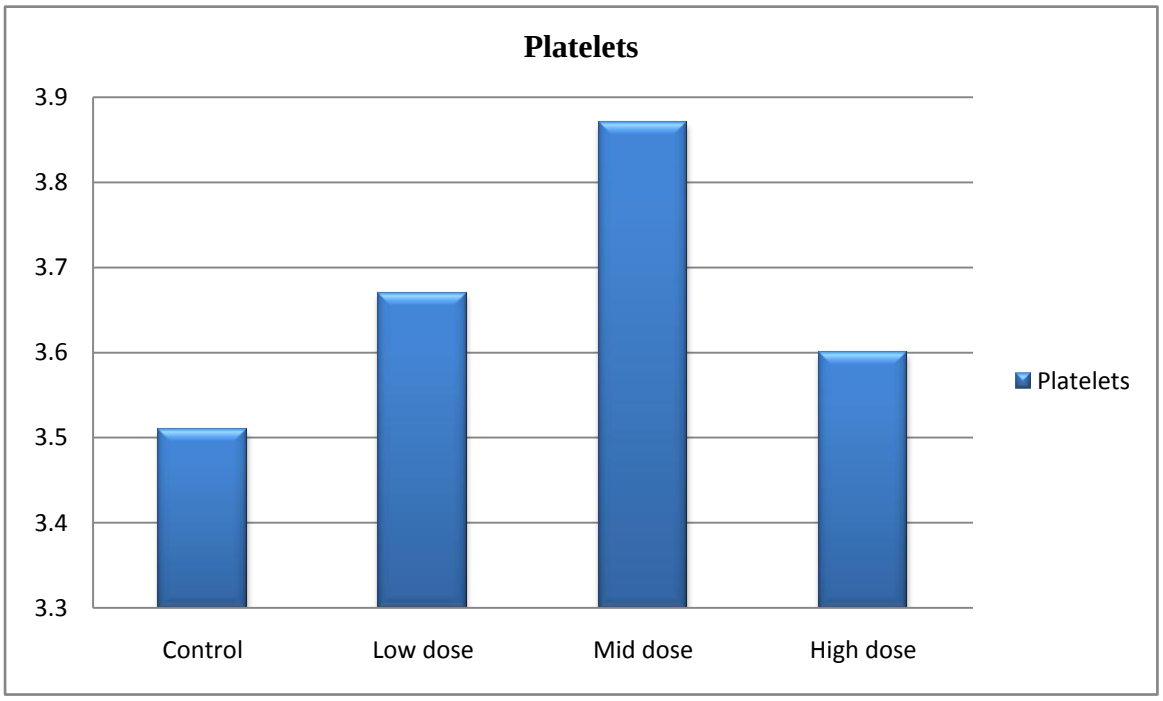


Chart 16: The mean value of Neutrophils and Lymphocytes of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool* for 90 days

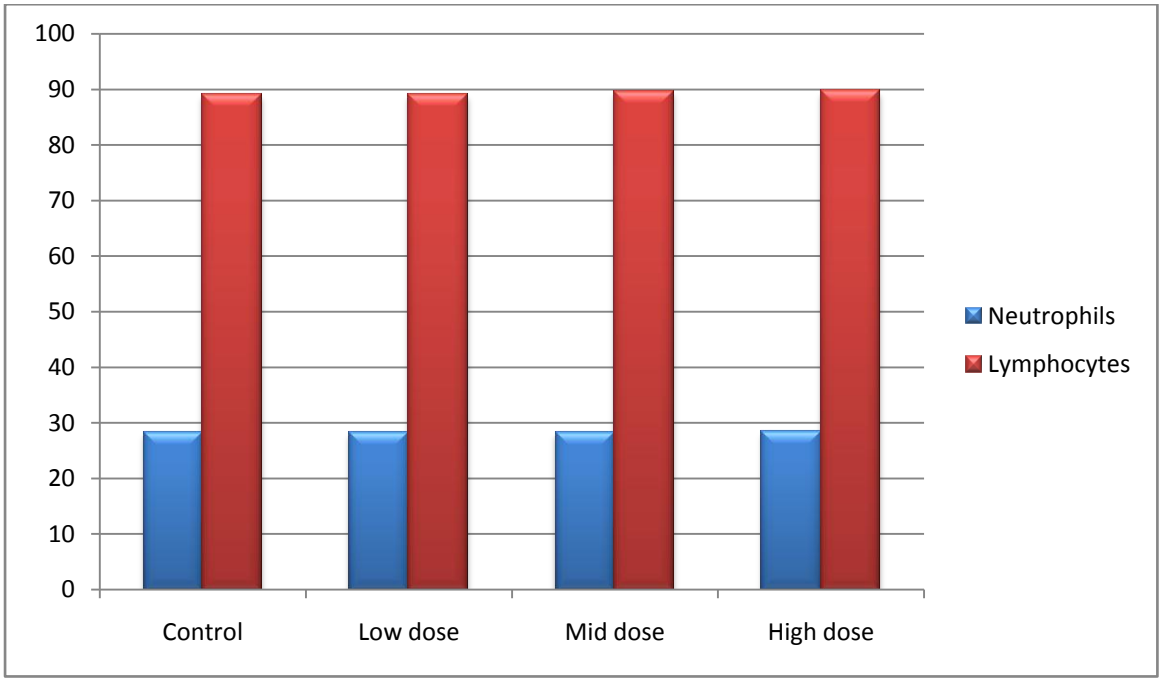


Chart 17: The mean value of Monocytes and Eosinophils of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool* for 90 days

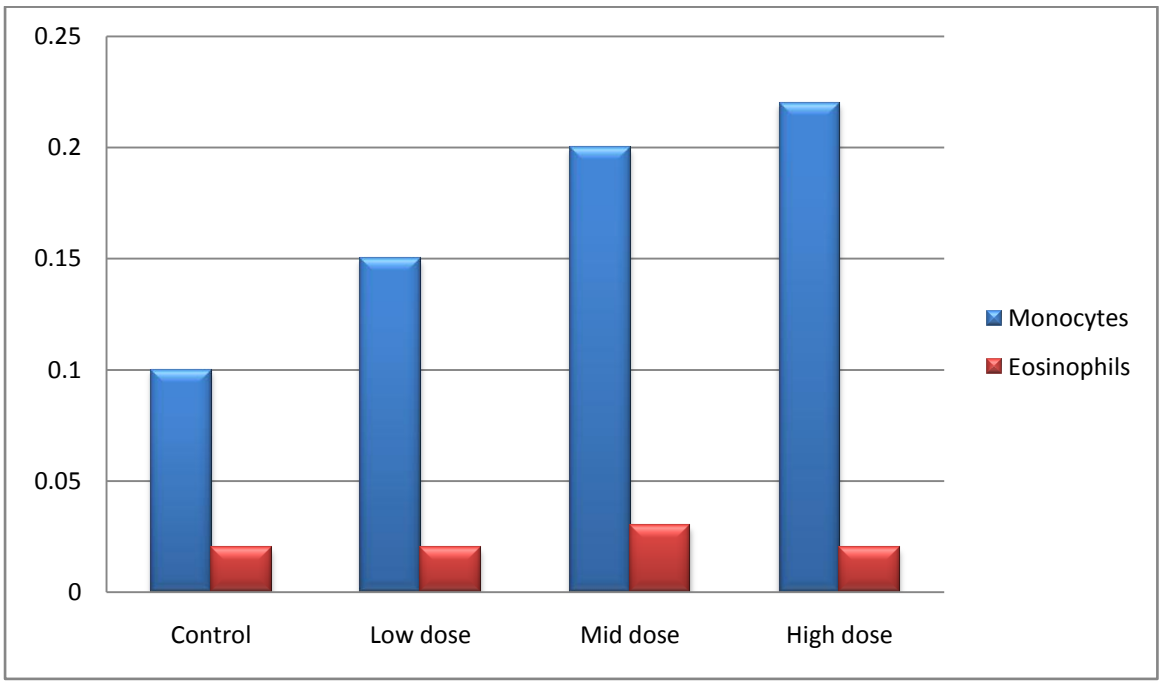


Chart 18: The mean value of PCV, MCHC, MCV, and MCH of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool* for 90 days

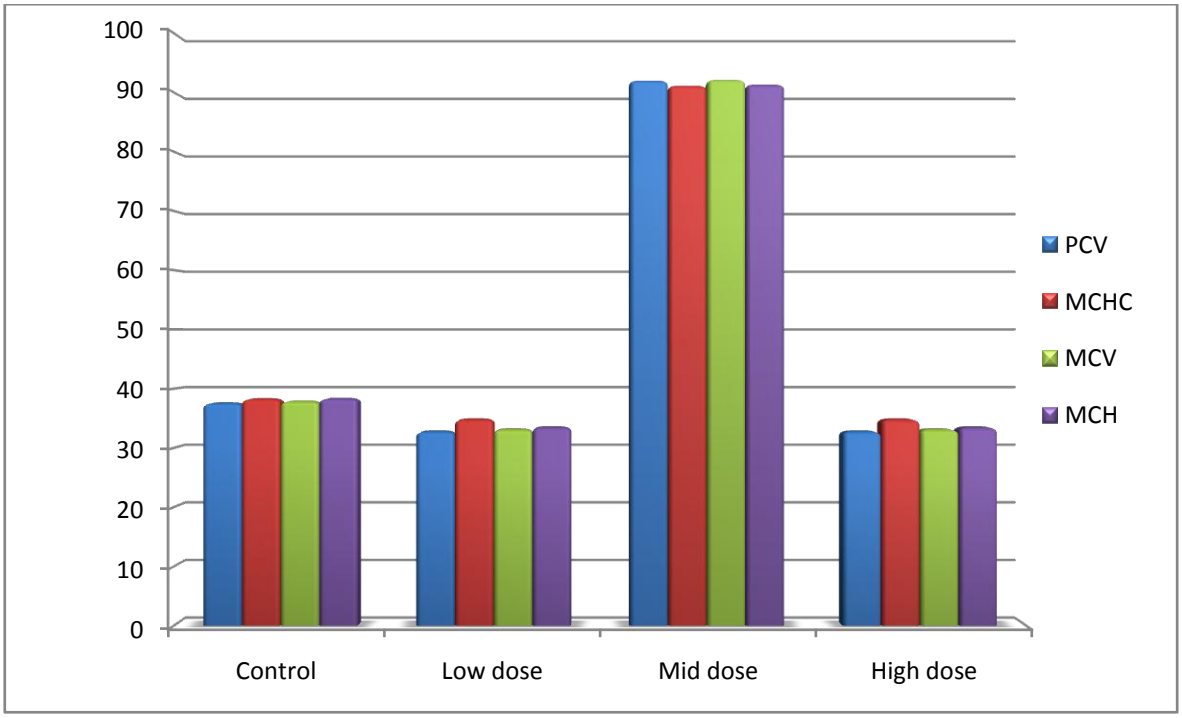


Table 21: Blood sugar test of Wistar albino rats group exposed to *Kalladaippu Thool*for 90 days

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
Bl.sugar (mg/dl)	90.30±8	89.5±6.28	91.6±16.1	91.89±20.9	N.S.

N.S- Not Significant, **($p < 0.01$), *($p < 0.05$), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test).

Chart 19:The mean value of Blood sugar of control and treated groups of wistar albino rats exposed to*Kalladaippu Thool*for 90 days

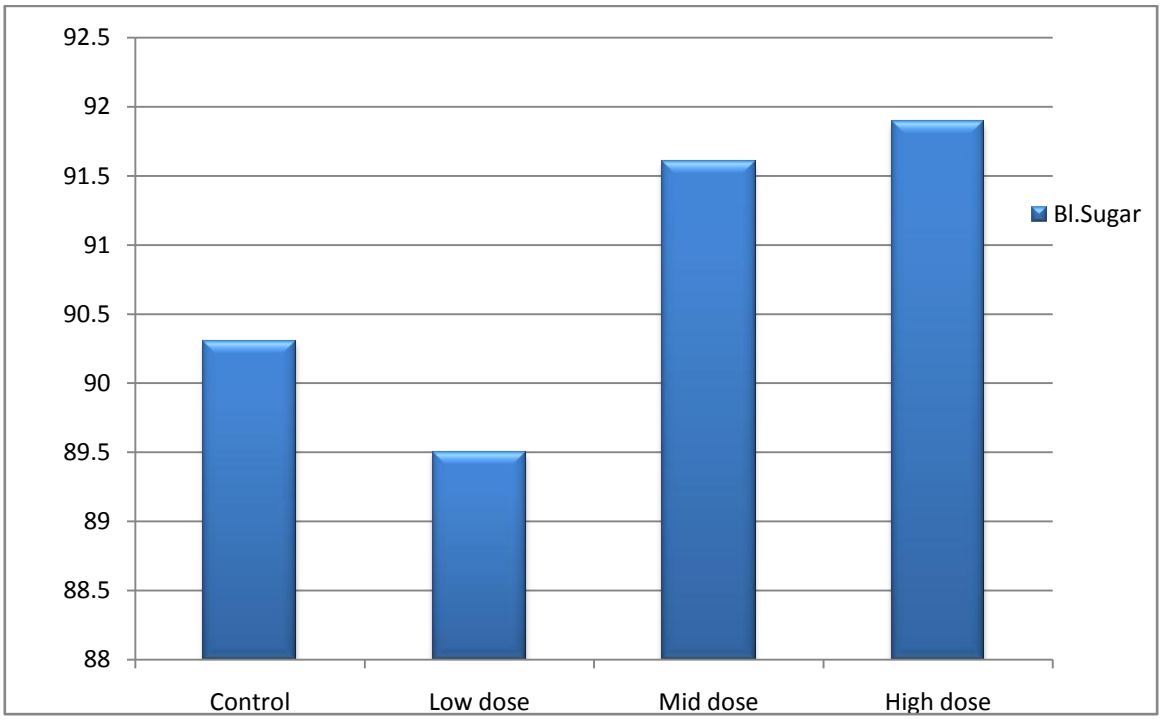


Table 22: Lipid profile test of Wistar albino rats group exposed to *Kalladaippu Thool*for 90 days

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
Sr.TC(mg/dl)	108.±8.3	114.83±12.93	115.6±8.28	112.83±6.76	N.S
Sr.TG(mg/dl)	129.83±2.4	136.55±9.35	4132.5±9.73	136.5±9.31	N.S

N.S- Not Significant, **($p < 0.01$), *($p < 0.05$), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 20:The mean value of Sr.T.Cholesterol, Sr.Triglyceride of control and treated groups of wistar albino rats exposed to*Kalladaippu Thool* for 90 days

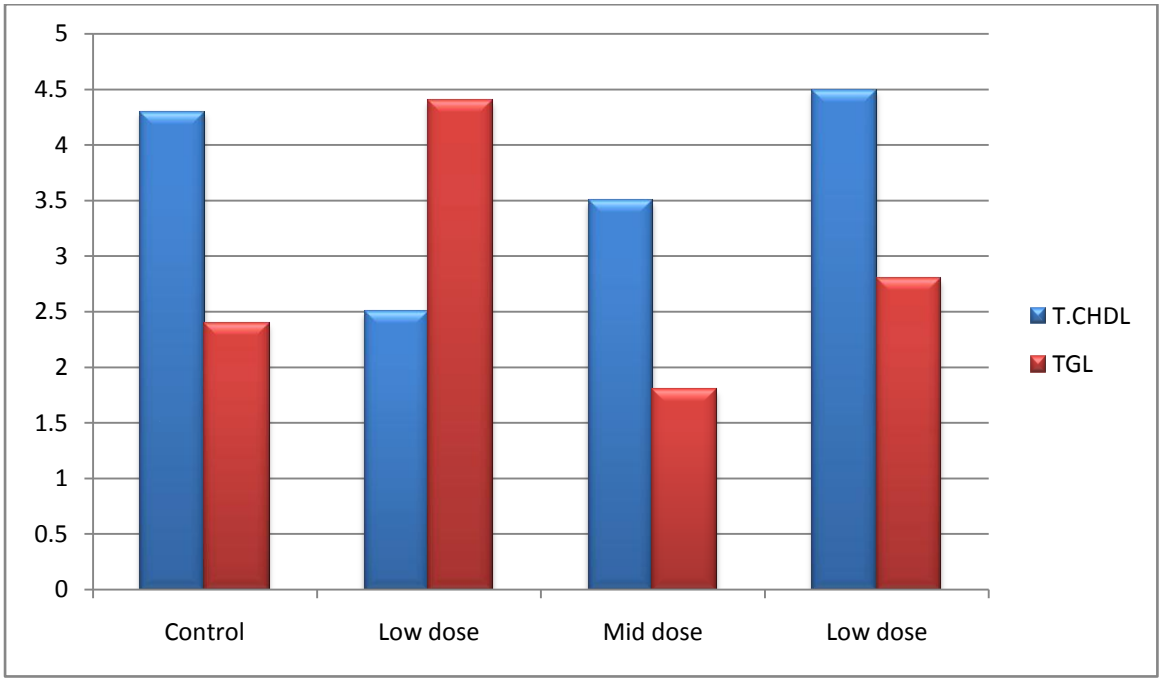


Table 23: Liver Function Test of Wistar albino rats group exposed to *Kalladaippu Thool*for 90 days

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
T. Bilirubin(mg/dl)	0.86±0.04	0.79±0.1	0.75±0.1	0.80±0.13	N.S
SGOT(IU/L)	21.6±5.08	22.5±9.97	27.6±9.09	26.15±2.04	N.S
SGPT(IU/L)	27±5.9	30.8±5.45	28.5±3.72	31.57±10.9	N.S
ALP (IU/L)	82.83±17.56	79.8±12.09	79±15.7	75.3±17.84	N.S
T.Protein (g/dl)	6.7±0.48	6.5±0.28	6.6±0.84	6.8±0.75	N.S
Albumin	3.9±0.57	3.6±0.67	3.6±0.59	3.4±0.36	N.S

NS- Not Significant, ** ($p < 0.01$), * ($p < 0.05$), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 21: - The mean value of T. Bilirubin of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 90 days

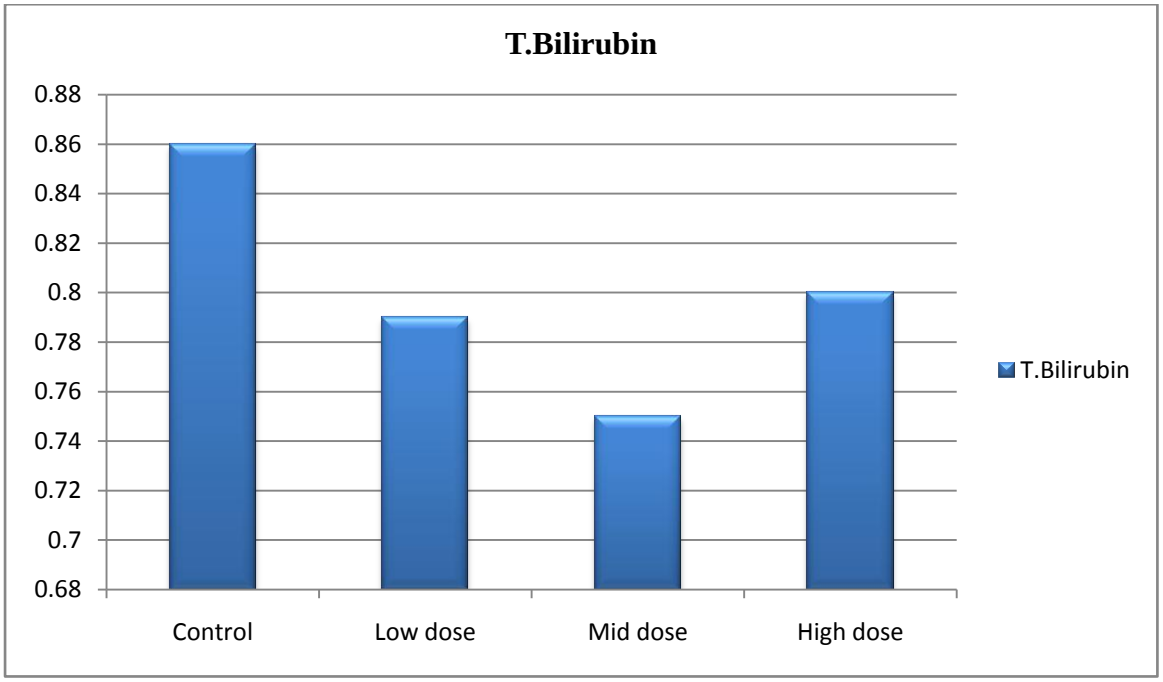


Chart 22:The mean value of SGOT, SGPT and ALP of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 90 days

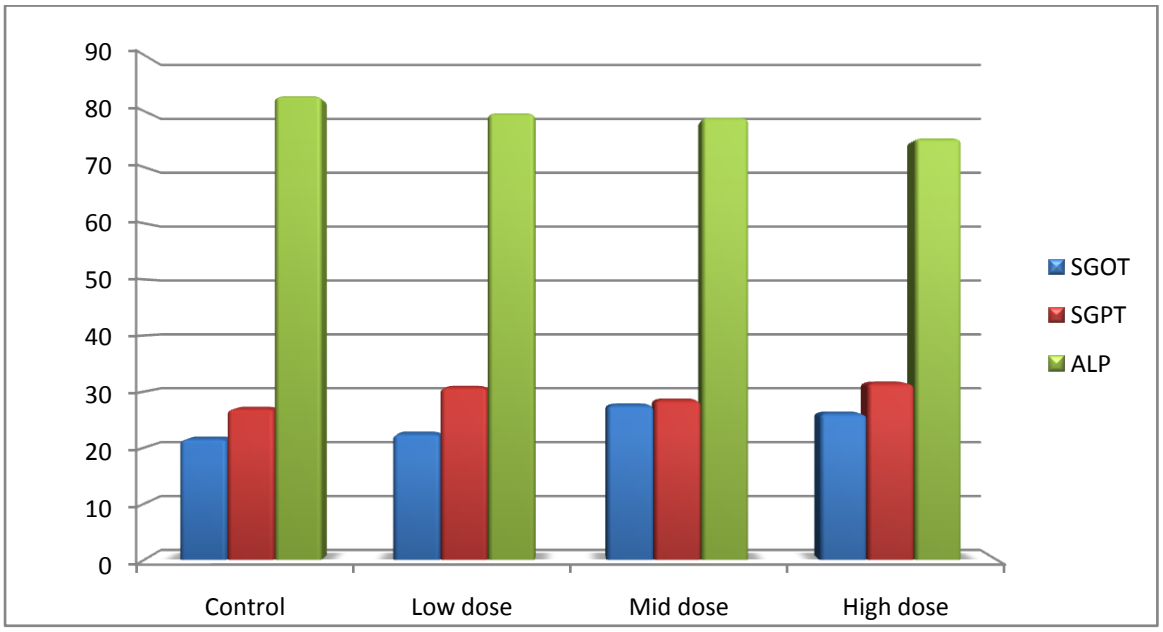


Chart 23:The mean value of T. Protein and Albumin control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 90 days

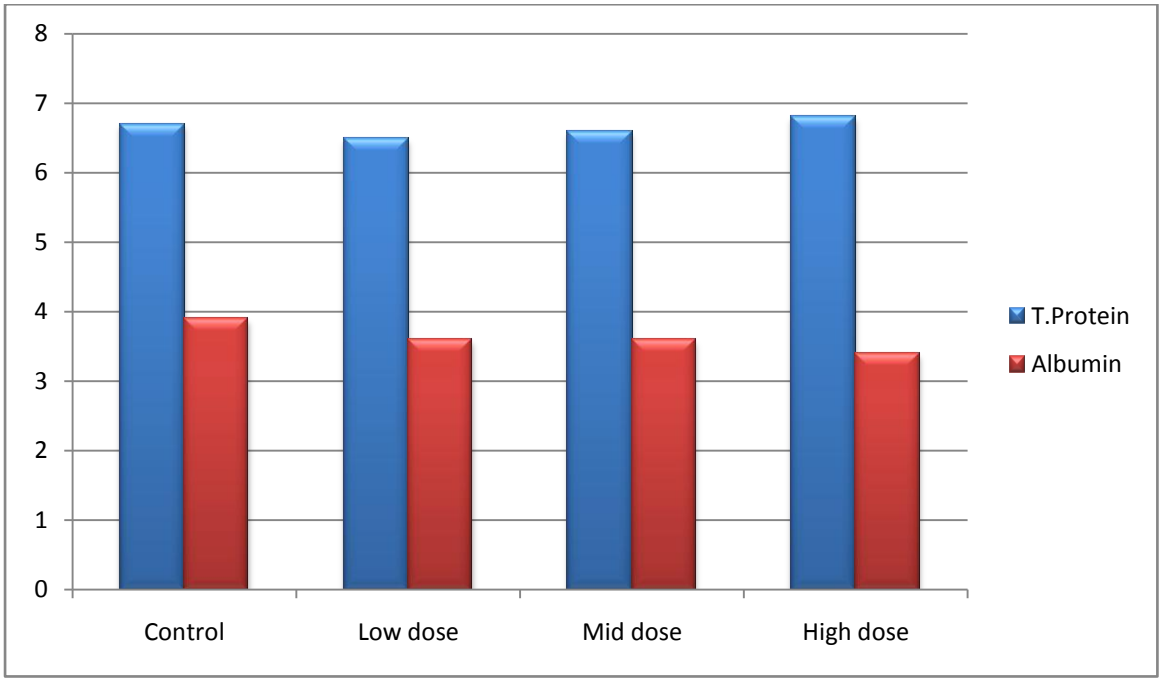


Table 24: **Renal Function Test of Wistar albino rats exposed to*Kalladaippu Thool*for 90 days**

Parameters	Control	Low dose	Mid dose	High dose
Urea (mg/dl)	32	30.16	28.3	26.5
Creatinine (mg/dl)	0.48	0.5	0.51	0.53
Blood Urea Nitrogen (mg/dl)	20.01±0.82	19.78±0.34	19.12±0.67	18.03±0.73

NS- Not Significant, ** ($p < 0.01$), * ($p < 0.05$), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 24: The mean value of Urea and Blood Urea Nitrogen of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 90 days

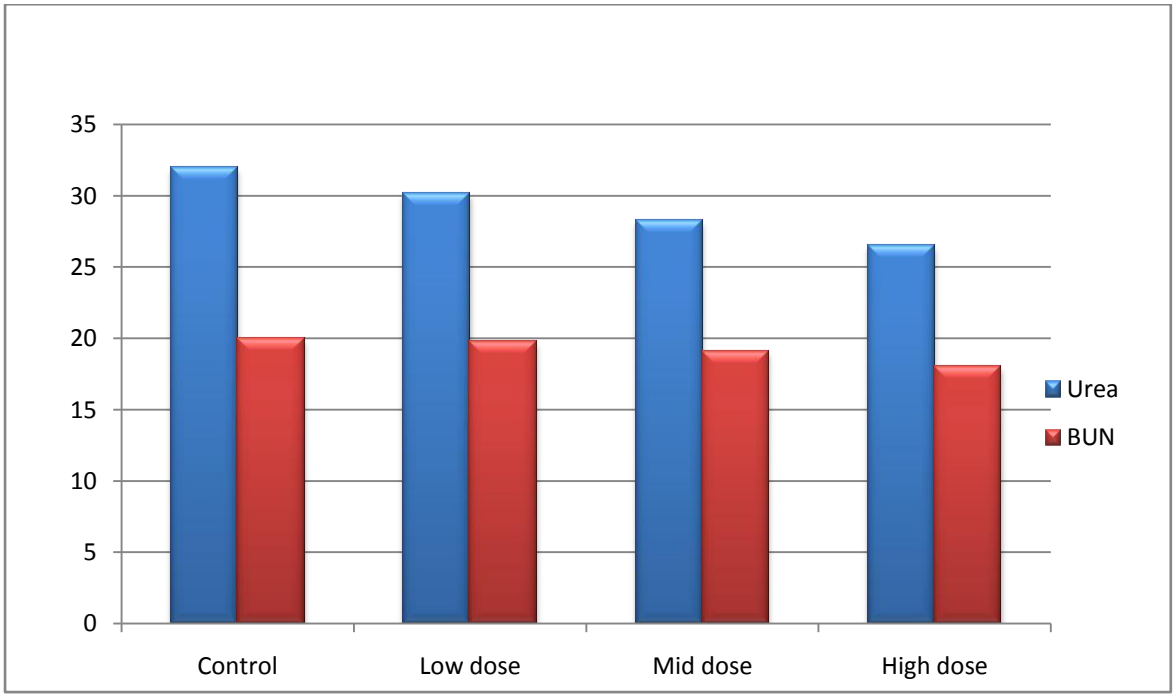
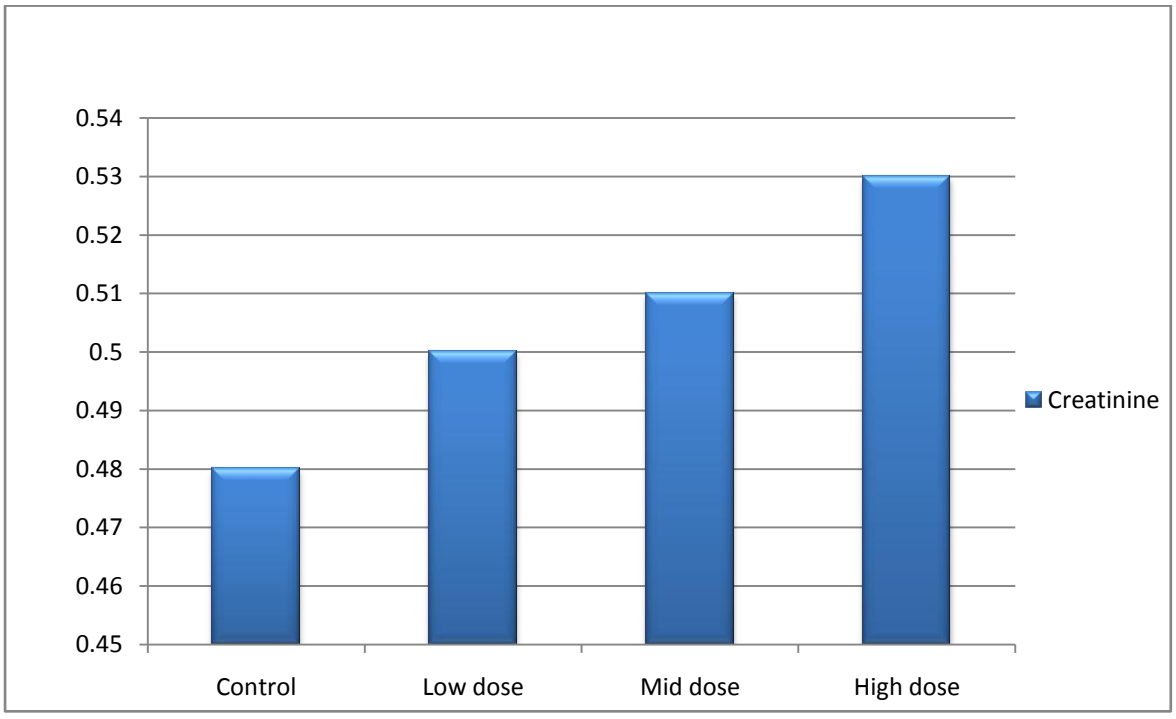


Chart 25:The mean value of Creatinine of control and treated groups of wistar albino rats exposed to *Kalladaippu Thool*for 90 days

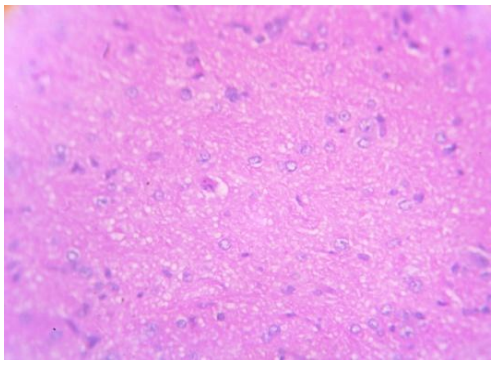
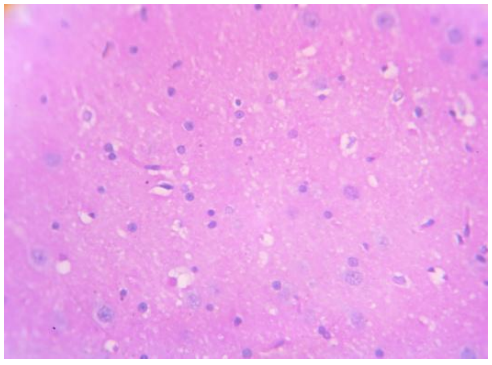


HISTOPATHOLOGICAL EXAMINATION

90-Days repeated dose oral toxicity study of *Kalladaippu Thool*

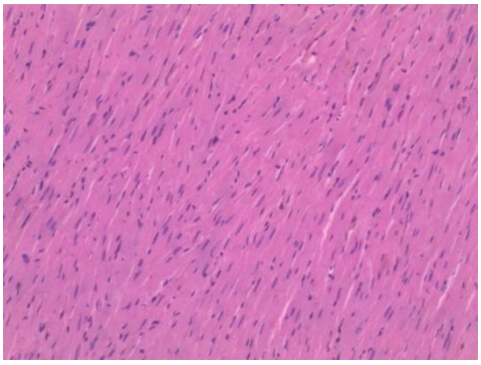
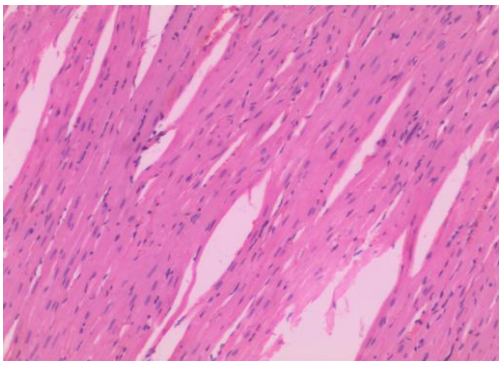
BRAIN

Control High dose

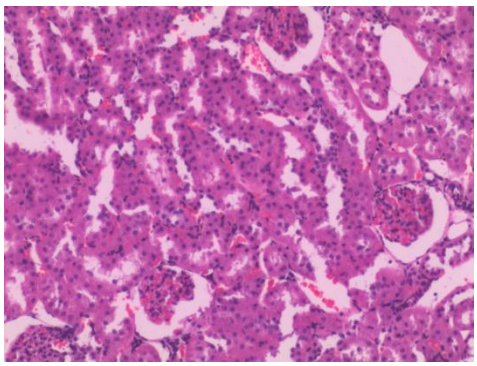
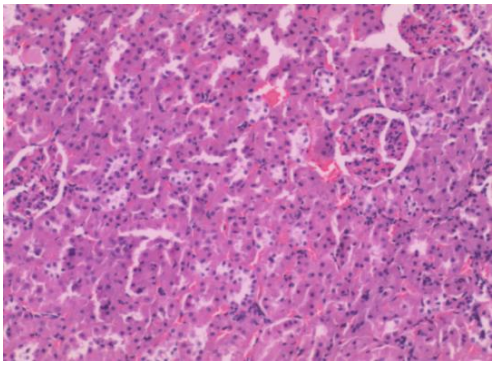
HEART

ControlHigh dose

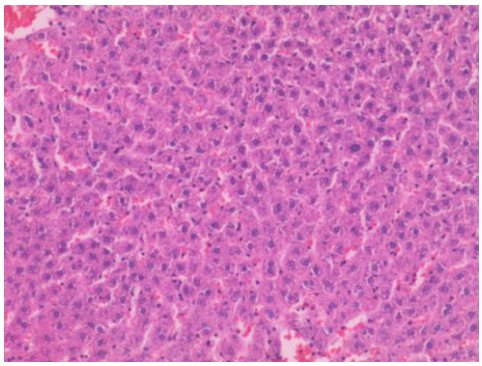
KIDNEY

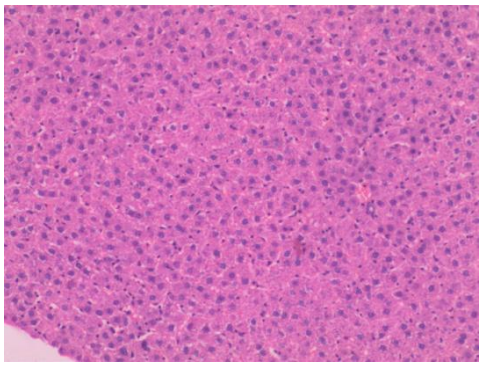
Control High dose

LIVER

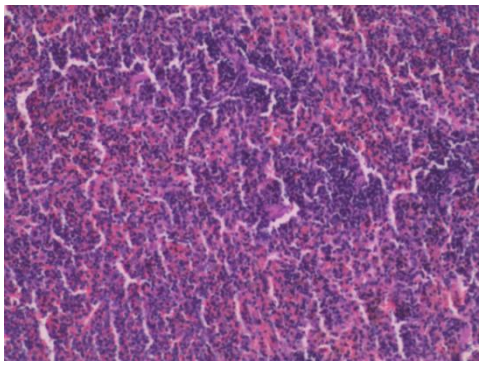
Control High dose

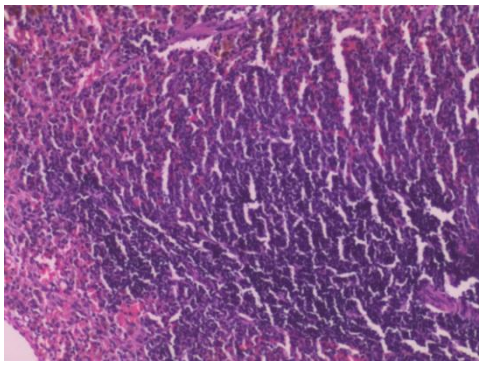




SPLEEN

Control High dose





Interpretation:

The above slides show the histopathology studies of sub-chronic toxicity. There is no toxicological abnormality seen in the vital organs after administration of the test drug *Kalladaippu Thool* .Thus the safety of the drug is revealed so that it can be administered for long time without any side effects.

Results of 90 days repeated dose oral toxicity study:

Repeated dose 90 days oral toxicity study of *Kalladaippu Thool*on rats were conducted. All animals from the treated dose survived throughout the dosing period of 90 days. No abnormal behavioural signs were observed during the study period.

The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited over all mild weight gain throughout the dosing period of 90 days.

The hematological and bio chemical investigations were conducted on 91st day after the repeated dose of the drug revealed there are no significant changes in the values of different parameters with that of the control.

Histopathological study of the organ such as brain, heart, kidney, liver and spleen were normal in control and test groups.

PHARMACOLOGICAL ACTIVITY

Anti-Urolithiatic Activity of *Kalladaippu Thool*

Table 25: Estimation of urinary electrolytes of normal and urolithiatic rats.

Groups	Oxalate(mg/dl)	Calcium(mg/dl)	Phosphate(mg/dl)
I Control	0.34±0.02	2.916±0.170	3.64±0.04
II Lithiatic control	2.10±0.08	8.150±0.33	7.29±0.06
III Standard (Cystone 750 mg/kg)	1.00±0.05**	3.916±0.250**	3.81±0.09**
IV KT- 100mg/kg	0.616±0.06*	2.966±0.128*	4.24±0.10*
V KT- 200 mg/kg	0.350±0.04**	2.983±0.185**	4.14±0.09**
VI KT- 400mg/kg	0.450±0.04**	3.00±0.146**	4.01±0.06**

The results were expressed as mean±SEM (n=6). Statistical analysis was performed by ANOVA test for multiple comparisons followed by Dunnett's test and $P<0.05$ was considered as significant.

Chart: 26 Estimation of urinary electrolytes of normal and urolithiatic rats

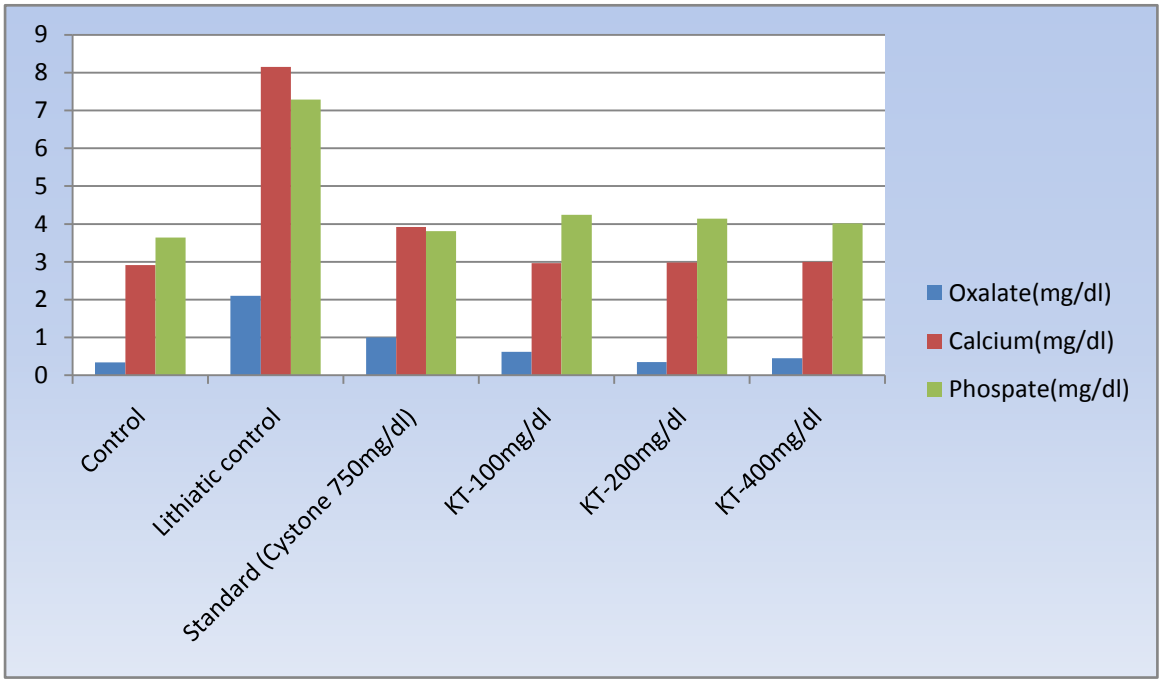
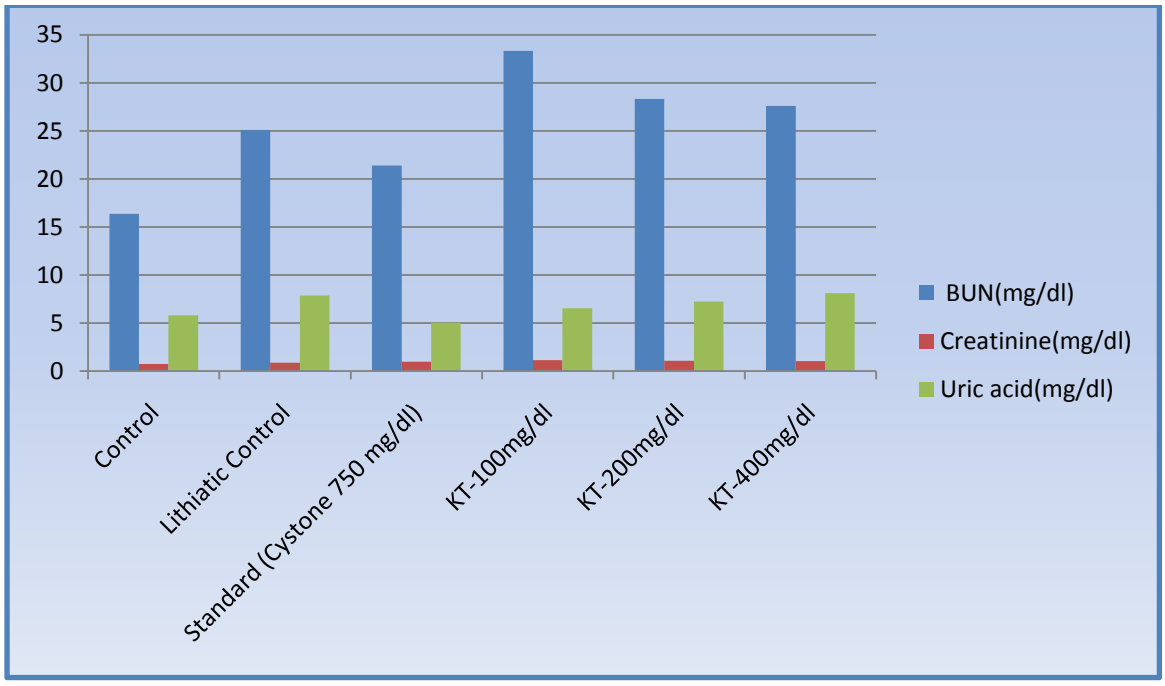


Table 26: Estimation of Kidney Serum Electrolytes of Normal and Urolithiatic Rats

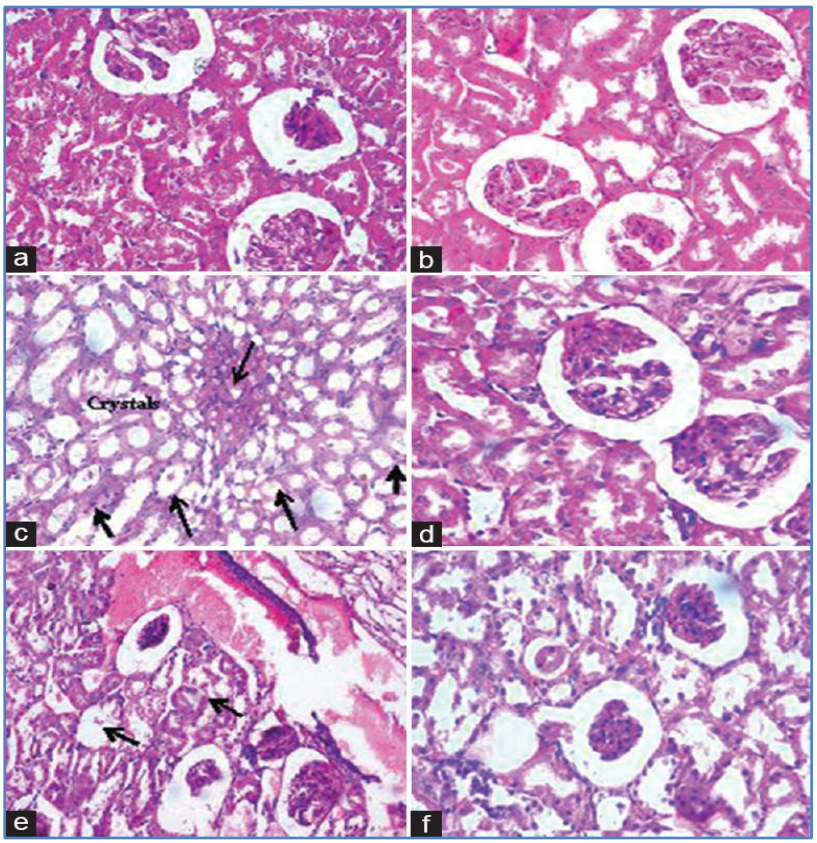
Groups	BUN(mg/dl)	Creatinine(mg/dl)	Uric acid(mg/dl)
I Control	16.369±0.30	0.721±0.01	5.80±0.12
II Lithiatic control	25.098±0.24	0.855±0.01	7.866±0.25
III Standard (Cystone 750 mg/kg)	21.398±0.39***	0.981±0.006***	5.033±0.08***
IV KT- 100mg/kg	33.328±0.73*	1.146±0.01*	6.533±0.14*
V KT- 200 mg/kg	28.328±0.60***	1.058±0.01***	7.233±0.08***
VI KT- 400mg/kg	27.588±0.71***	1.038±0.005***	8.116±0.10***

The results were expressed as mean±SEM (n=6). Statistical analysis was performed by ANOVA test for multiple comparisons followed by Dunnett's test and $P<0.05$ was considered as significant.

Chart 27: Estimation of Kidney Serum Electrolytes of Normal and Urolithiatic Rats



HISTOPATHLOGY EXAMINATION OF ANTI-UROLITHIATIC ACTIVITY



(a). Control group,

(b). Ethyl glycol treated group,

(c). ethylene glycol + Cystone treated (750 mg/kg) ,

(d). ethylene glycol + KT treated (100 mg/kg),

(e). ethylene glycol + KT treated (200 mg/kg),

(f). ethylene glycol + KTtreated (400 mg/kg),

Results and Discussion:

The chronic administration of 0.75%v/v ethyleneglycol aqueous solution to male rats resulted in hyperoxaluria. Results of study were represented in Table – 25, 26. It shows the urinary excreted values of calcium, Oxalate and phosphate were significantly ($P<0.01$) increased in 200mg/kg and 400 mg/kg of KT doses when compared to the control group and also the values of blood urea nitrogen, uric acid, creatinine in serum were significantly decreased 200mg/kg and 400 mg/kg of KT doses when compared to the control group.

Diuretic Activity and Saluretic Activity of *Kalladaippu Thool*

Table 27: Effect of *Kalladaippu Thool* on urinary volume in Wistar albino rats at 24h interval.

Group	Treatment and Doses	Urine volume(mL)	Diuretic action[a]	Diuretic activity[b]
I	Control	4.67±0.51	1.00	-
II	Furosemide(200 mg/kg)	8.90±0.45***	1.91	1.00
III	KT (100 mg/kg)	6.67±0.19*	1.43	0.75
IV	KT (200 mg/kg)	8.03±0.45***	1.72	0.90
V	KT (400 mg/kg)	10.17±0.95***	2.18	1.14

Values are expressed as mean ± SEM.n=6;
[a]Diuretic action - urine volume of test group / urine volume of control group.
[b]Diuretic activity - urine volume of test group / urine volume of control group.
[*]Significant change at P< 0.05 with respect to control rats.

Chart 28: Effect of Kalladaippu Thool on urinary volume in Wistar albino rats at 24h interval.

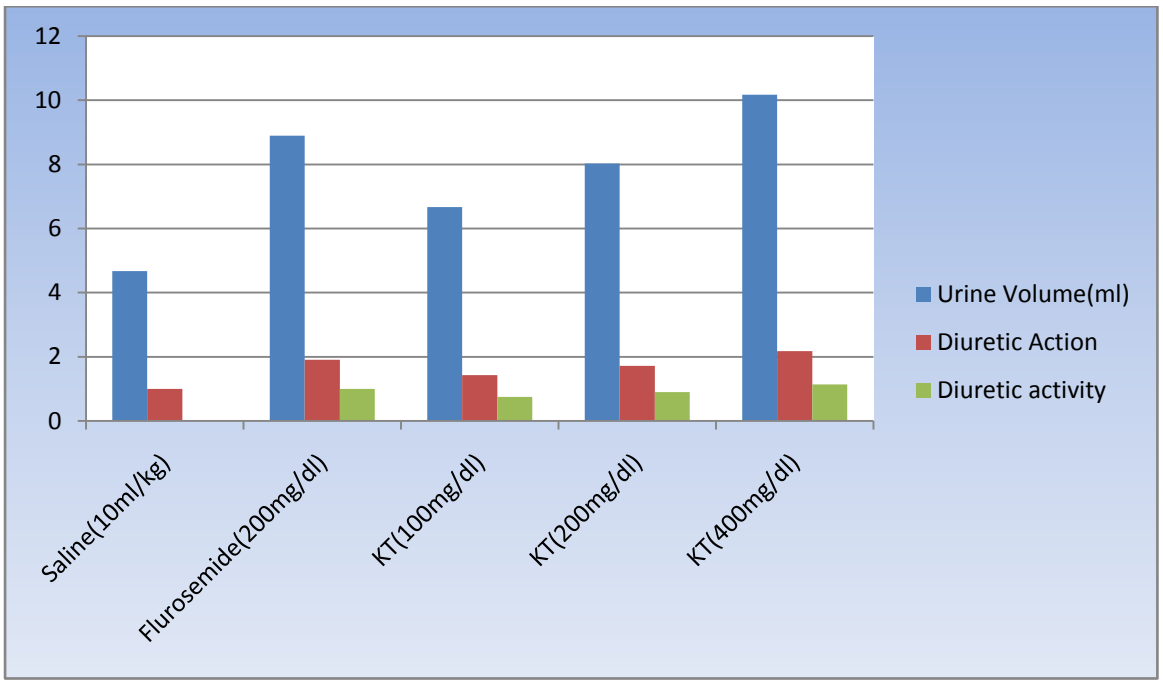


Table 28: Effect of Kalladaippu Thool on urinary electrolyte excretion of Wistar albino ratsat 24 h urine sample collection

Group	Treatment and Doses	Sodium (mMol/L)	Pottassium (mMol/L)	Chloride (mMol/L)
I	Control	110.14±9.12	58.49 ± 4.74	78.46± 4.29
II	Furosemide(200 mg/kg)	196.48±12.5***	119.84± 14.27***	141.75 ± 7.97***
III	KT (100 mg/kg)	141.29 ± 5.47*	75.38± 3.10*	87.11± 6.72*
IV	KT (200 mg/kg)	160.25± 6.12***	86.73 ± 3.70***	100.97± 4.99***
V	KT (400 mg/kg)	185.78±17.12***	104.91± 6.23***	114.89±5.43***

Values are expressed as mean ± SEM, n=6;
Index = excretion in test group/excretion in control group.
Significant changes at P<0.05 with respect to control rats.

Chart 29: *Kalladaippu Thool* on urinary electrolyte excretion of Wistar albino 24 h urine sample collection

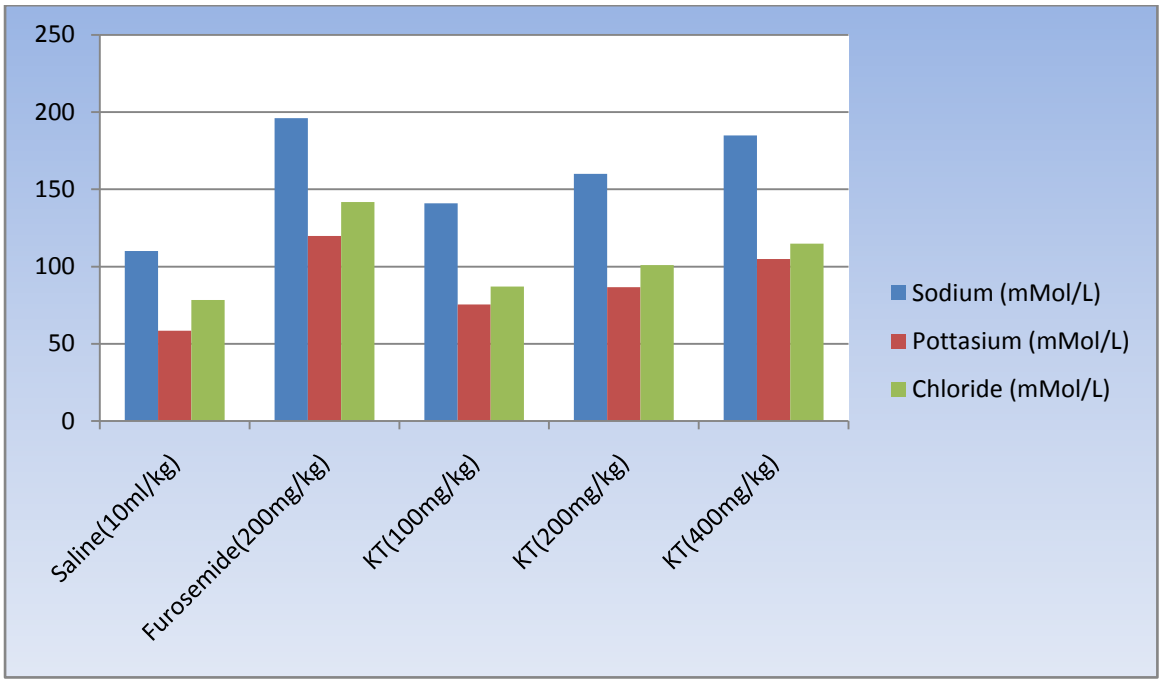


Table 29: Effect of *Kalladaippu Thool* on Saluretic effect and Natriuretic effect of Wistar albino rats at 24h of urine sample collection.

Group	Treatment Doses	Saluretic effect (Na+Cl)	Natriuretic effect (Na/k)	Saluretic index	Natriuretic index
I	Control	188.60 ±10.75	1.98 ± 0.28	1.00	
II	Furosemide(200 mg/kg)	338.23 ± 13.15***	1.79 ± 0.25	1.79	0.90
III	KT (100 mg/kg)	228.40 ± 10.09*	1.91 ± 0.15	1.21	0.96
IV	KT (200 mg/kg)	261.22 ± 1.31***	1.85 ± 0.04	1.37	0.93
V	KT (400 mg/kg)	300.68 ± 16.15***	1.81 ± 0.20	1.59	0.91

Interpretation:

Results of study were represented in Table: 28 - 29. They show the values of urinary volume and urinary electrolytes. The drug administrated groups showed significant increase in urinary output compared to Control.

Table -31, it explains the Saluretic effect of KT .The drug administrated group showed increased urinary sodium potassium and chloride levels compared to control groups. Significant differences were observed between standard and KT treated groups (Group IV, Group V) in excretion of urinary electrolytes. It has significant saluretic effect.The values greater than 2.0 indicate favourable natriuretic effect. As per that it has no natriuretric effect.

When compared to lipscitz value of *Kalladaippu Thooltreated* groups between 1- 1.50 as shown inTable-27, so it is considered as moderate Diuretic.

8. DISCUSSION

The drug *Kalladaippu Thool*was selected from the Siddha literature*"Anuboga vaidhiya navaneetham"*to validate the safety and its efficacy in the evaluation of Anti-Urolithiatic activity in ethylene glycol induced urolithiasis in Wistar albino rat model.

Various analyses such as physicochemical, chemical analysis were made. From the above analysis we came to know that the presences of active ingredients were responsible for its activity. Chemical analysis showed the presence of sulphate, iron and calcium. XRD analysis showed the presence of natroxlate, sodium chloride, sodium boron, potassium carbonate, potassium sulphide and boron carbide.Thus from these results we came to know that the effectiveness of the drug is due to the presence of these constituents and it has a synergistic effect in acting against the disease.

Toxicological studies of acute, sub-acute and sub chronic toxicity study were carried out in Wistar albino rat model according to the OECD guidelines. The test drug showed there was no mortality seen.

The pharmacological study was carried out in Wistar albino rat model. Three activities were seen in the drug *Kalladaippu Thool*.The activities were

- Anti-Urolithiatic activity
- Diuretic activity
- Saluretic activity

Anti-Urolithiatic activity was carried out in Ethylene glycol inducedurolithiasis in Wistar albino rat model. The test drug *Kalladaippu Thool*at the dose level of 200mg/kg and 400mg/kg b.wt exhibits significant lowering of the elevated levels of calcium, oxalate, phosphate, urea nitrogen, creatinine and uric acid excretion in urine samples. Thus this activity reveals the effect of the drug against Urolithiasis.

The Diuretic potential of *Kalladaippu Thool*was carried outin Wisteralbino rats withcomparison of control group**.** Test drug treated rats produced significant increase in urine output and electrolyte excretion in a dose-dependent manner when compared to control group. Furosemide and *Kalladaippu Thool* increase the urine flow significantly at 24 h when compared with control rats. The high dose excreted more than two fold the volume of urine as compared to control. The diuretic activity of the drug is considered nil if it is >0.72, little if

it is between 0.72 and 1.0, moderate if it is within 1.00-1.50 and good if it is above 1.50. In this respect, *Kalladaippu Thool* exhibits moderate diuretic activity. The diuretic responses with its electrolyte excretion potency of the *Kalladaippu Thool* where highly moderate in comparison to normal control rats. The *Kalladaippu Thool* at doses of 200 and 400 mg/kg showed a significant increasing sodium, potassium, and chloride excretion. Therefore the *Kalladaippu Thool* has been shown to possess significant saluretic effects. Thus the diuretic activity and saluretic activity of the drug is justified.

Thus by scrutinizing all the above mentioned factors it is concluded that the test drug *Kalladaippu Thool*isa safe and a potent anti-urolithiatic drug. It alsopossesses rich diuretic and saluretic activity which supports the effective treatment for managing renal calculi and its complications.

9. SUMMARY

- The test drug *Kalladaippu Thool*was selected from the siddha literature*"Anuboga vaithiya navaneetham"* for its Anti-urolithiatic, Diuretic and Saluretic activites.

- The test drug was prepared by the given procedure. All the ingredientswere identified and authenticated by the experts.

- Review of literature in various categories was carried out Siddha aspect, botanical aspect disclosed about the drug and the disease.

- The drug was subjected to analysis such as physicochemical, chemical and also instrumental analysis which provided the key ingredientspresent in the drug thus it accounts the efficacy of the drug

- Toxicological study was made according to OECD guidelines comprising acute, sub-acute and sub chronic toxicity study. It screens the safety of the drug whichattributes its utility in long time administration.

- Pharmacological study was done. It revealed that the Anti-urolithiatic, Diuretic and Saluretic activities of Kalladaippu *Thool*in Wistar albino rat model.

- Results and discussion gives the proper justifications to prove the potency ofthe drug.

- Conclusion gives a compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.

- Thus the herbo-mineral formulation *Kalladaippu Thool*is validated for itssafety and efficacy for treating *Kalladaippu* and it would be a great drug ofchoice.

10. CONCLUSION

From the literature evidence, physicochemical analysis, chemical analysis, Toxicological evaluation and Pharmacological studies, the drug *Kalladaippu Thool* have anti-urolithiatic, diuretic and saluretic activity. It was concluded that the *Kalladaippu Thool* can be used in the management of *Kalladaippu* (Urolithiasis).

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

69,Anna Salai,Guindy,Chennai - 600 032.

This Certificate is awarded to Dr/~~Mr/Mrs~~.....K. Vijayalakshmi.....

for participating as ~~Resource Person~~ / Delegate in the Seventeenth (XVII) Workshop on

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Prof. **Dr.D.SHANTHARAM,** M.D., D.Diab.,
VICE - CHANCELLOR

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs used in the Siddha formulation **"Kalladaippu Thool"** taken up for Post Graduation Dissertation studies by **Dr.K.Vijayalakshmi**, M.D.(S), II year, Department of Gunapadam, 2016, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

Achyranthes aspera Linn. (Amaranthaceae), Whole plant
Borassus flabellifer Linn. (Arecaceae), Flowers
Solanum melongena Linn. (Solanaceae), Whole plant
Musa paradisiaca Linn. (Musaceae), Stem sheath
Uncaria gambir Roxb. (Rubiaceae), Leaf extract

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

Certificate No: NISMB2562016

Date: 20-10-2016

Authorized Signatory

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

CERTIFICATE OF AUTHENTICATION OF MINERAL SAMPLES

Certified that the mineral submitted for identification by Dr.,K.Vijayalakshmi 2nd year PG Scholar, Department of Gunapadam, National Institute of Siddha, Tambaram sanatorium, Chennai-47 were identified as Alum,Borax,Sodium Chloride Impura,Sodium Carbonate with below microscopic and macroscopic characteristics based on Rutlys and Danas mineral descriptions.

M. Suresh Gandhi

Dr.M.Suresh Gandhi

Assistant Professor

Department of Geology

Guindy Campus, Chennai-600025

Date: 25/8/2016

Dr. M. SURESH GANDHI, M.Sc.,M.Phill.,Ph.D.,
Assistant Professor
Department of Geology
University of Madras
Chennai-600 025.

C.L.BAID METHA COLLEGE OF PHARMACY

(An ISO 9001-2000 certified institute)

Jyothi Nagar, Old Mahabalipuram Road

Thoraipakkam, Chennai – 600 097

CERTIFICATE

This is to certify that the project entitled, **Pharmacological and Toxicological study of KALLADAIPPU THOOL** in rats submitted in partial fulfilment for the degree of **M.D. (siddha)** was carried out at C.L. Baid Metha college of Pharmacy, Chennai-97, in the Department of Pharmacology during the academic year of 2015-2016. It has been approved by the **IAEC No: IAEC/XLIX/18/CLBMCP/2016**

C.L. Baid Metha College of Pharmacy ★ CHENNAI-97 ★ Thoraipakkam

(Dr.P.Muralidharan)

IAEC Member Secretary