SAFETY AND PHARMACOLOGICAL PROFILE OF *VELLAI PARPAM*

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INTRODUCTION

1. INTRODUCTION

In siddha system of medicine is profunded by *Agathiyar* and his clan of 18 siddhars. The siddha system is *amalgam* and inseparable from tamil.

The department of *gunapadam* is doing continues research, This drug evaluation is done by animal study, ICP OES, SEM analysis for heavy metals and particle size respectively, and to determine Acute, Sub Acute And Sub Chronic Study.

The selected drug *Vellai Parpam* was tried in animal models. The various dose of the drug acute [5mg/kg up to 2000mg/kg (5, 50, 300, 2000mg/kg)], sub acute (200 mg/kg and 400 mg/kg for 28 days) and sub chronic toxicity study were tried. The animals were validated to routine parameters of haematology, urology, lipid profile, diuretic, anti urolithiasis and anti inflammatory studies were studied.

The selected drug *Vellai Parpam* is a *sasthric* preparation authored by *Agathiyar Vaithiya Kaaviyam 1500.*published by V. R.Madhavan. The standard operative procedure were standardized and chemical analysis were carried out. The dose calculation was converted human dose to animal dose.

Low dose = Human dose 400 mg × 0.018(200 gms of rat)
= 7.2 mg
Mid dose = low dose × 5
= 7.2 ×5 = 36 mg
High dose = low dose × 10
= 7.2 × 10 = 72 mg.

The doses were calibrated use in standard scientific procedure subjected by pharmacologist and toxicologist in our institute. All the protocols and the experiments conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and Supervision of Experiments on Animals **(KKCP/2015/4529)** .Animal experimentation protocols are approved by Institutional Animal Ethical Committee.

As per the chemical analysis observation of the drug composition has diuretic, anti urolithiasis and anti inflammatory activities and animal study reviews the drug through histopathology study of the drug has no adverse reaction and no psychological abnormalities. The blood parameters showed has no erythrolysis, cumulative toxicities. So the drug may be tried for clinical study in future.

AIM AND OBJECTIVES

2. AIM AND OBJECTIVES

Aim:

To Evaluate The Safety And Pharmacological Profile Of The Test Drug *"Vellai Parpam"* In The Animal Models.

Objective:

- Review of various information (Siddha and modern) relevant to the study.
- Preparation of the drug as per classical Siddha literature.
- Standardization of the prepared drug

  - Physicochemical analysis
  - Chemical analysis to evaluate acidic and basic radicals.
  - ICP-OES – Inductively Coupled Plasma Optical Emission Spectrum
  - SEM – Scanning Emission Microscopy

Toxicity studies:

- Acute oral toxicity study by OECD – 423 Guideline.
- Repeated dose 28 days oral toxicity study by OECD – 407 Guideline.
- Repeated dose 90 days oral toxicity study by OECD – 408 Guideline.

Pharmalogical activities in wister albino rats

- Anti-Urolithiatic activity by ethylene glycol induced urolithiasis method
- Diuretic activity by lipschitz method
- Anti-inflammatory activity by carrageenan induced paw edema method

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Standard Operative Procedure

Drug selection:

- ***Vellai parpam*** were taken as a trial drug from the Siddha literature ***"Agathiyar vaithiya kaaviyam- 1500",*** Author:R,Madhavan

Ingredients:

Purified *Karpoora silasathu* (Selenite) - 1 *palam* (35 gm)

Purified *Porikaram* (Borax) - 1 *palam* (35 gm)

Decoctions:

Paruthi ver (Gossypium herbaecum) **- 1 *palam* (35 gm)**

Athiver thol (Ficus racemosa) **- 1 *palam* (35 gm)**

Egg white - Q.s

Collection of the Plant materials

All plant materials were freshly collected from in and around Gingee, Villupuram Dt, Tamilnadu.

Some 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.

The identity and authenticity of the mineral drugs were confirmed by Dr.E.Sasikala, Pharmacognosist, Siddha Central Research Institute, Chennai.

Purification of the drugs

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

- Roots of *Ficus racemosa* and *Gossypium herbaecum* was washed in the running tap water to remove the soil and impurities.
- The *karpoora silasathu* was purified by boiling it in tender coconut water until the water evaporates. The drug is removed, washed and then dried.
- *Vengaram* was fried using heat until the removal of its entire water content.

Ingredients before and after purification

VENGARAM

KARPOORA SILASATHU

ATTHI VER

PARUTHI VER

EGG WHITE

VELLAI PARPAM

Preparation of the Drug

Procedure

- The decoction will be prepared from *Athiver thol* and *Paruthiver*
- The *karpoorasilasathu* and *porikaram* Will be powdered and ground with the above decoction and made in to *villai*, which is subjected to *putam* process with 5 cow dunk cakes
- The same process will be repeated with egg white finally the *parpam* will be collected and stored in air tight container.

Labelling:

Name of the preparation	:	*Vellai Parpam*
Date of preparation	:	8/6/2014 and 15/2/2015
Therapeutic dose	:	200 mg twice a day
Adjuvant/Vehicle	:	Honey
Indication	:	*Kalladaippu, Mahotharam,Neer chiruppu*.
Date of expiry	:	100 years

REVIEW OF LITERATURE

SIDDHA REVIEW

4. LITRATURE REVIEW

4.1. GUNAPADAM REVIEW

1. **அத்தி -** ***Ficus glomerulata roxb***

வேறு பெயர் :

அதம், அதவு, உதும்பரம், கோளி, சுப்பிரதஷ்டம்.

பயன்படும் உறுப்பு :

வேர்.

சுவை	:	துவர்ப்பு
தன்மை	:	தட்பம்
பிரிவு	:	இனிப்பு
செய்கை	:	துவர்ப்பி குருதி பெருக்கி

குணம் :

"வீறு கடுப்பிரத்தம் வெண்சீத ரத்தமொடு
நாறுவிர ணங்களெலாம் நாடாவாம்-கூறுங்கால்
அத்திதரு மேகம்போம் ஆயிழையே! எஞ்ஞான்றும்
அத்திப்பாற் பட்டைக் கறி".

- அகத்தியர் குணவாகடம்.

கீழ்வாய்க் கடுப்பு, குருதிப்போக்கு, சீதக்கழிச்சல், நாற்றமுள்ள புண்கள், வெள்ளை ஆகியவைகளைப் போக்கும்.

பயன்

- அத்தி பால் - பித்தகோபம், நீரிழிவு, இரத்த மூத்திரக்கிரிச்சரம் போக்கும்.
- மரவேரிலிருந்து இறங்கும் கள்ளில், சீனியேனும், பேயன் வாழைக்கனியேனுங் கூட்டி நாடோறும் விடியற்காலையில் உட்கொள்ள, உட்சூடு, பித்த மயக்கம், நீர்வேட்கை முதலியவைகள் தணிக்கும்.

சேரும் பிற மருந்துகள் :

உள் மருந்துகள்

மிளகு தைலம் :

தீரும் நோய் : ஊதல் நோய், அசீரணம்.

4.2. பருத்தி –*Gossypium herbaecum.Linn*

வேறு பெயர் :

ஆச்சாத நபலை, பரி, உத்திரி, காற்பாசம், பன்னல்.

பயன்படும் உறுப்பு :

வேர்ப்பட்டை.

சுவை : துவர்ப்பு, இனிப்பு.

தன்மை : வெப்பம்

பிரிவு : கார்ப்பு

செய்கை :

சிறுநீர் பெருக்கி

ருதுவுண்டாக்கி

உள்ளழலாற்றி

பயன் :

- சீழ்வெள்ளை, குருதி வெள்ளை, குருதியழல் நோய், புண், வீக்கம் இவைகளும் நீங்கும்.
- பஞ்சிலிருந்து, " கன் காட்டன் " உம், அதிலிருந்து கொலோடீன் உம் செய்யலாம். இம்மருந்தை வெட்டுக்காயங்களின் மீது தடவ, அந்த இடம் சீக்கிரம் வறண்டுபோம். அவ்விடத்தில் ஒருவகைச் செதிள் போன்ற ஒன்று மூடிக்கொண்டு, புறக்காற்று, நுண்புழு முதலியன உட்புகாதபடி காக்கும்.

சேரும் பிற மருந்துகள் :

உள் மருந்துகள் :

பூரணாதி இளகம் :

தீரும் நோய்கள் : அழல் நோய், மூலவாய்வு.

மகாவில்வாதி இளகம் :

தீரும் நோய்கள் : வீக்கம், உப்பிச நோய், 40 வகை அழல் நோய்கள்.

கற்பூர சிலாசத்து

உபரசம் -120 ல் ஒன்று. சிலாசத்து என்பது சீலை+சத்து என பிரிந்து மலையினுடைய சத்தைக் குறிக்கும்.

கிடைக்கும் இடம் & தோற்றம்:

"முதுவேனிற் பருவந்தன்னில் மொய்க்கதிர் வெப்பம் தாக்கம்
வெதிர் செறியி மயமாதவியன் வரைக் கணிக லோகம்
மதுவென அருகி நாளும் மானுறு பிசினை போன்ற
பதிகிலா வெளியிற் றோன்றும் பண்புடை ரசம் சிலாசத்து"

போகர் 7000 -3ம் காண்டம்.

- முதுவேனிற் பருவத்தில் சூரிய வெப்பத்தினால் மலைகளிலுள்ள பொன், செம்பு, வெள்ளி போன்ற உலோகங்கள் உருகி, பிசின் போல் கசிந்து வருவது சிலாசத்து.
- நேபாளத்தில் துவர்ப்பூமியிலிருந்தும் எடுக்கப்படுகிறது.

சுவை, தன்மை, & பிரிவு :

சுவை	-	இனிப்பு
தன்மை	-	தட்பம்
பிரிவு	-	இனிப்பு

சத்துரு & மித்துரு சரக்கு :

சத்துரு	-	சீனம், காந்தம், வெண்காரம், இரும்பு
மித்துரு	-	நாகம்

பஞ்ச பூதத்தில் சிலாசத்து :

பிருத்திவியின் கூறு

தேர்வு செய்யும் முறை :

1. நெருப்பின் மீது வைத்தால் புகையாமல் இருக்க வேண்டும்.
2. கம்பி கம்பியாக பிரிய வேண்டும்.
3. தண்ணீரில் போட்டால் தண்ணீருக்குள் கயிறு போல் நீள வேண்டும்.

குணம் :

கற்பூரத்தின் மணமும், வெண்மை நிறமும் கொண்டு இருக்கும்.

பொது குணம் :

"கல்லடைப்பு மேகங்கனதூலம் வித்திரதி
சொல்லடைக்கு நீரருறுகற் சோணிதக்கான் -மெல்லிடையார்க்
கில்லகச்சத் தில்லையெனு மிந்திரிய நட்டமுமாங்
கல்லகச்சந் தில்லையெனுங் கால்".

கல்லடைப்பு, சீமேகம், அதிதூலம், **வித்திரதி**, **மூத்திரக்கிரிச்சரம்**, சோணிதவாதம் போகும்.

சேரும் பிற மருந்துகள் :

உள் மருந்துகள்

கற்பூரசிலாசத்து பற்பம் :

தீரும் நோய் : மேல் எரிவு, பித்த வெட்டை, நீர்க்கடுப்பு.

பவழ சிலாசத்து பற்வம் :

தீரும் நோய் : வெட்டை, வெள்ளை, நீர்சுருக்கு.

காரசூடசத்து பற்பம் :

தீரும் நோய் : நீரடைப்பு, கல்லடைப்பு, சதையடைப்பு, நீர்க்கட்டு.

வெங்காரம்

வெங்காரம் காரசாரம் 25 ல் ஒன்று ஆகும்.

வேறு பெயர் :

பொரிகாரம், வடங்கம், உருக்கினம், காரம், உருக்குமித்திரன், டங்கணம், தூமத்தையடக்கி.

போகர் நிகண்டு - 1200 யில்,

சுந்தாணி, குடோரி, ஆங்காரி, மணிகாரி.

கிடைக்கும் இடம் :

கலிபோர்னியாவிலுள்ள கிளியர் ஏரியிலும், பெரு என்ற இடத்திலும், இந்தியாவில் திபெத், நேபாளம் முதலிய இடங்களிலும் உள்ள ஏரிகளில் நீர்வற்றினால் உப்பு உறைந்து கிடைக்கிறது.

குணங்கள் :

- வெண்மையாக சில கோணங்களுடன் மினுமினுப்பாக இருக்கும்.
- நீரில் கரையும்.
- சாராயத்தில் கரையாது.
- காற்றுப்படும்படி வைத்தால் உப்பின் மேல் வெண்ணிறத் தூள் படியும்.
- பொரித்தால் அதிலிருந்து நீர் சுண்டிப் பொரிந்து அதில் சிறிய துவாரங்கள் தோன்றும்.

சிறப்பு குணம் :

- 64 சரக்கையும் கட்டும்.
- உபரசம் 120 யும் சத்தாக்கும்.
- காரத்தைக் கட்டும்.
- களங்கு, செந்தூரம், குரு இவைகளுக்கு ஆதியாகும்.

சத்துரு சரக்குகள் :

சிலாசத்து, அண்டம், சுண்ணம், பூரம், சூடன்.

அகத்தியர் பரிபூரணம்-1200

மித்துரு சரக்குகள் :

பாடாணம், உபரசம், உலோகம்.

வெங்காரத்தின் சுவை மற்றும் வீரியம் :

சுவை :

துவர்ப்பு, இனிப்புடன் கூடிய துவர்ப்பு.

வீரியம் :

வெப்பம்

செய்கை :

உள்ளாட்சி:

- குளிர்ச்சி உண்டாக்கி
- **சிறுநீர் பெருக்கி**
- **கல்கரைச்சி**
- பிரசவகாரி

வெளியாட்சி :

- சமனகாரி
- உடல்தேற்றி
- அழுகலகற்றி
- துவர்ப்பு

பொது குணம் :

"சொறிபுடையெண் குன்ம நமை சோரி யாசம்
பறிகிரகணி கல்லானம் பண்ணோம் - நெறியைத்
தடங்கணங்க பங்கிருமி சர்ப்பவிடங் சந்நி
யிடங்கனங்க லக்கிற்போ மெண்."

சொறி, புடை, குன்மம், தினவு, இரத்த மூலம், பல்நோய், **நாளவழியைத் தடுக்கின்ற மூத்திர கிரிச்சரங்கள்** முதலியவை நீங்கும்.

வழக்கு முறைகள் :

- பொரித்த வெங்காரத்தை 5 முதல் 10 குன்றி இளநீரில் போட்டுக் கொடுக்க **நீர்க்கட்டு** நீங்கும்.
- **வெங்கார நீர் : வாய்ப்புண், தொண்டைப்புண், அக்கரம்** முதலியவைக்கு வாய் கொப்புளிப்பதற்கு பயன்படுத்தலாம்.

சேரும் பிற மருந்துகள் :

உள் மருந்துகள்

சிந்தாமணிக் குளிகை :

தீரும் நோய் : நீர்க்கட்டு, மலக்கட்டு, அழல் நோய்.

நவக்கிரக பூபதி :

தீரும் நோய் : நீராம்பல், வல்லை.

சிந்து வல்லாதி மெழுகு :

தீரும் நோய் : நீரடைப்பு, கல்லடைப்பு, அண்டவாய்வு, சதையடைப்பு, பெரு நீர்க்கோவை

BOTANICAL REVIEW

4.2. BOTANICAL REVIEW

1. *Ficus glomerata roxb.,*

Synonyms

Ficus glomerata, Ficus lucescens, Ficus racemosavar.elongata.

Common Name

Udumbara, *Gular* fig, Cluster fig, Country fig, Cluster Fig Tree, Goolar Fig

Vernacular name

Tamil : Atti

Hindi : Goolar

Chemical constituents

Major : Lupeol, Friodelin.

Bark – CerylBehenete, Lupeol, A – Amyrin, Gluanol – Oac, B-SitosterolAnd A Ketone, Stigmatosterol.

Leaves – Gluanol – Oac–β – Amyrin and –β-Sitosterol.

Fruit - Lupeol-Oac, B-Sitosterol and A Sterol, tiglicacid, Ester of Taxaxasterol and Glucose.

Uses

- The root is useful in hydrophobia. Bark is cooling, acrid, galactogogue, good for gravid uterus. Unripe fruit is acrid and astringent to bowels, tonic, **styptic**, useful in *kabam*, biliousness, leucorrhoea and **blood disorders.**
- Ripe fruit is acrid sweet, **cooling, useful in blood disorders, burning sensation, urinary discharges**, thirst, leprosy, menorrhagia, nose bleeding and intestinal worms.
- Bark - useful in asthma and piles given as an **astringent** and as a wash for wounds.
- Leaves - astringent to bowels and good for bronchitis. The fresh juice of the ripe fruit is used as an adjuvant to a metallic preparation which is given in diabetes and other **urinary diseases.**

2. Gossypim herbaceum.Linn

Synonyms **:** *Erioxylum Rose & Standl, Selera Ulbr, Ultragossypium Roberty.*

Common name : Cotton plant.

Vernacular name : Karpash tula, Tula.

Tamil : Paruthi

Hindi : Kapas

Botanical Classification

Kingdom : Plantae

Division : Angiosperms

Order : Malvales

Family : Malvaceae

Genus : *Gossypium*

Species : *herbaecum*

Chemical constituents

Gossypol, Gossyfulvin, Gossypurpurin, Gossycaeulin, Gossyoin, Gossypetin, Gossypitrin ,Herbacetin, Herbacitrin, Lecuodelphinidine, Betine, Quercetin, Isoquercetin, Quercimeritrin, Phytosterols.

Root : Cycloartenol, Euphorbol, Teraxerone, Tinyatoxin.

Properties

All parts are Cooling, Sweet, Acrid, Anti-dysenteric.

Uses

- **Root** decoction is given for **Urinary problems.**
- Tincture of the fresh inner root bark is used to treat amenorrhea, dysmenorrhea.
- Used to treat abscess of the labia.
- The root is used to stop bleeding, especially internally.
- Externally, used for rheumatism, a dressing for freckles, herpes, scabies,Neuralgia, chronic headache, ulcers, sores, swellings and wounds.

MINERALOGICAL REVIEW

4.3. MINERALOGICAL REVIEW

1. Karpoora silasathu:

English : Asphalt ; Mineral pitch ; Plaster of paris

Hindi : Silajita

Source

Ejected out of rocks during hot weather in the lower Himalayas, Vidhya and other mountain tracts and Nepal where iron abounds, naturally flowing out from between the fissures in the rocks or it may be a tar formed in the earth from the decomposition of vegetable substances.

Constituents

Silasathu contains an oil which when distilled is known as ichthyol. Benzoic acid and benzoates which are present in silasathu in large quantities are considered by Chopra to be the main active principles.

Action

Locally antiseptic, anodyne, parasiticide, and antiphlogistic. Internally **diuretic, lithontriptic,** alterative, tonic, slightly laxative, respiratory stimulant, expectorant, intestinal stimulant.

Physical Properties of Gypsum *(karpoora Silasathu)*

Lustre :

Vitreous, Silky, Dull

Transparency :

Transparent, Opaque, Translucent

Colour :

Colourless to white

Tenacity :

Flexible

Uses:

- It is specially employed in **genito – urinary diseases** and in diabetes.
- It is mainly used in Gall stones, Jaundice, Enlarged spleen, Anasarca, **Renal stone** and **Bladder calculi**, **Anuria.**
- It is also used in **Ascites, Uraemia.**
- Silasathu is used as an external application for inflammatory swellings, arthritis.
- In strangury or **painful micturation** silasathu is used with other diuretic and demulcents.

Supportive articles:

1. The *karpoora silasathu* is one of the ingredients of the drug *jalamanjari chendooram* is an effective and significant hyponatraemic, hypochloraemic, hypokalaemic and diuretic activity is present.

2. The *karpoora silasathu* is one of the ingredients of *karasoodasathu parpam* is indicated for **urolithiasis** and diuretic activity.

3. The drug *silasathu paavanai* contains essential elements which are considered to be the inhibitors of stone formation. Moreover the drug has good anti microbial activity against E.coli which causes the commonest associated urinary tract infection.

Crystallography of Gypsum *(karpoora Silasathu)*

Crystal System :

Monoclinic

Class :

2 / m - Prismatic

Morphology :

Thin and thick tabular crystals.

Crystals may have wraped surfaces, or be bent or twisted.

Rosette – like clusters of lenticular crystals are common.

4.3.2) BORAX *(VENGARAM)*

Borax (sodium borate) is a naturally occurring mineral composed of sodium, boron, oxygen and water.

Tam : *Vengaram*, *Venkaram*

Eng : Sodium Biborate, Borax

Sans : Tankana

Tel : Velligaram

Mal : Ponkaram

SOURCE

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.

Taste :

Sweet with astringent.

Potency :

Heat

Pirivu :

Pungent

Action :

Diuretic

Astringent

Antacid

Local sedative

Anti-septic.

Physical Properties of Vengaram

Chemical Formula	**:**	$Na_2B_4O_7.10H_2O$
Composition	**:**	Hydrous sodium borate
Colour	**:**	Colourless, white, light grey. Also in light tints of blue, green and yellow.
Streak	**:**	White
Hardness	**:**	2 - 2.5
Transparency	**:**	Transparent to opaque
Specific Gravity	**:**	1.7
Luster	**:**	itreous to dull
Other ID Marks	**:**	1.Has a sweetish, metallic taste 2 . Dissolve in water
In Group	**:**	Borates; Hydrous Borates

Boraxs healing and preventative properties :

- Borax protects against the accumulation of fluorides in the body; is effective as an antidote in fluoride toxicity; and can remove fluorides from the body.
- **Anti-microbial :**
  Borax is toxic to insects, parasites, protozoa and bacteria.
- **Fungicide :**
  Effective against moulds and fungi, internally and externally.
- **Immune system enhancer :**
  Promotes healing of wounds.
  Reduction and control of **inflammation.**
- **Toxin removal :**
  Protection from heavy metals.
- **Stabiliser** of calcium, silicon, copper and magnesium levels, inhibits **calcification.** Boran sufficiency normalises calcium levels, preventing both abnormal calcium deposition and bone weakness.
- Obesity.

- **Cancer.** Boron may be a preventative for prostate cancer.
- **Antiseptic.** Very effective for bladder infection and **urinary tract infection.**

Uses :

- *Vengara parpam* cures *pitha* disease like burning micturation and *kalladaippu*. Borax is given internally in doses varying from 10-30 grains in acidity of stomach, dyspepsia and intestinal organism. It commonly mixed in decoction for *kalladaippu*.
- In small doses it is given to children as a laxative.
  It is also used in loss of appetite, painful dyspepsia, cough, asthma and diarrhoea.
- Externally borax is used in lotion in acne, freckles, chloasma.
- Boro-glycerine is useful as an antiseptic lotion in purulent ophthalmia and diphtheria.

Supportive articles :

1) The *Vengaram* is one of the ingredients of the drug *jalamanjari chendooram* is an effective and significant hyponatraemic, hypochloraemic, hypokalaemic and diuretic activity is present.

2) Toxicity study of *Vengaram* and *Silasathu* :

   Acute oral toxicity study; NK and VSP at the dose of 2000mg/kg/po did not exhibit any mortality in rats. **Repeated oral toxicity for 28 days;** Test drug NK and VSP at the dose 500mg/kg/po when administered for 28 days in rats orally did not show toxicity in hematological parameter.

ANALYTICAL STUDY
OF
THE DRUG

5. ANALYTICAL STUDY OF *VELLAI PARPAM*

Analytical study of the drug brings the validation to be used as a medicine by subjecting the drug into many analysis and determining its quality and effectiveness. Characterization is essential to achieve analytical study of a drug. Through analytical studies such as screening of organoleptic analysis, physico chemical analysis and also to assess the active principles and elements present in the drug. Thus analytical study brings the efficacy and potency of the drug. Analytical study of the drug includes:

- **Organoleptic characters**
  - Colour
  - Odour
  - Taste

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

- **Chemical analysis**
  - Preliminary Basic and Acidic radical studies

- **Heavy metal analysis**
  - ICP OES
- **Analysis of partical size**
  - SEM

5.1. ORGANOLEPTIC CHARACTER

The organoleptic characters of the sample drug were evaluated. 1gm of the test drug was taken and the colour, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result was noted.

PHYSICO CHEMICAL ANALYSIS

5.2. PHYSICOCHEMICAL ANALYSIS

Physicochemical studies of the trial drug *Vellai Parpam* have been done according to the WHO guidelines.

Determination of Ash Values

Total Ash

3g of the test drug was accurately weighed and incinerated in a crucible dish at a temperature not exceeding 450 ° C until it was 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 with 25ml of water for 5mins. The insoluble ashes were collected using filter paper. It was then washed with hot water and transferred to the silica crucible. It was then ignited for 15minutes at temperature not exceeding 450^{o}C. For determination of weight of the water soluble ash the silica crucible and residue were weighed until constant weight was attained. The weight of the water soluble ash is determined by subtracting the weight of insoluble ash from the weight of total ash.

MATERIALS AND METHODS

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 were collected using filter paper and washed with hot water. It was then transferred to the silica crucible and ignited for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue were weighed until constant weight is attained.

Determination of Extractive Value

Alcohol Soluble Extractive Value

3g of test drug powder was weighed and macerated with 100ml of ethanol in a closed container for 24 hours. The resulting solution was shaken continuously for 6 hours. It is then allowed to stand and soak for 18 hours. The solution was filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C. Then the content was cooled and weighed.

Water soluble Extractive value

3g of test drug powder was weighed and macerated with chloroform and water respectively, at 80^{o}C for 24 hrs. The resulting solution was shaken continuously for 6 hours and allowed to stand and soak for 24hrs then filtered. The solution from both chloroform and water respectively was filtered and evaporated of the filtrate in a flat bottomed shallow dish. It was dried at 105^{o}C then cooled and weighed.

Determination of Loss on Drying at 105°C

The powdered drug was taken and dried in the oven at 105°C to constant weight. The result was noted.

CHEMICAL ANALYSIS

5.3 CHEMICAL ANALYSIS OF *VELLAI PARPAM*

The chemical analysis of *VELLAI PARPAM* was carried out in Bio chemistry Lab, National institute of siddha.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Physical Appearance of extract	Light white in colour	
2.	**Test for Silicate** a. A 500mg of the sample was shaken well with distilled water.	Fully soluble.	Absence of Silicate
3.	**Action of Heat:** A 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:** A 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	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	Absence of sodium

Preparation of Extract:

5gm of *Vellai Parpam* was taken in a 250ml clean beaker and added with 50 ml 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:** 2 ml of the above prepared extract was taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution	Cloudy appearance Presence.	**Presence of Sulphate**
2.	**Test For Chloride:** 2 ml of the above prepared extract was added with 2ml of dil-HCl is added until the effervescence ceases off.	Cloudy appearance present.	**Presence of Chloride**
3.	**Test For Phosphate:** 2ml of the extract were treated with 2ml of dil. ammoniummolybdate solution and 2ml of con.Hno3	No cloudy yellow appearance	**Presence of Phosphate**
4.	**Test For Carbonate:** 2ml of the extract was treated with 2ml dil. magnesium sulphate solution	Cloudy appearance presence.	**Presence 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 is evolved	Absence of nitrate
6.	**Test For Sulphide:** 1gm of the extract was treated with 2ml of con. HCL	No rotten egg smelling gas is evolved	Absence of sulphide
7.	**Test For Fluoride & Oxalate:** 2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil.calcium chloride solution and heated**.**	No cloudy appearance.	Absence of fluoride and oxalate

8.	**Test For Nitrite:** 3drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil. benzidine solution is placed.	No characteristic changes	Absence of nitrite
9.	**Test For Borate:** 2 Pinches (50mg) of the extract was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	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 iodide solution.	No Yellow precipitate is obtained	Absence of lead
2.	**Test For Copper:** **a.** One pinch (25mg) of extract was made into paste with con. HCl in a watch glass and introduced into the non-luminuous part of the flame.	No blue colour precipitate	Absence of copper
3.	**Test For Aluminium:** To the 2ml of extract dil. sodium hydroxide was added in 5 drops.	No characteristic changes	Absence of Aluminium
4.	**Test For Iron:** To the 2ml of extract add 2ml of dil. Ammonium thiocyanate solution. b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added	No red colour appeared	Absence of Iron
5.	**Test For Zinc:** To 2ml of the extract dil. sodium hydroxide solution was added in 5 drops to excess and dil. ammonium chloride is added.	No white precipitate	Absence of Zinc

6.	**Test For Calcium:** 2ml of the extract was added with 2ml of 4% dil. ammonium oxalate solution	Cloudy appearance and white precipitate is obtained	**Presence of calcium**
7.	**Test For Magnesium:** To 2ml of extract dil. sodium hydroxide solution was added in drops to excess.	White precipitate is obtained	**Presence 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 colour	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 is obtained	Absence of potassium
10.	**Test For Sodium**: 2 pinches (50mg) of the extract is made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved.	Absence of sodium
11.	**Test For Mercury**: 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	No yellow precipitate is obtained	Absence of Mercury
12.	**Test For Arsenic:** 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	No brownish red precipitate is obtained	Absence of arsenic
III. Miscellaneous			
1.	**Test For Starch:** 2ml of extract was treated with weak dil. Iodine solution	No blue colour developed	Absence of starch

2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes were noted.	No brick red colour is developed	Absence of reducing sugar
3.	**Test For The Alkaloids**: 2ml of the extract was treated with 2ml of dil. picric acid	No yellow colour was developed	Absence of Alkaloid
4.	**Test For Tannic Acid:** 2ml of extract was treated with 2ml of dil.ferric chloride solution	No Blue-black precipitate is obtained	Absence of Tannic acid
5.	**Test For Unsaturated Compound:** To the 2ml of extract 2ml of dil.Potassium permanganate solution is added.	Potassium permanganate is not decolourised	Absence of unsaturated compound
6.	**Test For Amino Acid:** 2 drops of the extract was placed on a filter paper and dried well. 20ml of Burette reagent is added**.**	No violet colour	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 colour was developed No red colour was developed No violet colour was developed No Blue colour was developed.	Absence of oxyquinol epinephrine and pyrocatechol Antipyrine, Aliphatic amino acids and meconic acid are absent. Apomorphine salicylate and Resorcinol are absent. Morphine, Phenol cresol and hydrouinone are absent.

ELEMENTAL ANALYSIS

5.4. ELEMENTAL ANALYSIS:

HEAVY METAL ANALYSIS

The analysis of heavy metals and trace elements were estimated by using Inductively coupled plasma optical emission spectrometry (ICP- OES). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

ICP-OES INDUCTIVELY COUPLED PLASMA OPTICAL EMISSIOS SPECTROMETRY (ICP OES)

Introduction

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

Principle

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

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

Sample preparation – Microwave Digestion

- Weight 0.25 g of test sample and transfer into a liner provided with instrument.
- Slowly add 9ml of Nitric acid or sulphuric acid such that no piece of sample sticks on the slide.
- Mix thoroughly and allow reacting for few minutes.
- Place the liner in the vessel jacket.
- Close the screw cap hand- tight in clockwise direction.
- Seal the vessel and placed in the rotor fixed in microwave.
- Set temperature to 180°C for 5 minutes, hold at 180°C for least 10 minutes. Allow the vessels to cool down to a vessel interior temperature below 60°C and to a vessel surface temperature (IR) below 50°C before removing the rotor.
- The digested sample was made up to 100ml with Millipore water. If visible insoluble particles exist, solution could be filtered through whatmann filter paper.
- Transfer the digested solution into plastic containers and label them properly. Perkin Elmer Optima 5300DV was used for standard ICP-OES analysis. The optimized operating conditions are given in table 1, the wavelength of analytical lines are given in table and the test *Velai Parpam* underwent microwave digestion for sample preparation.

Table 1 : ICP- OES Operating Conditions

Rf frequency	40 M Hz
Range	165 – 782 nm
Detection limit	Up to ppm level using SCD detector

ANALYSIS OF PARTICLE SIZE

5.5 ANALYSIS OF PARTICAL SIZE

SCANNED ELECTRON MICROSCOPY (SEM)

The partical size of the *Vellai Parpam* was determined using High resolution scanning electron microscopy (HR SEM). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

Experimental procedure :

A SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross-sections. The primary electron beam interacts with the sample in a number of key ways :-

- Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen.
- Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast.
- Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection. The X-ray emitted are characteristic of the elements in the top few μm of the sample.

The SEM is carried out by using FEI Quanta FEG 200-High Resolution Instrument.

Resolution : 1.2 nm gold particle separation on a carbon substrate

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

Method :

A representative portion of each sample was sprinkled on to a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination.

Sample preparation :

Sample preparation can be minimal or elaborate for SEM analysis, depending on the nature of the samples and the data required. Minimal preparation includes acquisition of a sample that will fit into the SEM chamber and some accommodation to prevent charge build-up on electrically insulating samples.

TOXICITY STUDIES

ACUTE TOXICITY

6. TOXICOLOGICAL STUDY

6.1. SAFETY AND PHARMACOLOGICAL PROFILE OF SIDDHA DRUG *"VELLAI PARPAM"*

AIM: Effect on the siddha drug "*vellai parpam*" its Acute and sub acute toxicity studies in wistar rats

Test drug: To Evaluate the acute and sub acute toxicities of siddha drug *vellai parpam* in wistar albino rats.

Drug and preparation of stock solution

The aqueous suspension of *vellai parpam* was prepared in 1% CarboxyMethylCellulose (CMC) solution in distilled water prior to oral administration to animals. It was used within seven days and stored at 8ºC .While for further use freshly prepared solution were used .The vehicle alone served as control.

Experimental animals:

Albino rats (wistar rats) of either sex, weighing (150-250 g) were procured from animal housing facility, K, K college of pharmacy, Gerugambakkam, Chennai. All animals were placed in a polypropylene cages in a controlled room temperature 24ºC ±1ºC and relative humidity of 60-70 % in animal house. The animals were maintained in standard pellet diet and water ad libitum. They were acclimatized to laboratory condition for seven days before commencement of the experiment.
All the protocols and the experiments conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and Supervision of Experiments on Animals **(KKCP/2015/4529)**. Animal experimentation protocols are approved by Institutional Animal Ethical Committee.

Acute oral toxicity study:

The Acute toxicity studies were performed in accordance with the OECD 423 guidelines. Female wistar rats weighing 100-250gm were selected and divided into 5 groups containing three animals in each group. The single dose of *vellai parpam* the dose starting from 5mg/kg up to 2000mg/kg (5, 50, 300, 2000mg/kg) was administered orally. The drug treated animals were carefully observed individually for the toxicity signs and mortality. The parameters such as changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system, behavioural pattern, convulsions, tremors, salivation, lethargy, diarrhoea, sleep and coma were observed. From the maximum dose 1\5th or 1\10th of the dose was considered as therapeutic dose for further study.

REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY

6.2. SUB ACUTE TOXICITY STUDIES OF *"VELLAI PARPAM"* IN WISTAR ALBINO RATS - (OECD – 407 guidelines)

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into 3 groups of 10 animals (5 male and 5 female). *Vellai Parpam* was administered to rats at the dose of 200mg/kg and 400mg/kg 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 animals 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 haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum. Serum was stored at 20°C until analyzed for biochemical parameters.

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

Justification for Dose Selection:

The results of acute toxicity studies in rats indicated that *Vellai Parpam* was non toxic and no behavioural changes was 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. From the maximum dose 1\5th or 1\10th of the dose was considered as therapeutic dose for further study.

Preparation and administration of dose:

Vellai Parpam at two doses level 36mg/kg and 360mg/kg respectively were prepared. The test substance was freshly prepared every 7 days for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals:

The rats randomly divided into three groups. In each group contains 10 animals. Animal's acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliporous and non-pregnant.

OBSERVATIONS:

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

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 percent body weight gain were calculated. (table -9)

ii) Food and water Consumption:

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

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

Haematological Investigations:

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

Biochemical Investigations:

Serum and Urine was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, uric acid, creatinine, triglyceride, cholesterol and glucose levels by using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate amino transferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, spleen, heart, uterus and testes/ovaries were recorded. The relative organ weight of each animal was then calculated as follows;

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

Histopathology:

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 2000mg /kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the " L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

The organs included heart, kidneys, liver, spleen and pancreas of the animals were preserved they were subjected to histopathological examination.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were subjected to One-way Anova Followed by Dunnet t' test using a computer software programme.
(Graph Pad Prism 5.0)

REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY

6.3. REPEATED DOSE 90-DAYS ORAL TOXICITY STUDY OF *VELLAI PARPAM* IN WISTAR ALBINO RATS (OECD GUIDELINE - 408)

Test Substance	: ***Vellai Parpam***
IAEC Number	: NIS/IAEC - II/2016/07.
Animal Source	: King institute of technology, Guindy, Chennai.
Animals	: Wister Albino Rats (Male -12, and Female-12)
Age	**:** 6-8 weeks
Body Weight	**:** 150-200gm.
Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid.
Diet	**:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** between 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 consist of 6 animals (Male -3, and Female-3). 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 are low dose (X), mid dose dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose **(400 mg) and the body surface area of the rat (0.018). i.e X dose is 7.2 mg/animal, 5X dose is 36 mg/animal,10X dose is 72 mg/animal.**

Preparation and Administration of Dose:

VELLAI PARPAM was suspended in Honey with distilled water to obtain concentrations of 200mg/ml. It was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 90 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 90 consecutive days.

OBSERVATIONS:

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

- **Body Weight:**

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

- **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 animal's 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 Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

- **Haematological Investigations:**

  Haematological parameters were determined using Haematology analyzer.

- **Biochemical Investigations:**

Biochemical parameters were determined using auto-analyzer.

- **Histopathology:**

Control and highest dose group animals 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 with Haematoxylin-eosin.

- **Statistical analysis:**

Findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were subjected to One-way ANOVA followed by dunnet't'test using a computer software programme - INSTAT-V3 version.

PHARMACOLOGICAL STUDIES

ANTI UROLITHIATIC ACTIVITY

7. PHARMACOLOGICAL STUDY OF *VELLAI PARPAM* ON WISTAR ALBINO RATS

7.1. ANTI UROLITHIATIC ACTIVITY

Stone induction– Kidney stones were induced by 0.75% of ethylene glycol in drinking water for 28 days ad libitum. After 28 days induction, the animals were used for the study

Table 2: Experimental design:

Groups	**Treatment**
Group I	Normal Control
Group II	Urolithiatic rats- 0.75% of ethylene glycol for 28 days
Group III	Urolithiatic rats + Vellai Parpam 200mg/kg for 28 days
Group V	Urolithiatic rats + Vellai Parpam 400mg/kg for 28 days

In the experiment a total of 24 rats (6 normal rats, 18 urolithic rats) were used. The rats were divided into 4 groups of 6 rats each. Group I (Gr I)-Normal control rats; Group II (Gr II)-Urolithic rats, (Gr III)-Urolithic rats given Vellai Parpam (200mg/kg for 28 days) and (Gr IV)-Urolithic rats given Vellai Parpam (400mg/kg for 28 days). At the end of 28 days, the animals were housed in metabolic cages and collected urine after 24 h.

Biochemical assays

The urine collected after 24 h was subjected to analyze calcium, oxalate, uric acid, citrate.

DIURETIC ACTIVITY

7.2. Diuretic Activity On Wister Albino Rats (Lipschitz Method)

Wistar rats were divided into five groups of six rats in the group (I) served as normal control (Vehicle) which received normal saline water (2 ml/kg orally) only. Group II received as furosemide (10mg/kg, p.o) Groups (III) to (IV) received *Vellai Parpam* respectively at dose of 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 a metabolic cages. A total volume of urine collected for 5 hr was measured at the end. During this period no food and water were made available to animals. Various parameters like total urine volume and concentration of sodium, potassium and chloride in the urine were measured and estimated respectively.

Estimation Of Urinary Electrolytes

Urine electrolytes (Na, K and Cl-) were determined by Ion selective Electrode method as described by the user instruction manual of the biochemical kits (Roche, Roche diagnostics Pvt. Ltd.,)

ANTI-INFLAMMATORY ACTIVITY

7.3. Anti – Inflammatory Activity On Wistar Albino Rats

(Carrageenan-Induced Rat Paw Edema)

The extract of *Vellai Parpam* (200 and 400 mg/kg) or indomethacin (10 mg/kg) was administered orally to different groups of rats. Acute inflammation was induced half an hour after above treatment by sub-planter injection of 0.1 ml freshly prepared 1% suspension of carrageenan in right hind paw in rats (Winter et al., 1962). The paw volume was measured initially and then at 1, 2, 3and 4 h after the carrageenan injection by using plethysmographic method of Harris and Spencer (1962).

RESULTS

8. RESULTS

Many studies have been carried out to bring the efficacy and potency of the drug *Vellai Parpam*. The study includes literary collections, organoleptic character, physicochemical, Chemical analysis, Elemental analysis and toxicological study and pharmacological study. The drug ***Vellai Parpam*** has been selected from the text ***"Agathiyar vaithiya kaaviyam 1500".***

- Botanical aspect explains the active principle and medicinal uses of the plants.
- *Gunapadam* review brings the effectiveness of the drug in treating renal stone.
- The pharmacological review explains about the methodology of Anti-Urolithiatic
- Activity and the drugs used.
- Modern and siddha aspect of the disease was also reviewed.

4. ANALYTICAL STUDY OF THE TEST DRUG

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

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

5.1. ORGANOLEPTIC CHARACTER

The following characters have been noted in *Vellai Parpam*

Table 3: organoleptic characters

Colour	White
Odour	Pleasant
Taste	Characteristic taste
Texture	Fine powder
Particle size	Completely pass through sieve no 120

5.2. PHYSICOCHEMICAL ANALYSIS

Determination of Ash Values

Table 4: Percentage **of Total Ash**

Parameter	**Percentage%**
Ash Value	90.94

Table 5: Percentage of **Acid insoluble ash** values

Parameter	**Percentage%**
Acid insoluble Ash	20.78

Interpretation for ash values

Ash: Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug. **Total ash**: Total ash value of herbo mineral drug indicated the amount of minerals and earthy materials present in the plant material. The total inorganic content (potassium, calcium, chloride, magnesium, etc.,) present in the drug is measured through the total ash value and it is of **90.94 %** for *Vellai Parpam.*

Acid insoluble ash: The acid insoluble ash value of the drug is **20.78%** for *Vellai Parpam.*

Loss on Drying

Table 6: Percentage **Loss in weight on drying**

Parameter	**Percentage%**
Loss on drying at 105^0C	0.49

INTERPRETATION

- The total of volatile content and moisture present in the drug was established in loss on drying.
- Moisture content of the drug reveals the stability and its shelf-life.
- High moisture content can adversely affect the active ingredient of the drug.
- Thus low moisture content could get maximum stability and better shelf life.

5.3. Results of acid radicals studies:

Table 7: Results of acid radicals studies:

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

Interpretation

The acidic radicals test shows the presence of **Sulphate, Chloride, Carbonate .**

Table 8: Results Of Basic Radicals Studies:

S.NO	Parameters	Observation	Result
1	Test for Lead	-	Negative
2	Test for Copper	-	Negative
3	Test For Aluminium	-	Negative
4	Test For Iron	-	Negative
5	Test For Zinc	-	Negative
6	Test for Calcium	Cloudy appearance and white precipitate is obtained	**Positive**
7	Test For Magnesium	White precipitate is Obtained	**Positive**
8	Test For Ammonium	-	Negative
9	Test For Potassium	Yellowish precipitate is Obtained	**Positive**
10	Test For Sodium	-	Negative
11	Test For Mercury	-	Negative
12	Test For Arsenic	-	Negative

Interpretation :

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

5.4. ELEMENTAL ANALYSIS:

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY

Table 9: ICP OES Study results of *Vellai Parpam*

S.NO	Elements	Wavelength in nm	Vellai Parpam mg /L
1	Arsenic	As 188.979	BDL
2	Calcium	Ca 315.807	222.370
3	Cadmium	Cd 228.802	BDL
4	Copper	Cu 327.393	BDL
5	Nickel	Ni 231.604	BDL
6	Mercury	Hg 253.653	BDL
7	Sodium	Na 589.592	404.310
8	Phosphorus	P 213.617	04.301
9	Sulphur	S 181.975	21.824
10	Lead	Pb 220.353	BDL

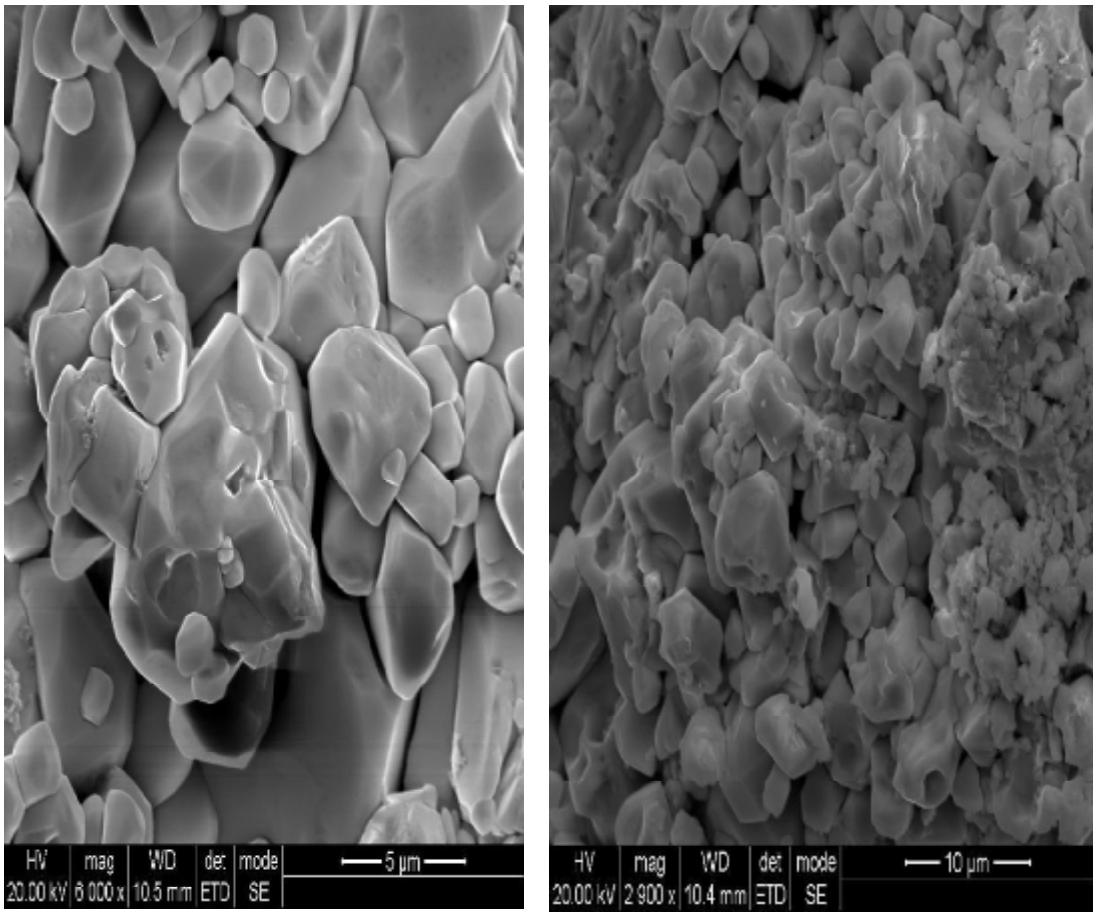
* BDL – Below Detection Limit

The results Shows the quantitative analysis of the elements present in ***Vellai Parpam.*** The heavy metals were found to be within normal limits. The presence of other elements shows the therapeutic value of **Vellai Parpam.** Hence the drug *Vellai Parpam* is considered as a safe drug.

5.5. ANALYSIS OF PARTICAL SIZE

SCANNED ELECTRON MICROSCOPY

Determination of Partical size of Vellai Parpam



The picture shows that the particles are stabilize, have irregular morphology and distributed in near nano range, ***Vellai Parpam*** has particle size of 5 to 10 microns.

6. TOXICITY STUDY ON VELLAI PARPAM IN WISTAR ALBINO RATS

6.1. Acute oral toxicity study of *VELLAI PARPAM*

Table 10: Dose finding experiment and its behavioural Signs of Toxicity

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	5	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	50	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	300	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	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

Interpretation:

The acute toxicity result shows no mortality rate up to 2000mg/kg. It showed changes in alertness, pile erection, grooming, touch response and decreased motor activity. The behavioral changes are normal. Hence the test drug ***Vellai Parpam*** is a safe herbal drug and can be used for long time administration

6.2. SUB-ACUTE ORAL TOXICITY

REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY IN WISTAR ALBINO RATS

Table 11:Body weight (g) of albino rats exposed to *vellai parpam* for 28 days

DOSE	DAYS				
	1	7	14	21	28
CONTROL	**153.6±22.683**	**169.4±29.315**	**170.8±28.870**	**172.8 ± 29.73**	**175.8±28.310**
LOW DOSE	**150.1 ±20.105**	**159.9 ± 20.88**	**161.5±21.706**	**159 ± 20.77**	**165.7 ± 12.83**
HIGH DOSE	**169.5± 28.75**	**181.7 ± 33.87**	**185.8 ± 32.48**	**192 ± 30.93**	**198 ± 26.74**
P value (p)*	**NS**	**NS**	**NS**	**NS**	**NS**

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 1:The mean weight of control and treated groups of wistar albino rat exposed to *Vellai Parapm*.

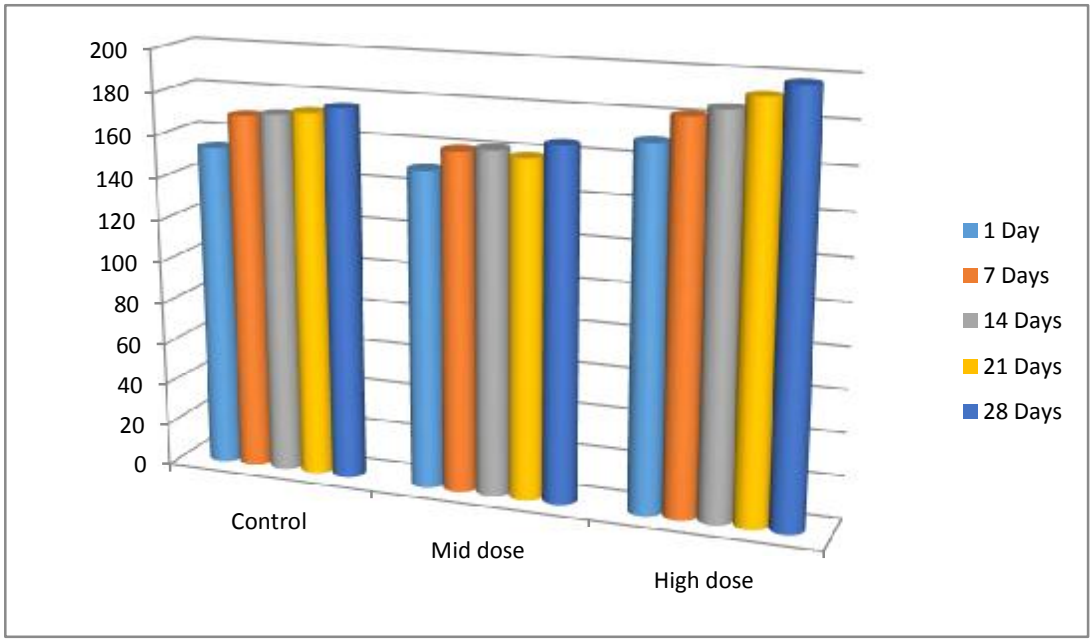


Table 12: Water intake (ml/day) of Wistar albino rats group exposed to *Vellai Parapm*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	**61.5 ± 8.95**	**61.2±6.23**	**58.5±6.23**	**60±8.096**	**61.5±4.56**
MID DOSE	**57.1±3.41**	**58.5±3.62**	**59.8±4.26**	**60.4±4.29**	**58.8±4.13**
HIGH DOSE	**58±2.44**	**58.2±2.14**	**58.9±2.13**	**58.2±2.82**	**58.9±2.96**
P value (p)*	NS	NS	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)

Chart 2: The average water intake of control and treated groups of wistar albino rat exposed to *Vellai Parapm*.

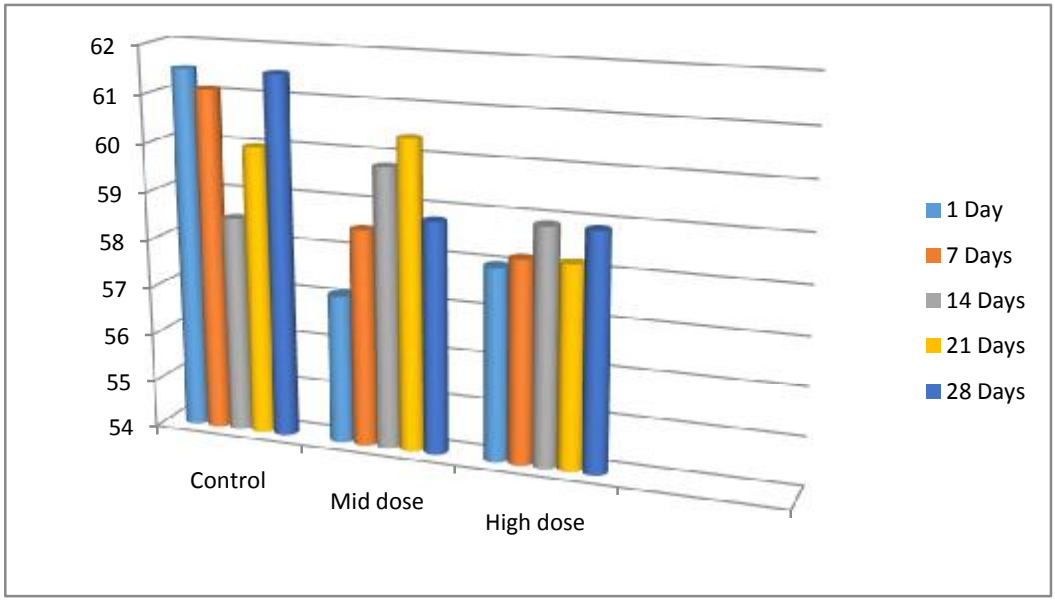


Table 13: Food intake (gms/day) of Wistar albino rats group exposed to *Vellai Parapm.*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	**37±5.37**	**38.5±4.11**	**39.5±4.37**	**38.5±3.37**	**37±4.21**
MID DOSE	**34.7±2.98**	**35.4±2.24**	**35.1±2.28**	**35.2±3.32**	**35.9±2.51**
HIGH DOSE	**40.2±3.45**	**40.3±3.88**	**39.9±3.78**	**40.3±3.40**	**40.1±3.84**
P value (p)*	**NS**	**NS**	**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)

Chart 3: The average food intake of control and treated groups of wistar albino rat exposed to *Vellai Parapm*.

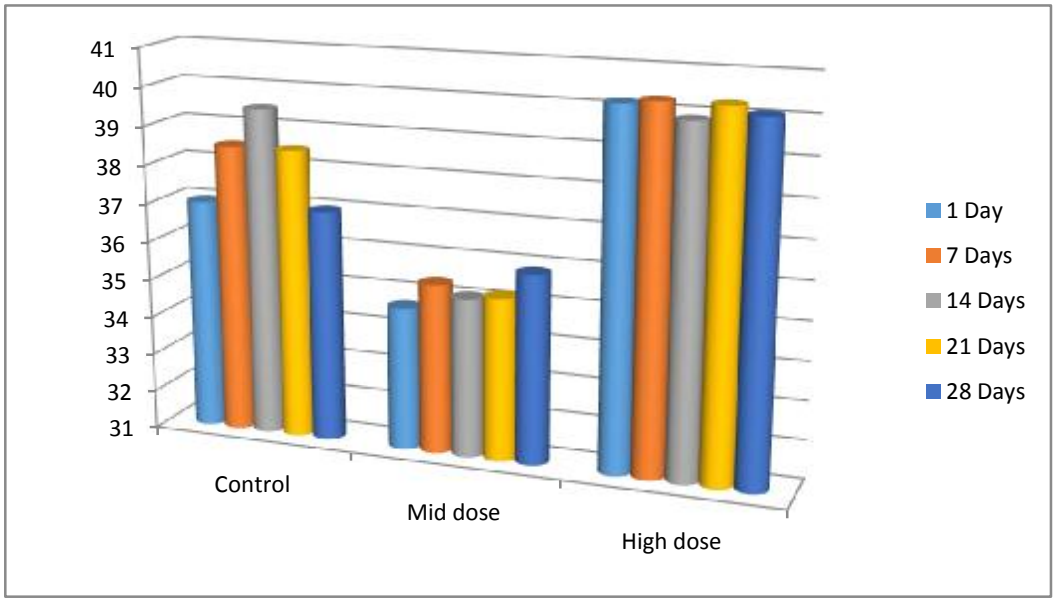


Table 14: Haematological parameters of Wistar albino rats group exposed to *Vellai Parpam*

Haematological parameters	Control	Mid dose	High dose
Total R.B.C. count ($\times 10^6$mm−3).	9.09±0.03	9.12±0.03	9.09±0.3
Total W.B.C. Count ($\times 10^3$ mm−3).	12.37±0.23	12.38±0.16	12.36±0.14
Haemoglobin (Hb) (g/dl)	15.42±0.24	15.45±0.17	15.35±0.17
Hematocrit (%).	42.52±1.52	42.19± 1.76	42.39±1.61
Platelets (×103 mm−3).	830.44±5.93	827.61±14.38	825.93±13.48
Lymphocytes(%).	84.21±0.40	82.96±1.50	82.30±1.01
Neutrophils (%).	20.23±1.35	19.23±0.59	18.83±0.45
PCV	37.61±0.01	37.83±0.05	37.80±0.10
MCV	52.13±0.23	52.36±0.33	52.46±0.33

Data are expressed as mean ± SEM

Chart 4: The mean value of T.WBC of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

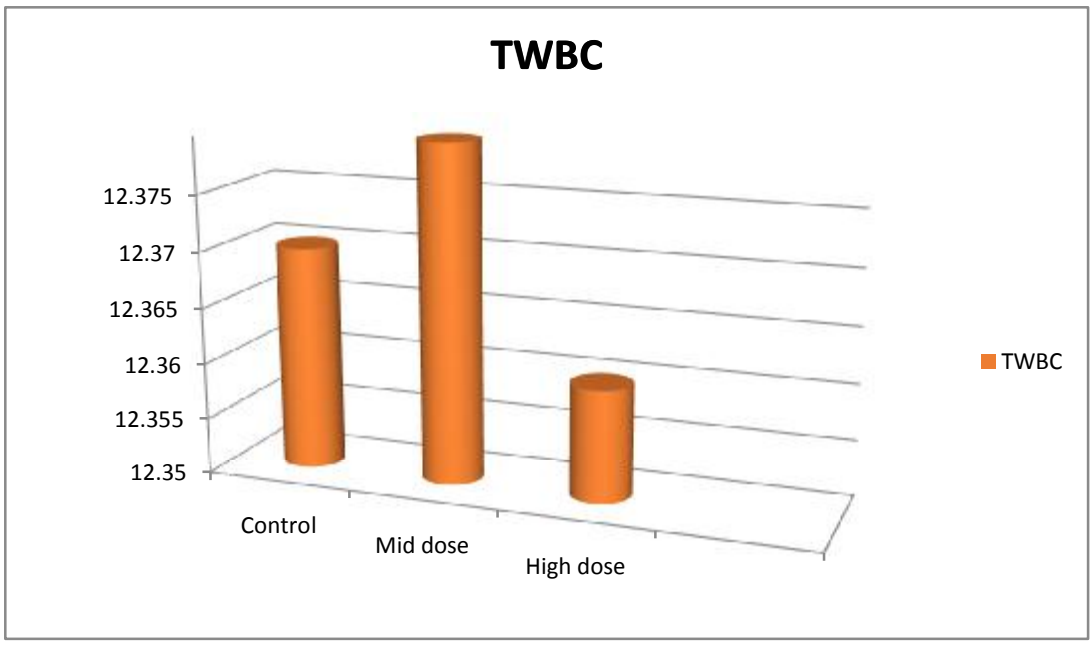


Chart 5: The mean value of Hb and T.RBC of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

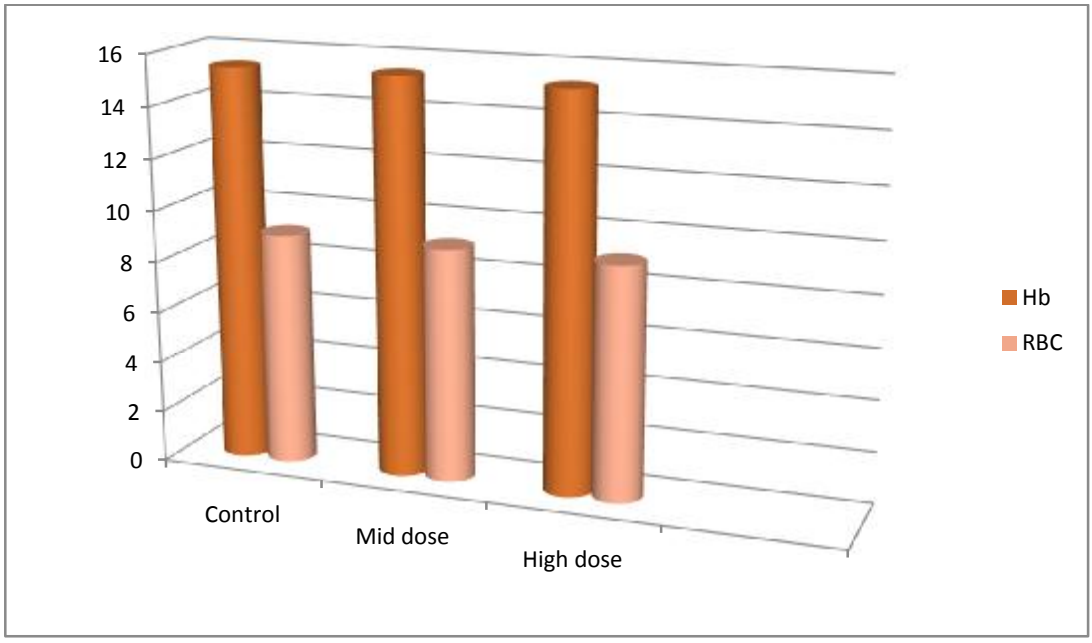


Chart 6: The mean value of Platelet of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

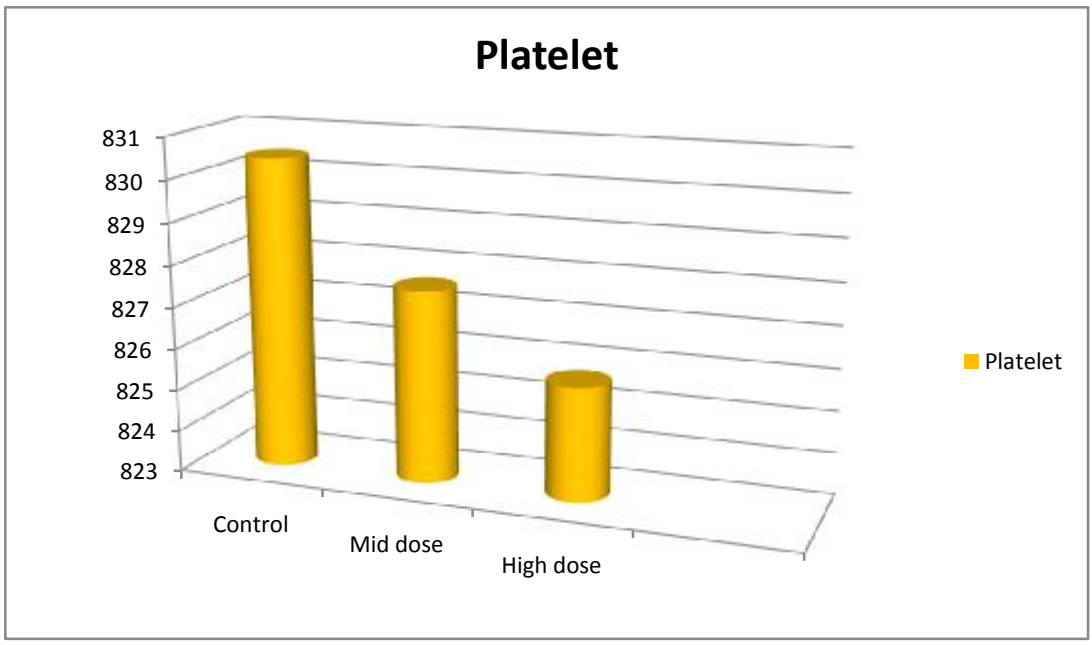


Chart 7: The mean value of Neutrophils and Lymphocytes of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

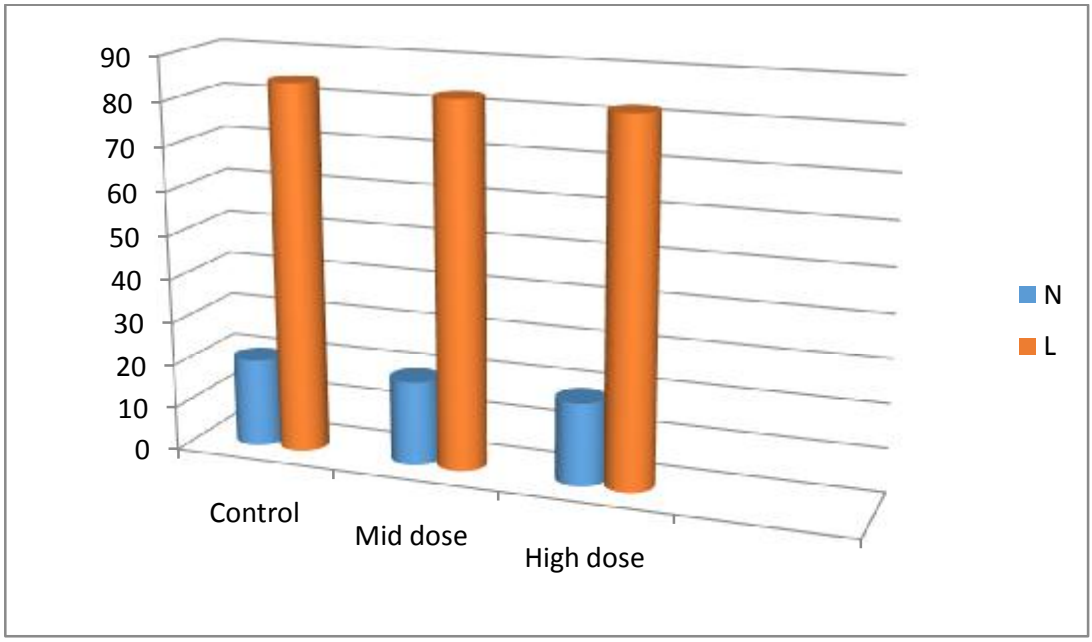


Chart 8: The mean value of Monocytes, Basophils and Eosinophils of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

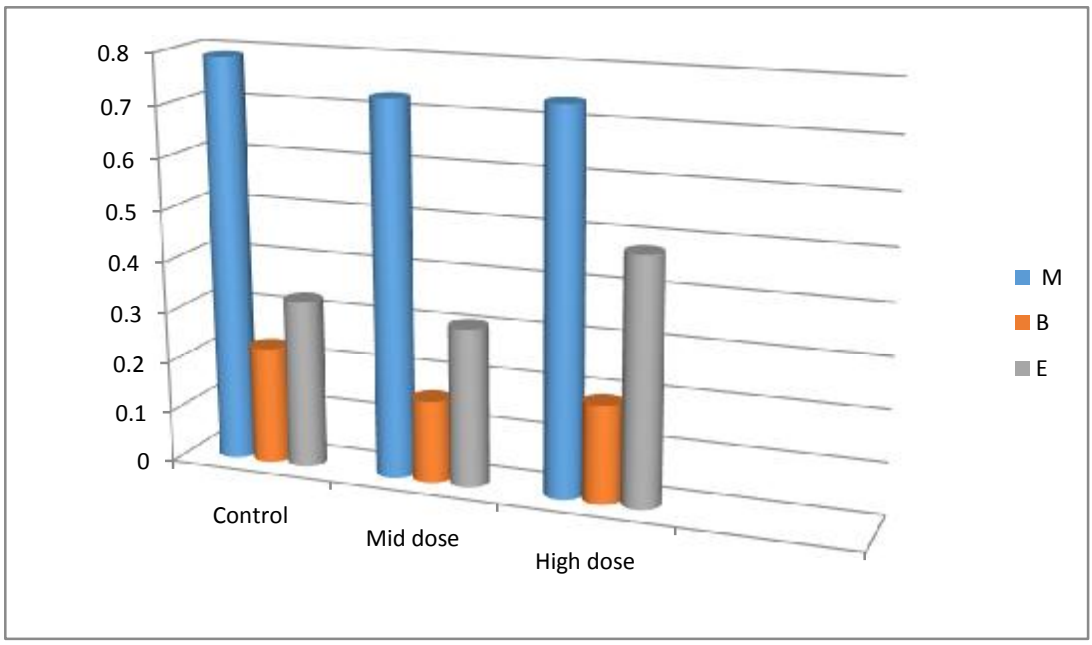


Chart 9: The mean value of PCV, MCHC and MCV of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

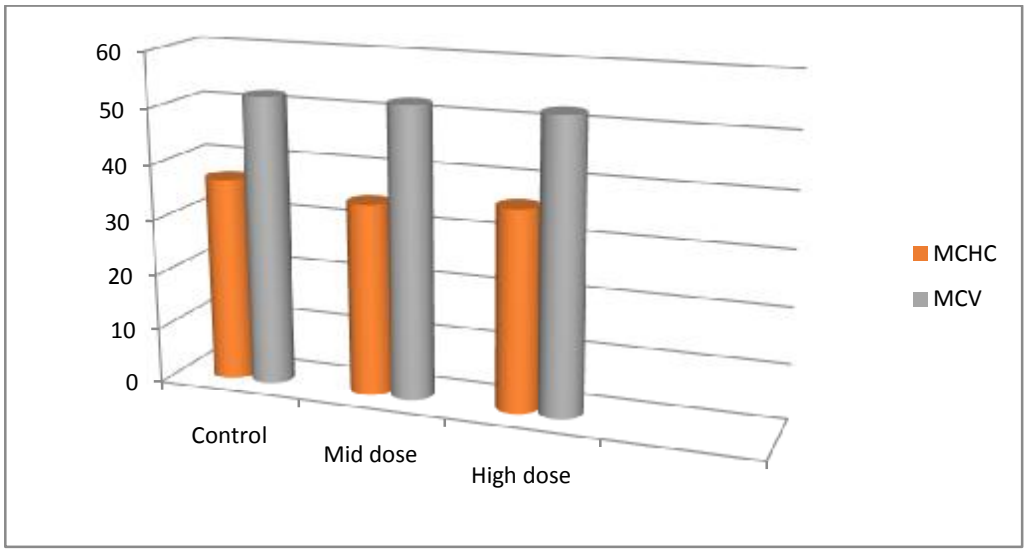


Table 15: Biochemical Parameters of of Wistar albino rats group exposed *Vellai Parpam*

BIOCHEMICAL PARAMETERS	control	Mid dose	High dose	P Value (p)*
GLUCOSE (R) (mg/dl)	110.83±0.98	111.83±0.40	112.06±0.16	N.S
T.CHOLESTROL(mg/dl)	92.45±1.36	92.38±1.68	89.42±4.12	N.S
TGL(mg/dl)	47.31±3.15	45.08±2.23	46.83±2.33	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 10: The mean value of Blood glucose, Total cholesterol and Triglycerides of control and treated groups of wistar albino rats exposed to *vellai parpam*

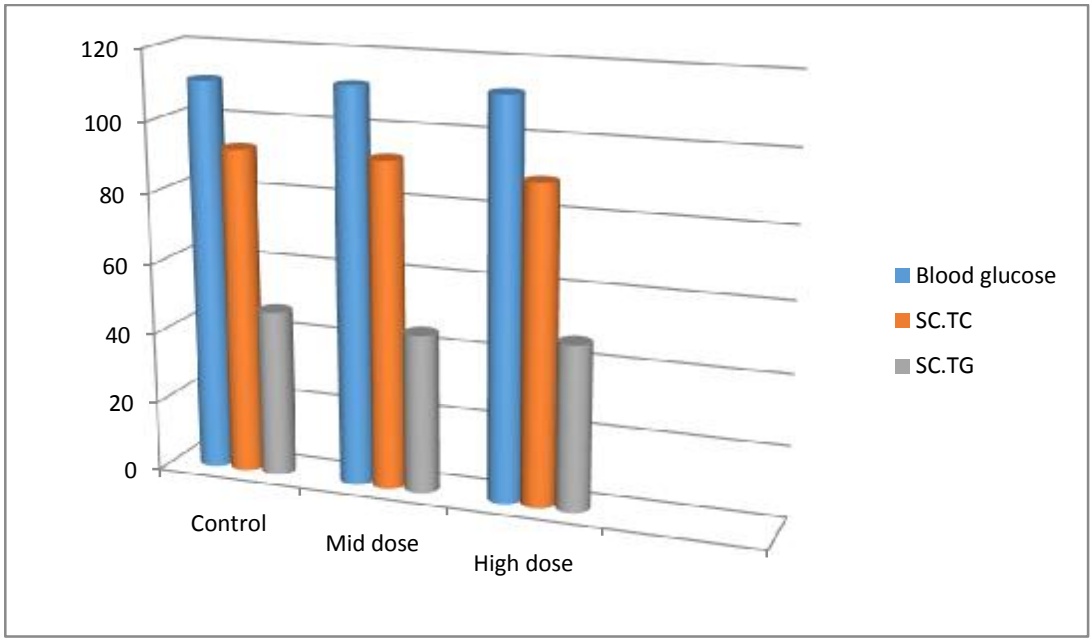


Table 16: Renal function test of of Wistar albino rats group exposed to *Vellai Parpam*

PARAMETERS	CONTROL	Mid dose	High dose	P Value (p)*
UREA (mg/dl)	15.30±0.47	14.00±0.45	13.9±1.258	N.S
CREATININE(mg/dl)	0.5890±0.079	0.63±0.04	0.684±0.11	N.S
URIC ACID (mg/dl)	1.2±0.01	1.2±0.02	1.2±0.02	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 and Uric acid of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

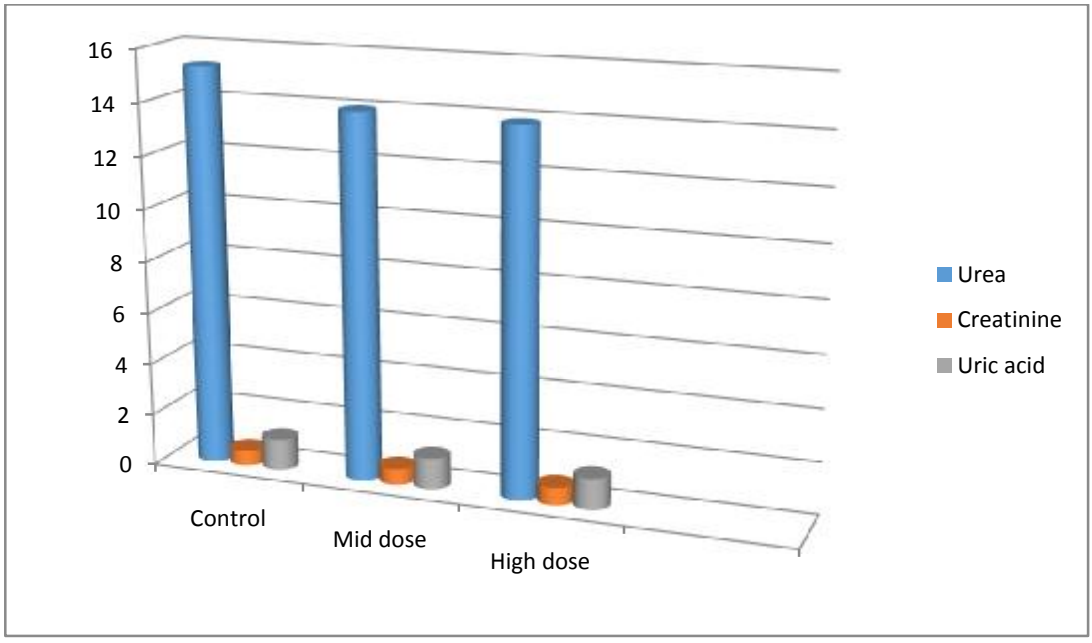


Table 17: Liver Function Test of of Wistar albino rats group exposed to *Vellai Parpam*

PARAMETERS	CONTROL	Mid dose	High dose	P Value (p)*
T.BILIRUBIN(mg/dl)	0.2569±0.32	0.254±0.69	0.254±0.969	N.S
AST(U/dl)	121.41±2.68	119.56±1.33	119.61±3.123	N.S
ALT(IU/dl)	69.40±1.57	69.712±2.121	69.72±1.35	N.S
ALP (IU/L)	112.6±4.67	115.01±1.029	116.41±4.154	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 *Vellai Parpam*

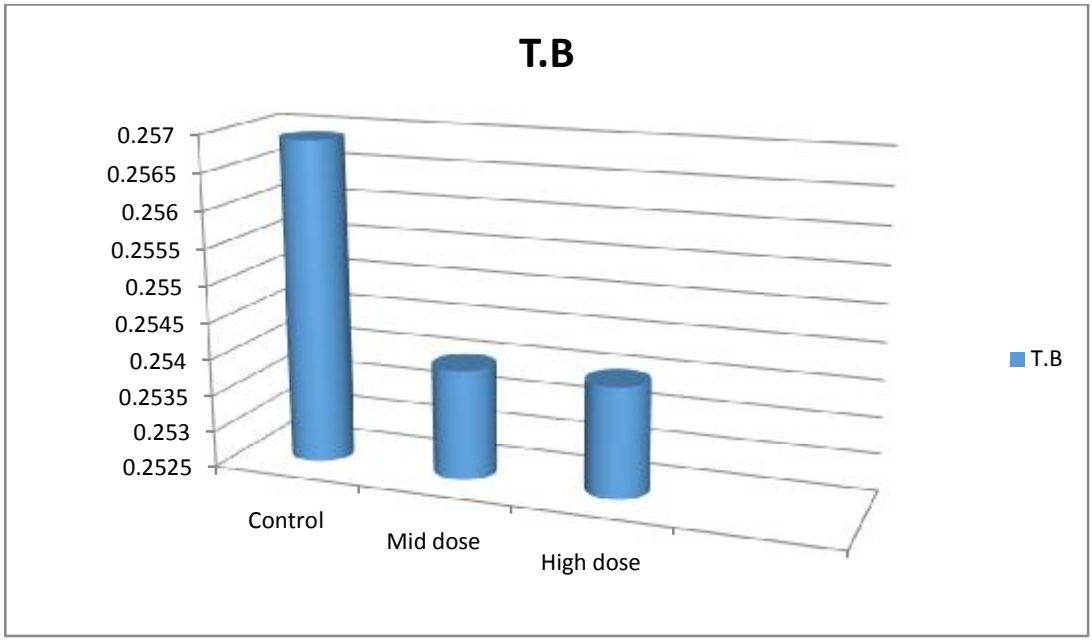
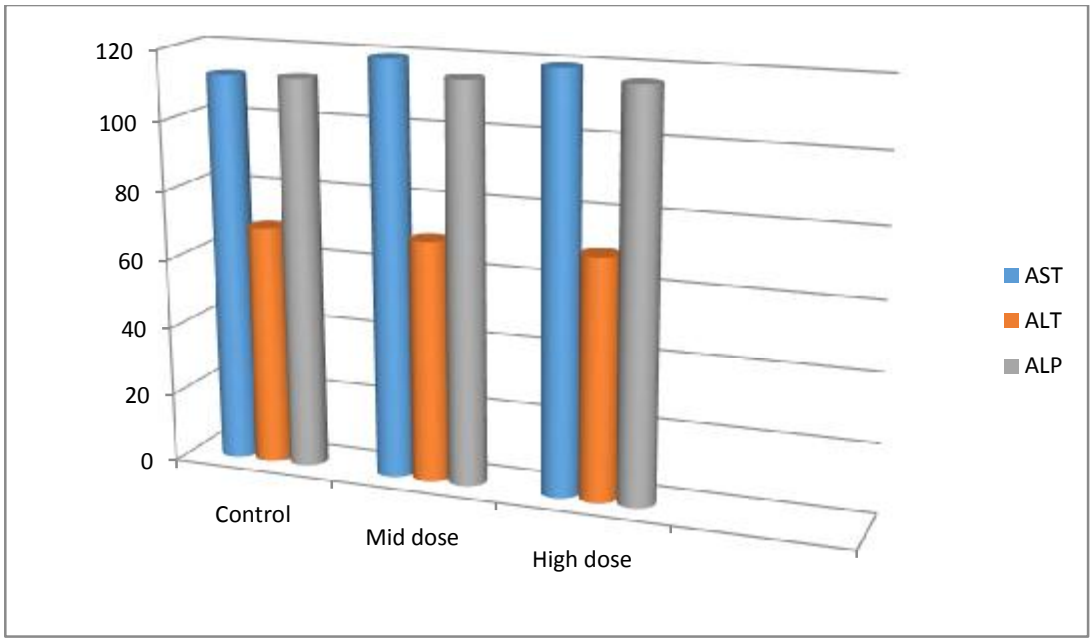


Chart 13: The mean value of AST, ALT and ALP of control and treated groups of wistar albino rats exposed to *Vellai Parpam*



Interpretation of Repeated dose 28 days oral toxicity study:

Sub-acute oral toxicity study repeated dose of ***Vellai Parpam*** 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 is discussed below.

Body weight:

The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited overall 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.

Interpretation of haematological investigation

The haematological investigation results of the rats conducted on 28th 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.

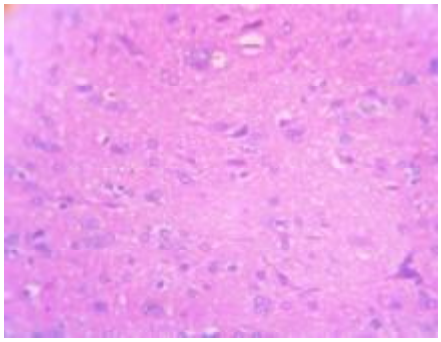
Interpretation of biochemical investigation

The biochemical investigations were conducted on 28^{th} day and the result is produced. The results revealed there is 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.

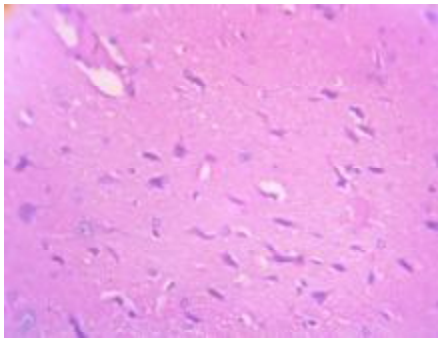
HISTOPATHOLOGICAL EXAMINATION

28-Days Repeated Dose Oral Toxicity Study Of *Vellai Parpam* In Wistar Albino Rats

BRAIN

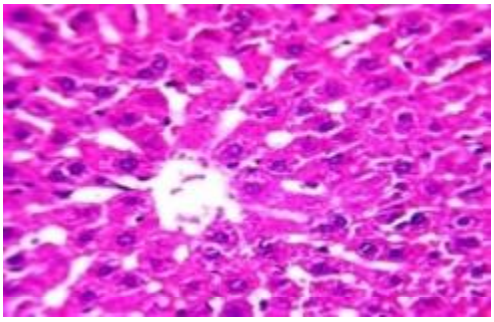


CONTROL

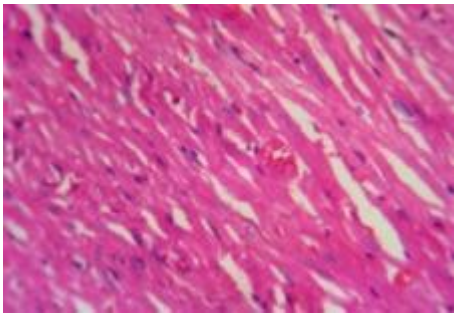


HIGH DOSE

HEART

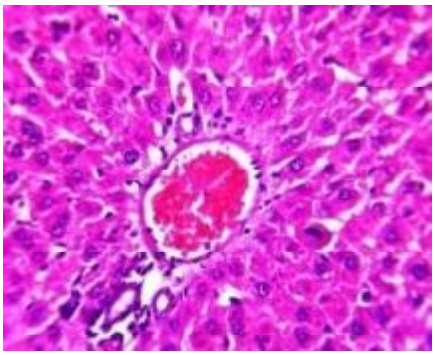


CONTROL

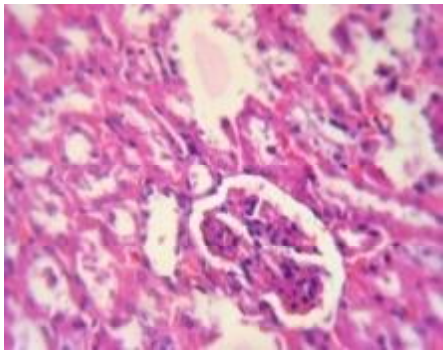


HIGH DOSE

KIDNEY

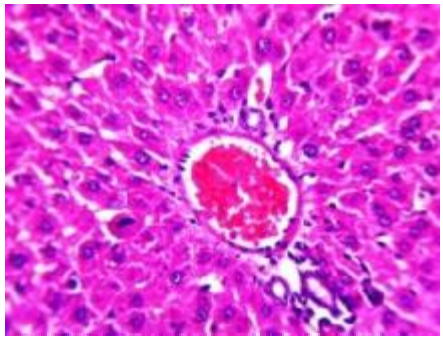


CONTROL

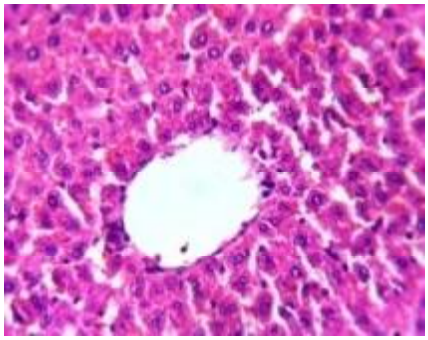


HIGH DOSE

LIVER



CONTROL



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 ***Vellai Parpam*.** Thus the safety of the drug is revealed so that it can be administered for long time without any side effects.

REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY OF *VELLAI PARPAM* IN WISTAR ALBINO RATS.

Table 18: Sub chronic toxicity study - Body weight of wistar albino rats group exposed to *Vellai Parpam*

DAYS	Weight(gms)/Days				P value (p)*
	Control	Low dose	Mid dose	High dose	
1	162.4±29.65	154.4 ± 21.83	155.6± 13.09	161.5± 28.17	NS
15	170.1 ± 28.49	162.8 ± 23.54	171.7 ± 29.83	179.8 ± 32.13	NS
30	184.4 ± 28.83	184.71 ± 14.76	182.8 ± 32.17	190.2 ± 28.55	NS
45	202.7± 27.81	205.6± 29.12	203.1± 19.07	207.9± 22.05	NS
60	226.6±33.47	232.6±23.91	230.6±33.61	226.6±23.63	NS
75	240.8 ± 26.76	244.8 ± 28.08	242.0± 28.70	240.8 ±26.87	NS
90	263.5± 27.94	266.9± 27.68	268.2± 27.31	266.1± 29.41	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 19: Haematological parameters of Wistar albino rats group exposed to *Vellai Parapm*.

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Total RBC cells/cu.mm)	7.27±0.25	7.25±0.34	7.22±0.46	7.22±0.25	N.S
Total WBC (cells/cu.mm)	11316.67±29	11033.33±51	11050±70	11366.67±87	N.S
Platelets cells/ul	3.33±0.19	3.52±0.33	3.78±0.35	3.45±0.65	N.S
Hb g/dl	12.75±0.33	12.56±0.30	13.51±0.78	12.81±1.30	N.S
PCV%	38.27±1.0	37.69±0.91	39.9±2.3	38.46±0.08	N.S
MCV fl	0.17±0.04	0.34±0.09	0.48±0.12	0.28±2.8	N.S
MCH pg	32.50±1.37	33.00±1.6	30.33±2.2	30.17±3.2	N.S
MCHC %	32.50±1.37	36.50±4.76	34.33±2.58	33.50±1.87	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 T.WBC of control and treated groups of wistar albino rats exposed to *Vellai Parapm*

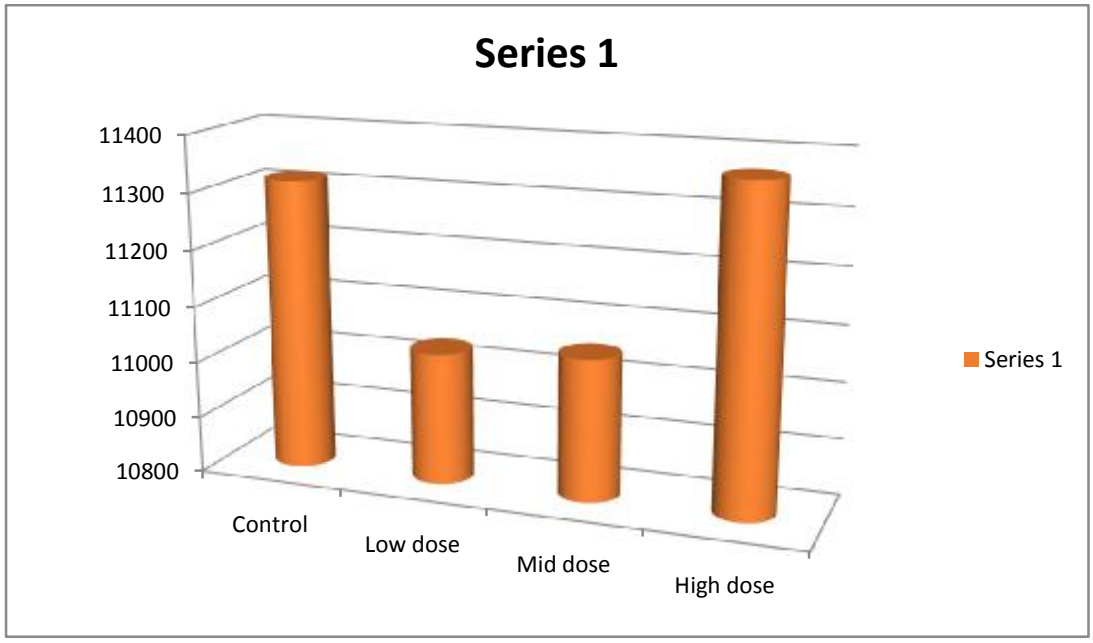


Chart 15: The mean value of Hb and T.RBC of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

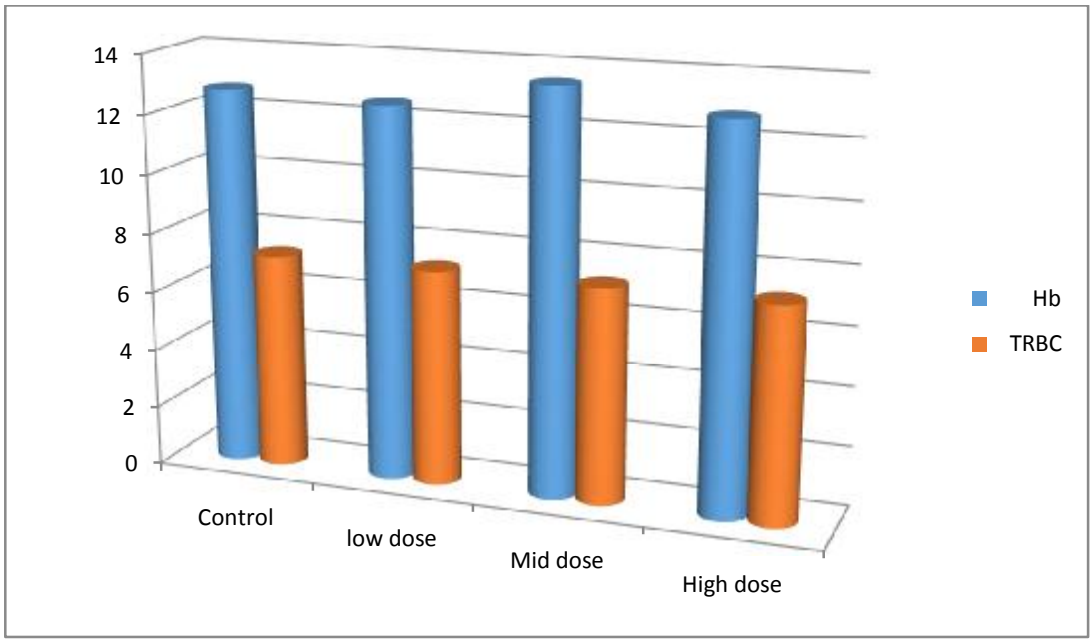


Chart 16:The mean value of Platelet of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

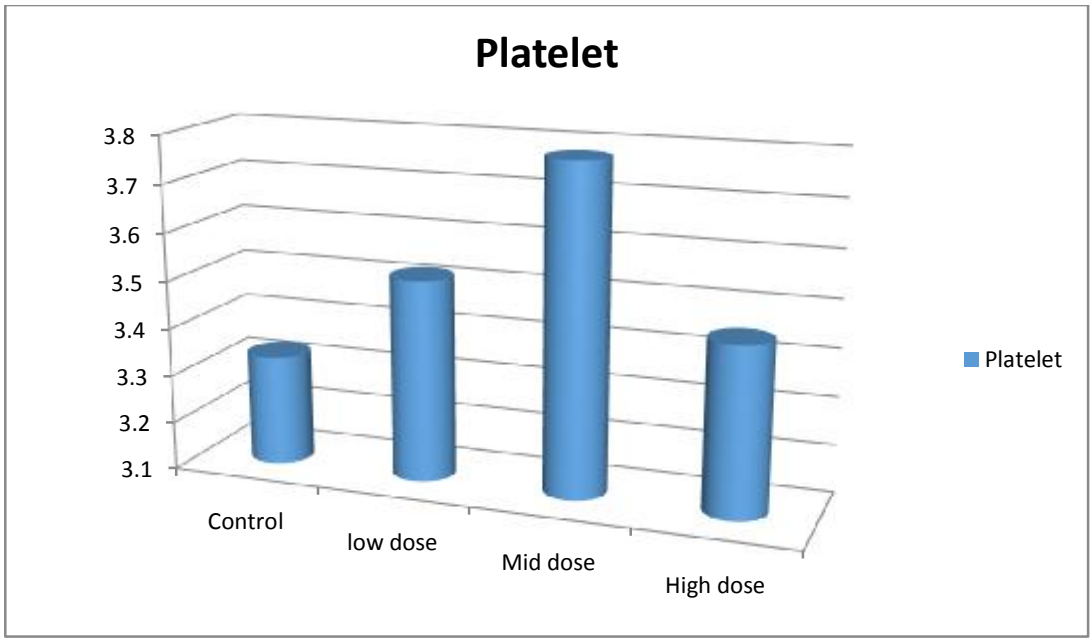


Chart 17: The mean value of PCV, MCH, MCHC, MCV of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

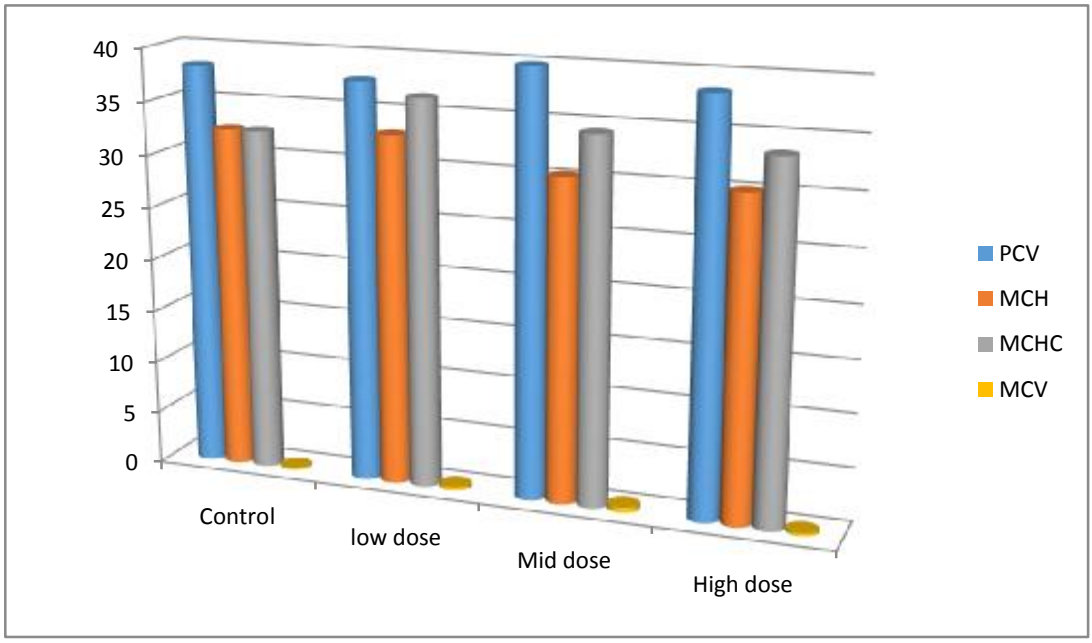


Table 20: Biochemical Parameters of of Wistar albino rats group exposed to *Vellai Parpam.*

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
GLUCOSE (R) (mg/dl)	121.67±5.6	122.37±10.1	121.33±10.8	120.97±11.8	N.S
T.CHOLOSTEROL(mg/dl)	105.17±11.89	120.00±4.73	110.50±6.50	119.67±8.52	N.S
HDL(mg/dl)	43.50±3.33	40.83±5.34	44.67±2.73	40.50±1.64	N.S
LDL(mg/dl)	42±10.7	52.17±10.8	38.67±5.95	42±11.3	N.S
VLDL(mg/dl)	29.60±1.85	26.93±3.77	27.20±1.67	23.30±2.32	N.S
TRIGLY(mg/dl)	148.00±9.25	134.67±18.85	136±8.36	116.50±11.6	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 18:The mean value of Total cholesterol and Triglycerides of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

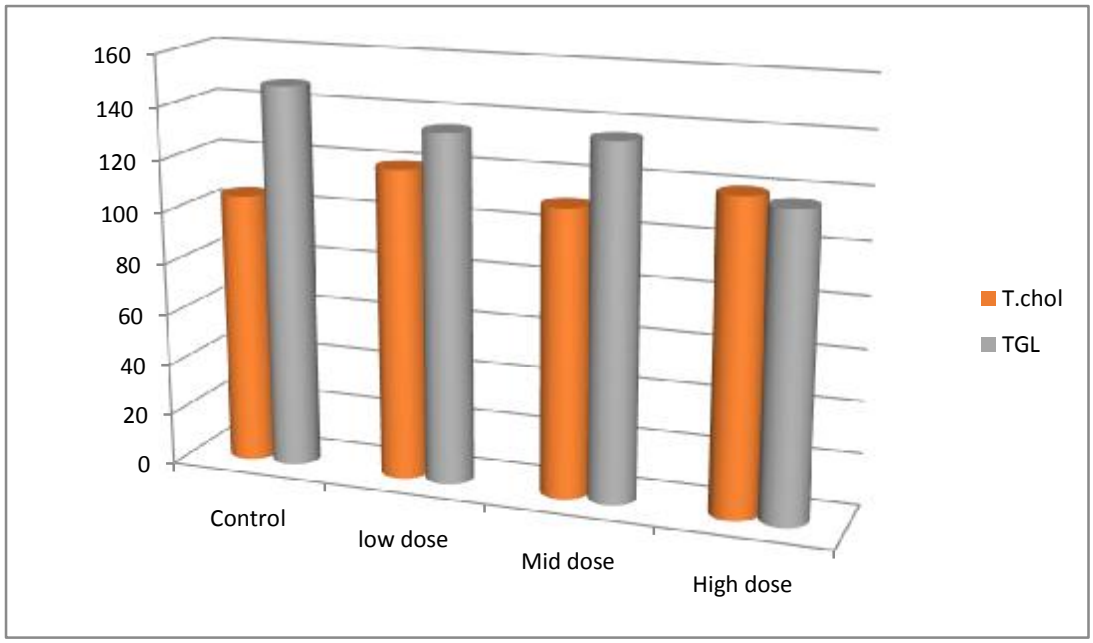


Chart 19: The mean value of Blood sugar of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

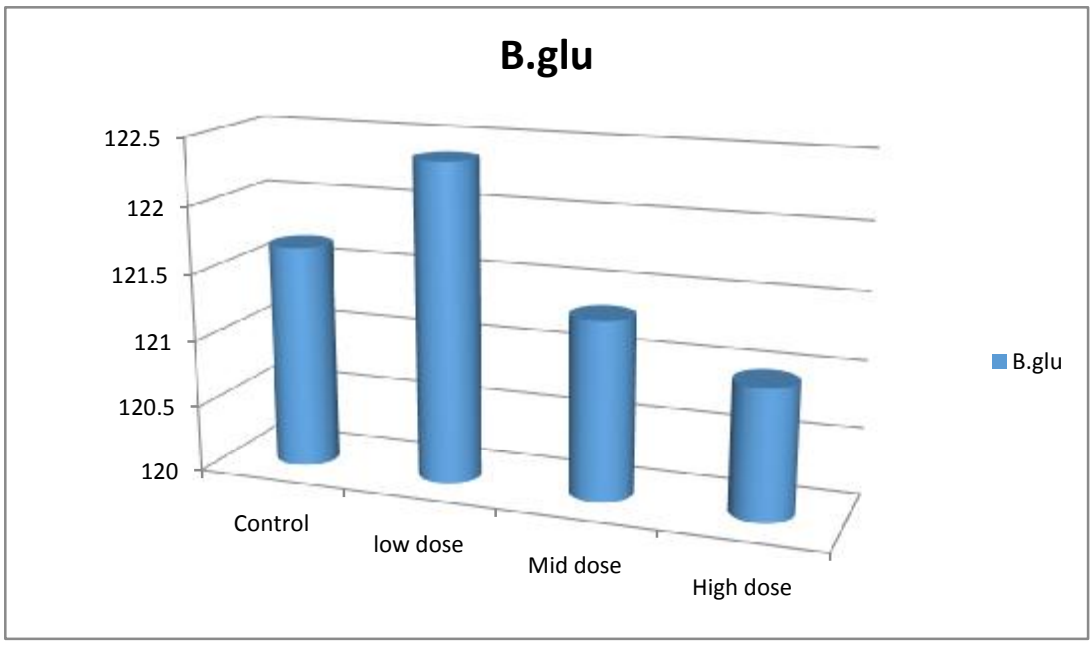


Chart 20: The mean value of HDL, LDL, VLDL of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

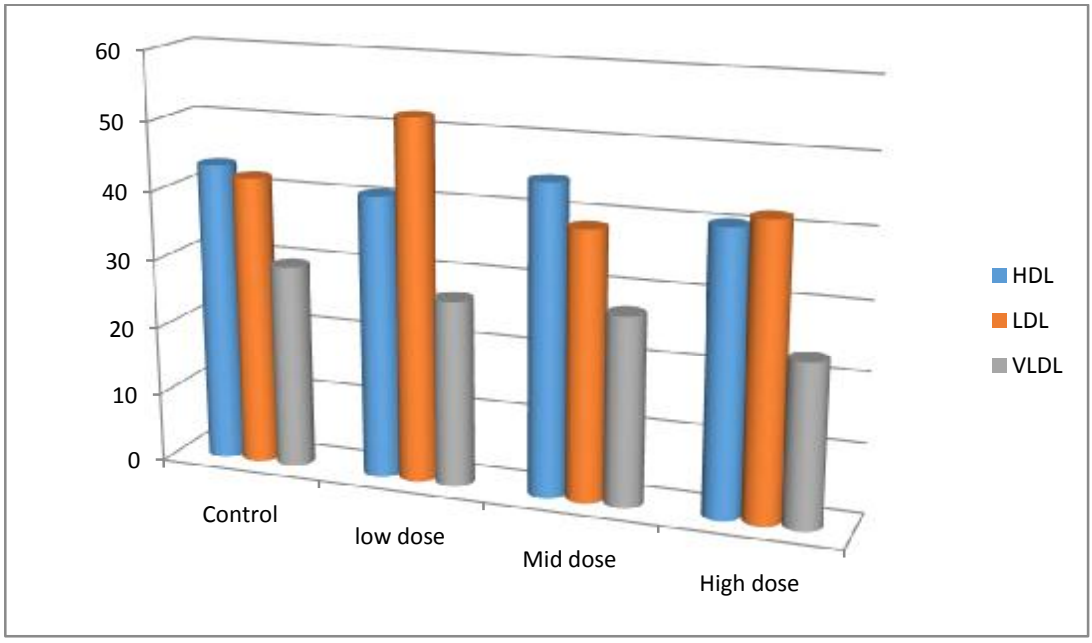


Table 21: Renal function test of of Wistar albino rats group exposed to *Vellai Parpam*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
UREA (mg/dl)	28.33±5.2	31.50±4.23	29.17±4.30	31.67±5.82	N.S
CREATININE(mg/dl)	0.567±0.13	0.517±0.172	0.550±0.137	0.483±0.116	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 Urea and Creatinine of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

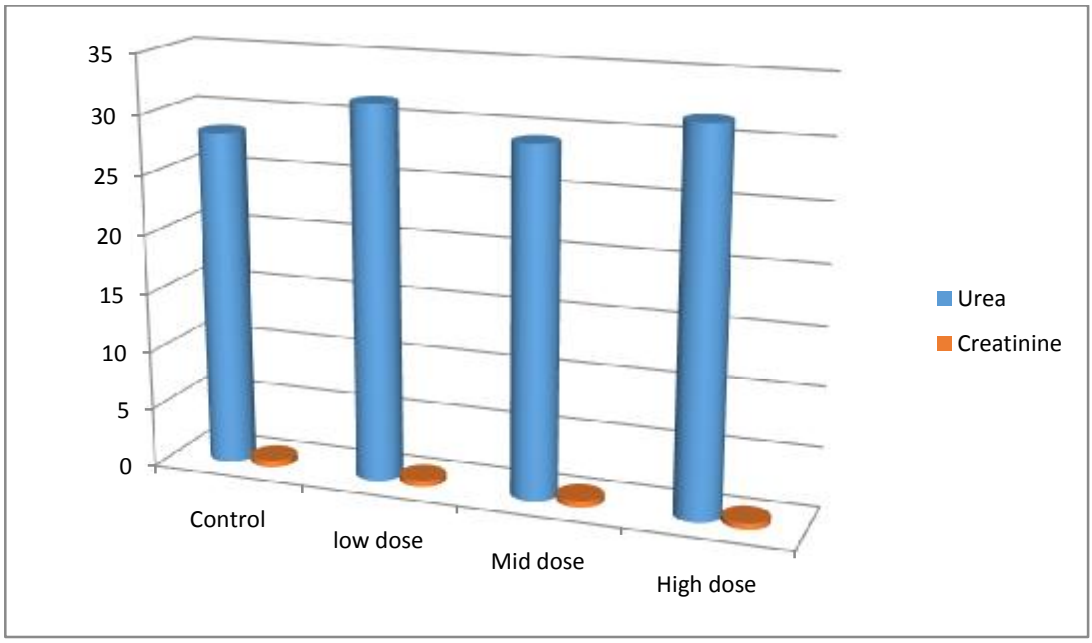
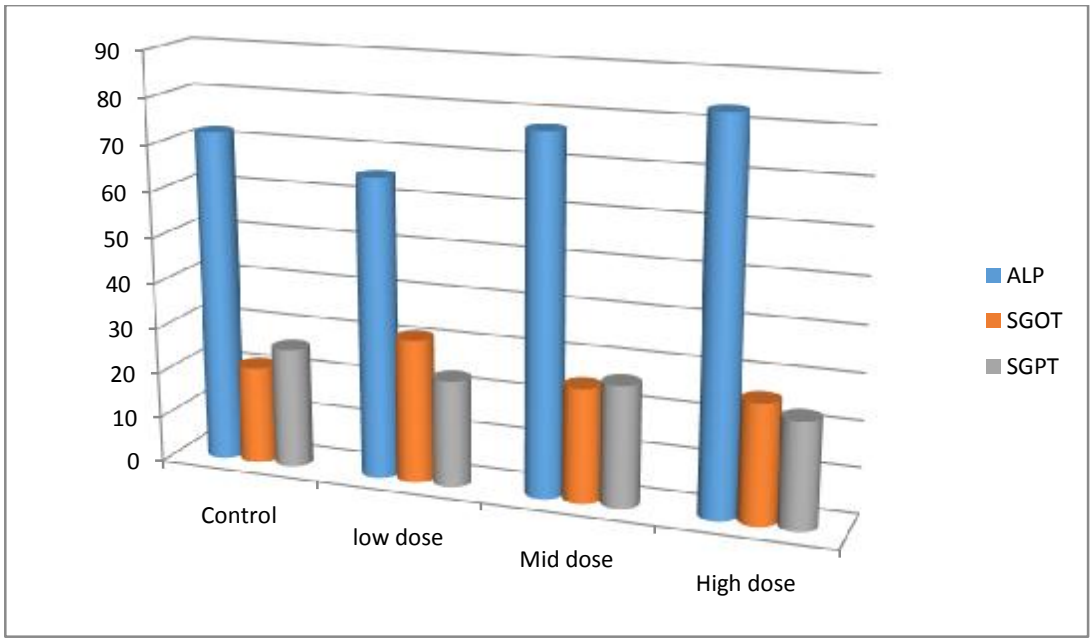


Table 22: Liver Function Test of Wistar albino rats group exposed to *Vellai Parpam*

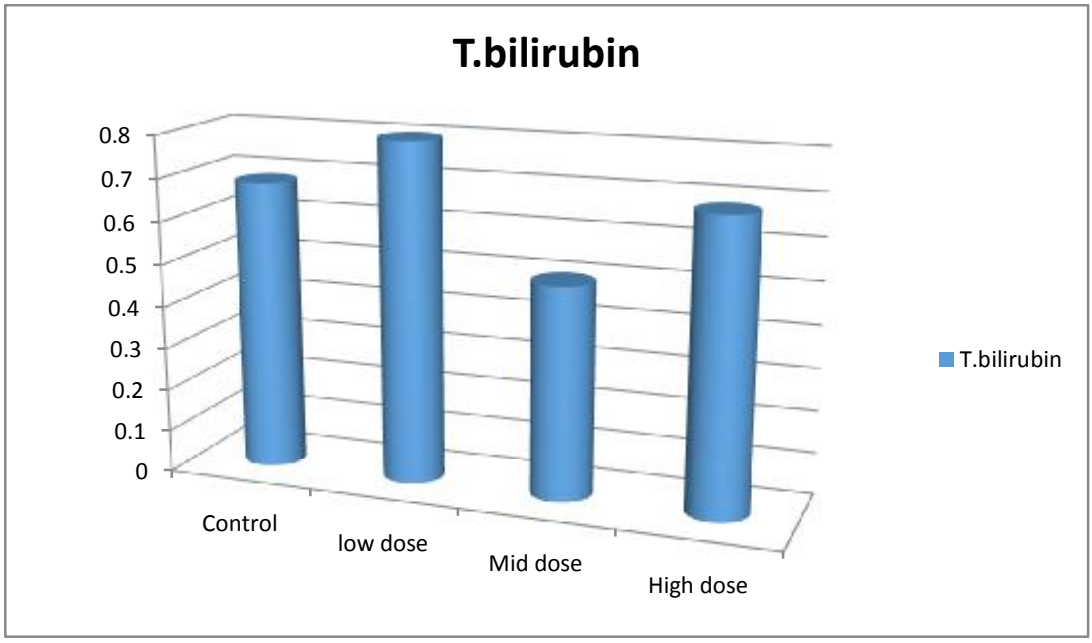
PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
T.BILIRUBIN(mg/dl)	0.683±0.14	0.800±0.08	0.500±0.632	0.683±0.147	N.S
SGOT(U/dl)	21.33±5.12	31.33±3.20	24.83±3.48	26.00±4.38	N.S
SGPT(U/dl)	26.33±7.09	23.33±3.20	26.33±3.77	23.17±4.62	N.S
ALP(U/dl)	72.33±21.3	65.33±16.56	77.33±19.0	83.83±19.77	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)

22: The mean value of SGOT, SGPT, ALP of control and treated groups of wistar albino rats exposed to *Vellai Parpam*



23: The mean value of T.bilirubin of control and treated groups of wistar albino rats exposed to *Vellai Parpam*



Interpretation of repeated dose 90 days oral toxicity study:

Repeated dose 90 days oral toxicity study of ***Vellai Parpam*** on rats were conducted. All animals from the treated dose survived throughout the dosing period of 90 days. Various parameters were studied and the interpretation of the study result is discussed below.

Body weight:

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

Interpretation of haematological investigation

The haematological investigation results of the rats conducted on 90th 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.

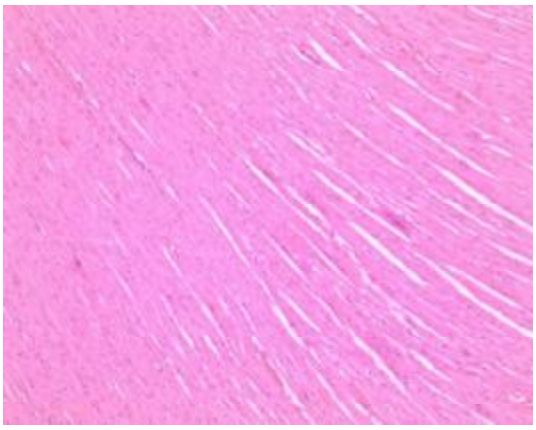
Interpretation of biochemical investigation

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

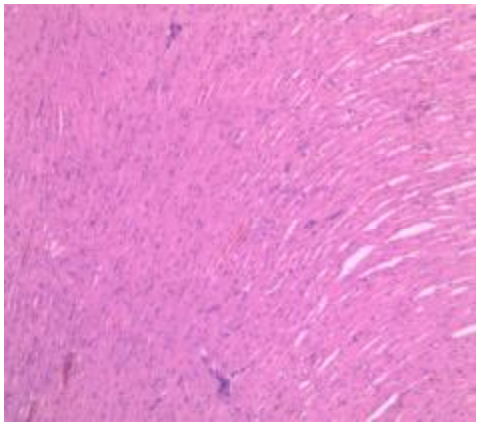
HISTOPATHOLOGICAL EXAMINATION

Repeated Dose 90-Days Oral Toxicity Study Of *Vellai Parpam* In Wistar Albino Rats showing histopathologic slides of sub-chronic toxicity of *Vellai Parpam*

HEART

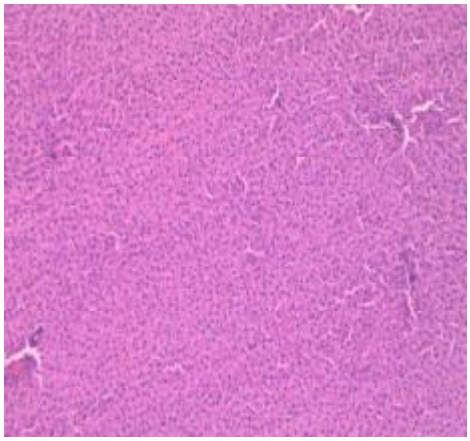


CONTROL

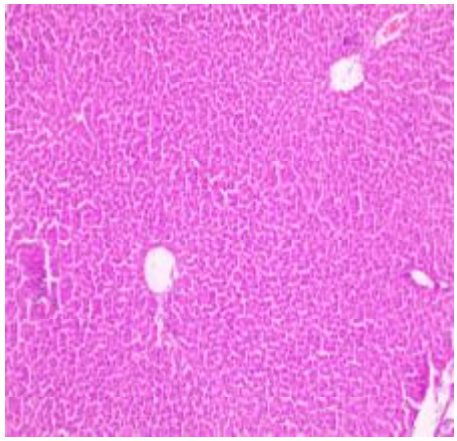


HIGH DOSE

LIVER



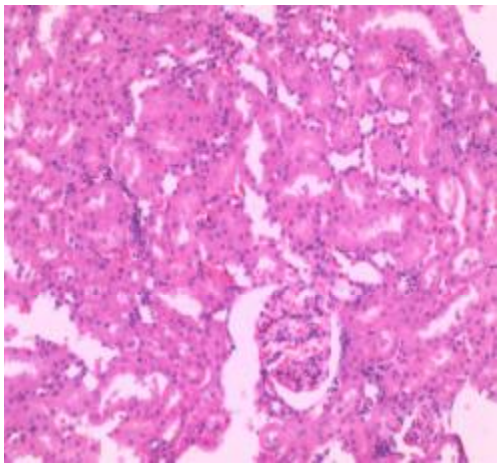
CONTROL

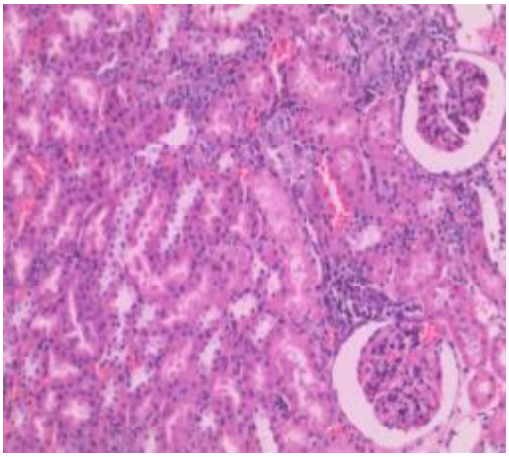


HIGH DOSE

showing histopathologic slides of repeated dose 90 days oral toxicity of *Vellai Parpam*

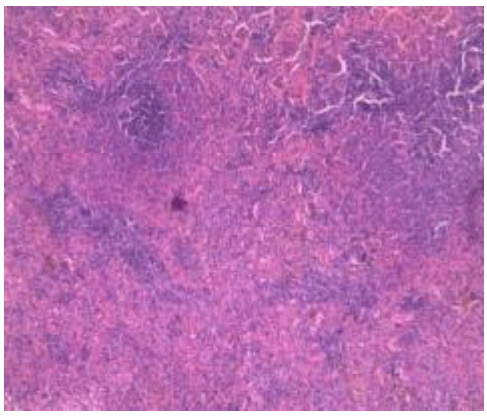
KIDNEY

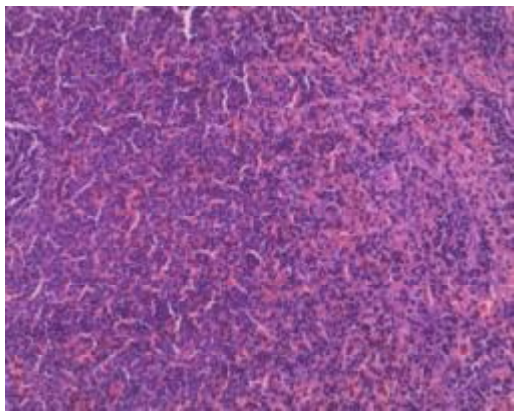




CONTROL **HIGH DOSE**

SPLEEN





CONTROL **HIGH DOSE**

Interpretation:

Kidney

- Renal sections of both the samples exhibited normal histology with distinct cytoarchitecture in both the cortex and medulla
- Appearance of glomeruli, tubules, interstitium and lumen was normal in both the samples with no signs of degeneration

Heart

- Regular arrangement of myocardial fibers with clear transverse striation and normal structure were observed.
- Appearance of cardiomyocyte was normal with dark nuclear region. The nuclei of muscle fibers appear central arrangement.

Liver

- Appearance of portal vein, bile duct and hepatic artery was normal
- Hepatocellular architecture, including hepatic sinusoid and hepatic cord was normal

Spleen

- Lymphoid follicles appears normal
- Marginal sinus (MS) of the rat and its sinus lining cells appears normal
- Erythropoietic cells (EP) are scattered throughout the red pulp of both the samples.

PHARMACOLOGICAL RESULTS

1. Urolithiatic activity

Table 23: Effect of *Vellai Parpam* on ethylene glycol induced urolithiatic activity in rats

Biochemical Assays	Gr-I	Gr-II	Gr-III	Gr-IV
Calcium	0.82±0.07	2.66±0.15[a]	1.60±0.14[b] ***	1.14±0.08 b ***
Oxalate	0.62±0.08	4.50±0.64 [a]	2.70±0.50 [b] *	2.05±0.06 [b] **
Uric acid	0.82±0.04	2.75±0.30 [a]	2.20±0.37 [a]*	1.42±0.25 [b] *
Citrate	2.42±0.20	0.76±0.05 [a]	1.27±0.14 [a]***	0.97±0.08[a]***

Calcium, oxalate, citrate, uric acid, mg/24 h urine. Superscript letters represent P <0.05 (Tukey's test). [a] As compared with group I, [b] As compared with group II.*P<0.05, **P <0.01, ***P<0.001.

Chart 24: Effect of *Vellai Parpam* on ethylene glycol induced urolithiatic activity in rats

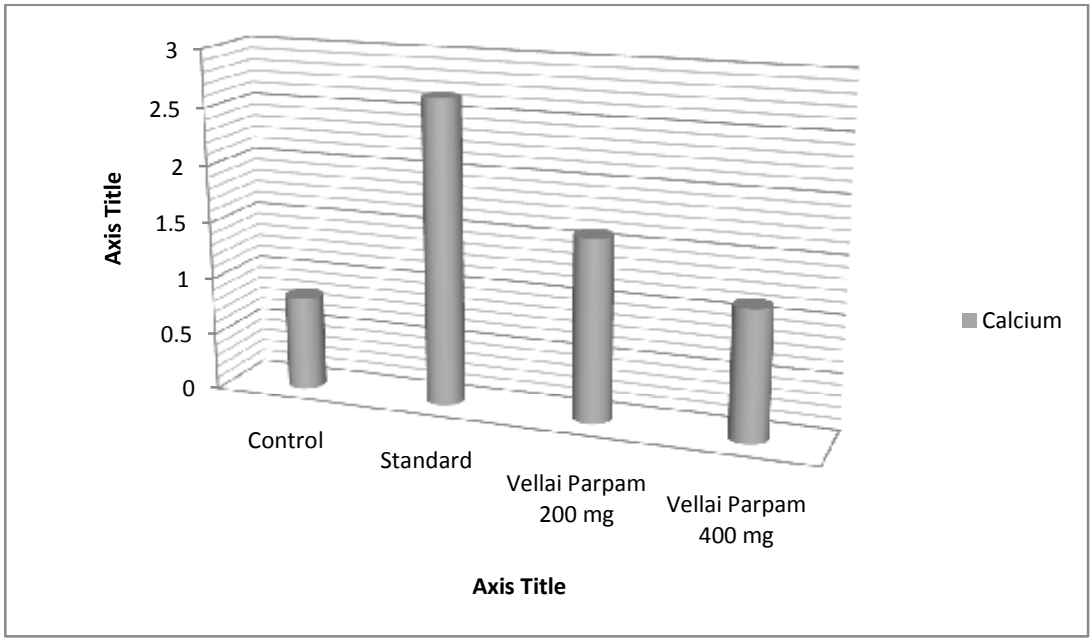


Chart 25: Effect of *Vellai Parpam* on ethylene glycol induced urolithiatic activity in rats

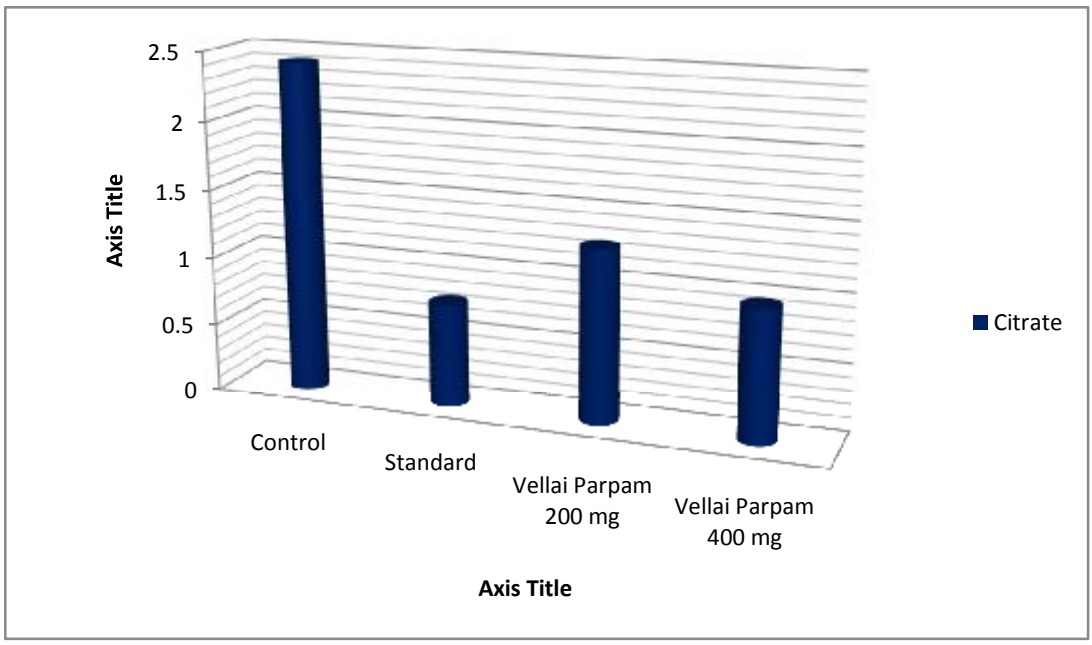


Chart 26: Effect of *Vellai Parpam* on ethylene glycol induced urolithiatic activity in rats

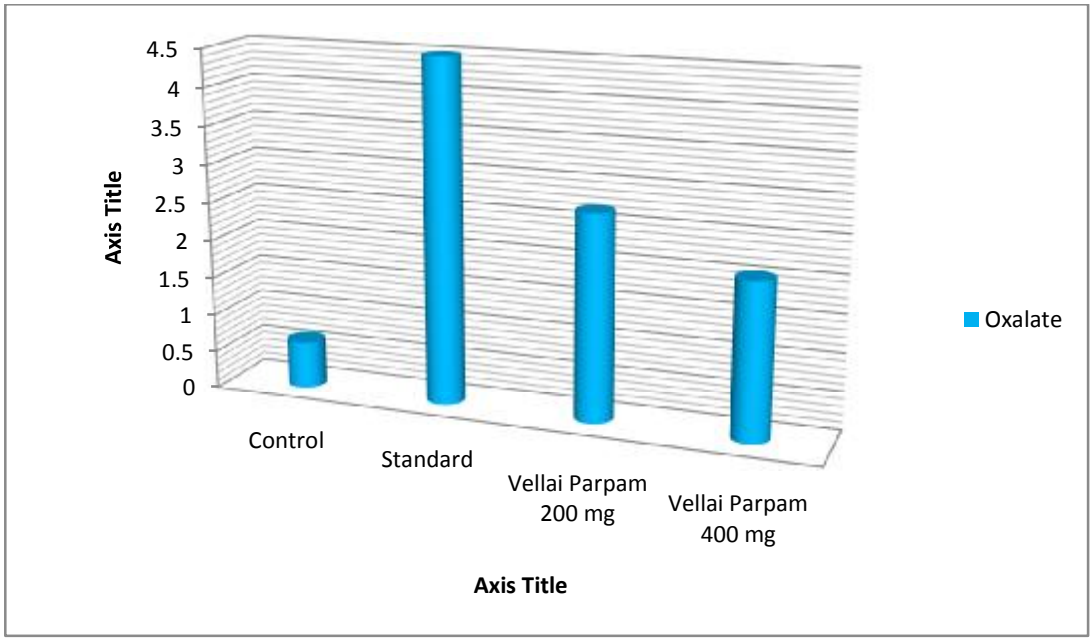
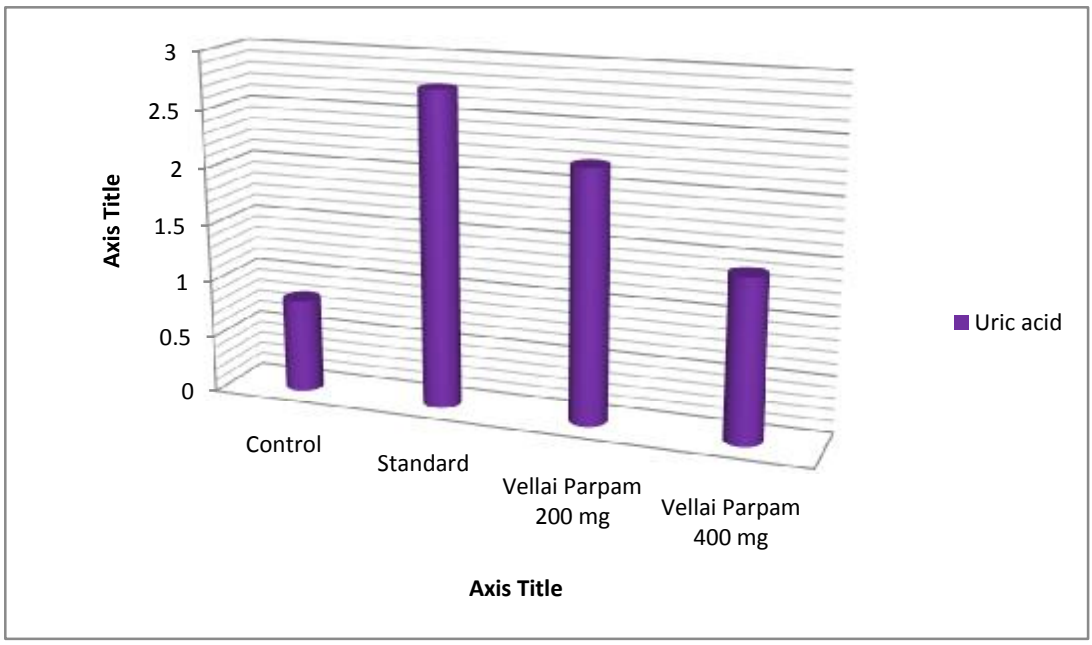


Chart 27: Effect of *Vellai Parpam* on ethylene glycol induced urolithiatic activity in rats



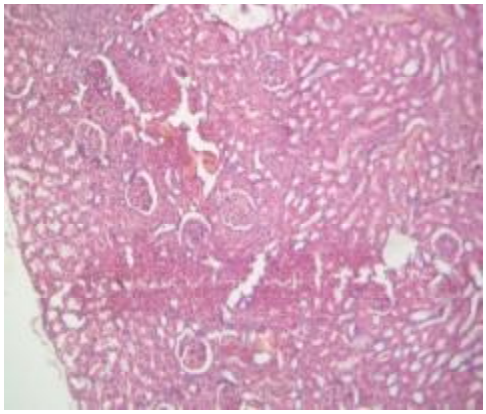
Histopathology:

GroupI- Normal saline

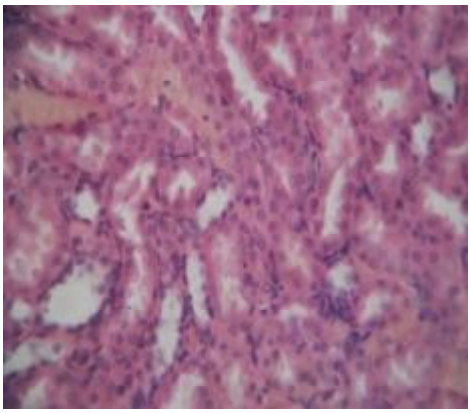
Group II- Ethylene glycol

Group III- Vellai Parpam 200 mg/kg

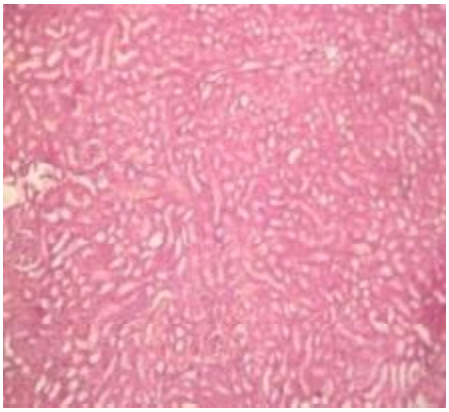
Group IV- Vellai Parpam 400 mg/kg



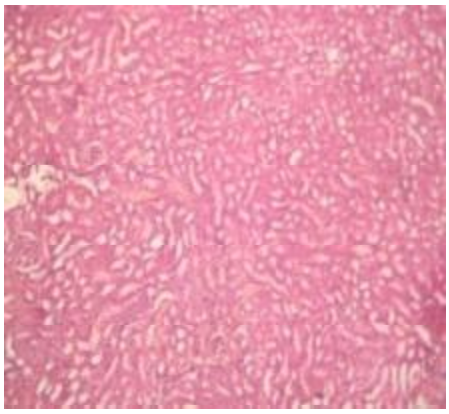
CONTROL



ETHYLENE GLYCOL



VELLAI PARPAM 200 mg



VELLAI PARPAM 400 mg

Interpretation:

The trail drug *vellai parpam* significantly increased the excretion of calcium, oxalate, citrate, uric acid in urinary output of rats compared with the standard drug cystone at the dose level of 200 mg and 400 mg/ Kg B.W. The drug acts more potent at the high dose 400mg/Kg than the low dose level of 200 mg/Kg. The histopathological results of the trail drug were also confirmed that there is excretion of stone in ethylene glycol induced rats. Thus the drug *vellai parpam* exhibits the anti-urolithiatic activity.

2. Diuretic Activity (Lipschitz Method)

Table 24: Estimation Of Urinary Electrolytes

Groups	Total Urine Vol (ml/kg/5hrs)	Na+ mmol/L	K+ mmol/L	Cl- mmol/L
Control (10ml/kg)	13.73± 0.37	102.0± 2.30	45.67± 2.02	72.33± 2.60
Furosemide (10mg/kg)	23.60± 0.83 ***	192.0 ± 2.30 ***	78.67± 1.45***	112.7± 5.81 ***
Vellai Parpam (200mg/kg)	14.23 ± 0.29 ###	119.3 ± 4.05 ###	52.00± 2.30###	76.67± 2.40 ###
Vellai Parpam (400 mg/kg)	15.60 ± 0.03 ###	138.0 ± 2.30 ###	60.00± 1.15 ###	86.67± 2.90 ##

Values are Mean ± SEM; n = 6 animals in each group: * P<0.05, ** P< 0.01, *** P<0.001 is considered significant when compared with group I; # P<0.05, ## P< 0.01, ###P<0.001 is considered significant when compared with group II by Tukey multiple comparison test.

Chart 28 : Estimation of Urinary electrolytes

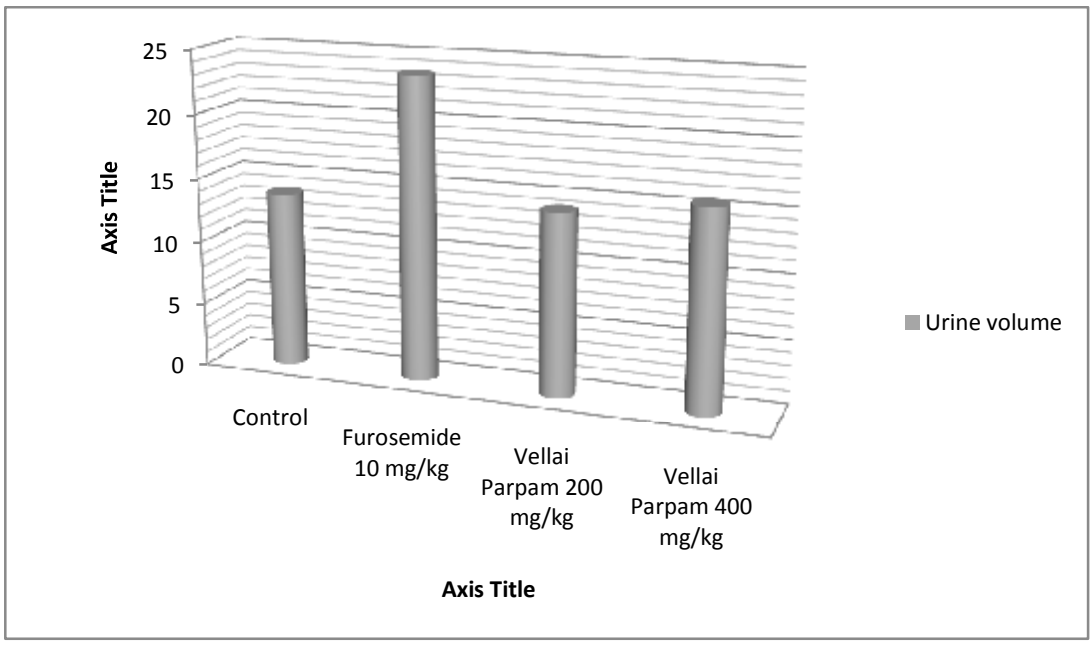


Chart 29: Estimation Of Urinary Electrolytes

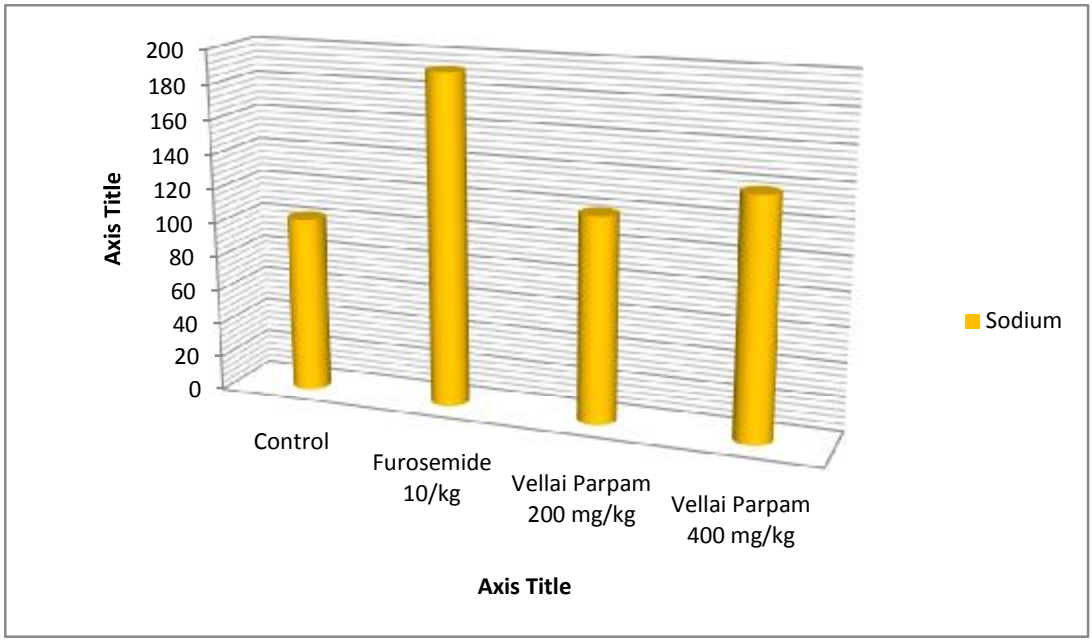


Chart 30: Estimation Of Urinary Electrolytes

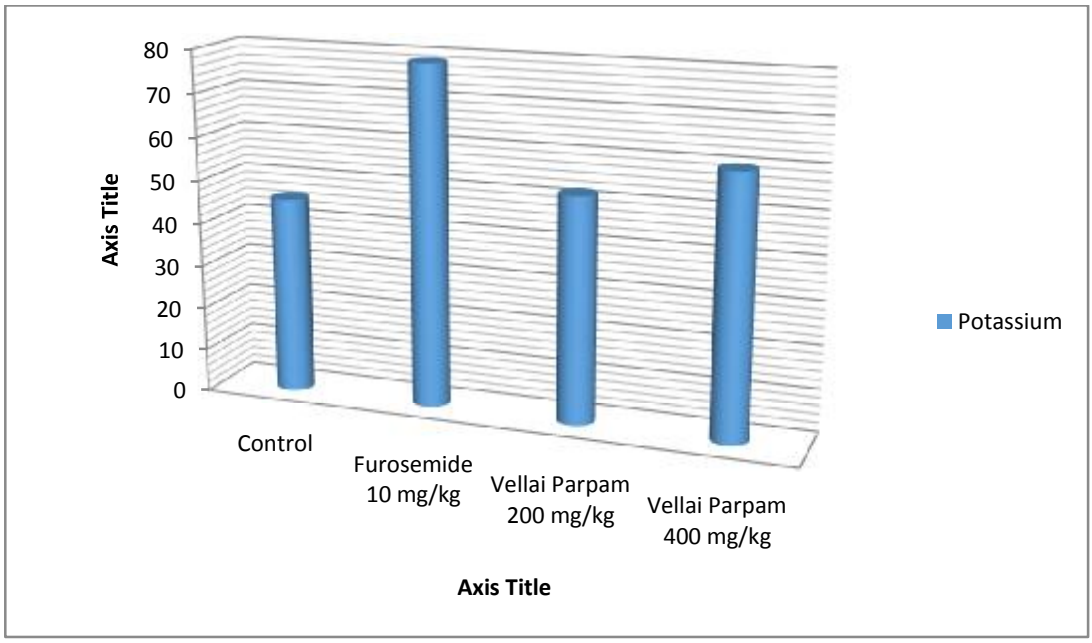
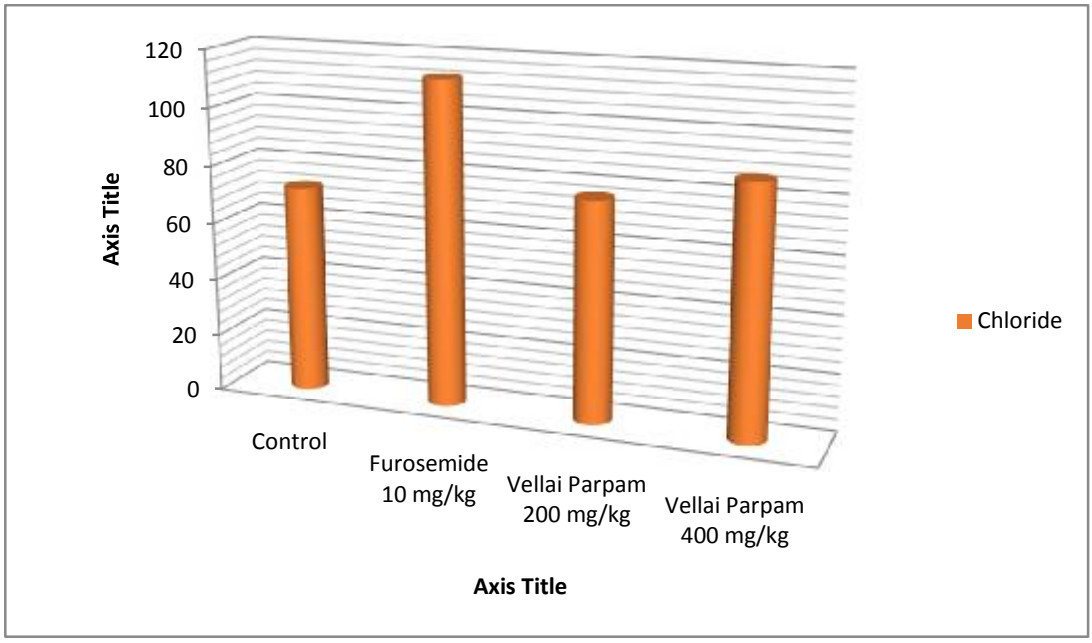


Chart 31: Estimation Of Urinary Electrolytes



Interpretation:

At the dose level of 200mg and 400 mg/Kg B.W. the trail drug *vellai parpam* showed the results of there is an increase in the urinary output along with an increase in concentration of sodium, potassium and chloride ions in urine. Thus the trail drug *vellai parpam* exhibits the greater diuretic activity when compared with the standard drug Furosemide.

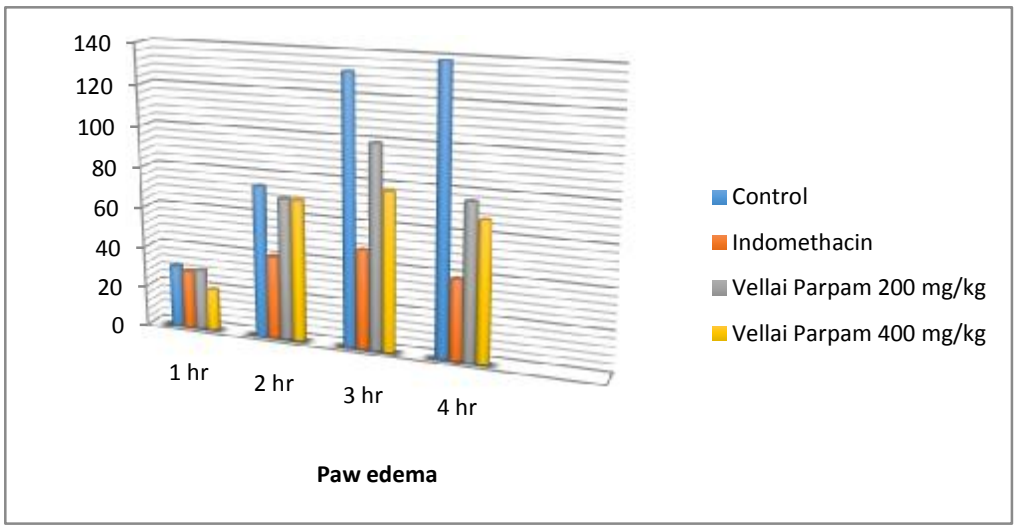
3. Anti – inflammatory activity

Table 25 : Carrageenan-induced rat paw edema

Treatment	Percentage inflammation after carageenan injection at hr			
	1	2	3	4
Control	30.10± 2.82	74.12± 10.14	131.14± 11.18	138.50± 4.50
Vellai Parpam 200mg/kg	29.00± 3.34	69.28± 1.14	99.34± 1.78 **	76.12± 8.08 **
Vellai Parpam 400mg/kg	20.00± 2.16	69.16± 3.14	77.50± 7.50 ***	68.00± 12.18 ***
Indomethacin 10mg/kg	27.18± 1.28	40.18± 8.98 **	48.07± 8.18 ***	39.00± 5.26 ***

Values are Mean ± SEM; n = 6 animals in each group: * P<0.05, ** P< 0.01, *** P<0.001 is considered significant when compared with control rats followed by Two way ANOVA.

Chart 32: Percentage inflammation after carageenan injection at hr's:



Interpretation:

The observed results exhibited the significant inhibition of paw edema in drug *indomethacin* treated group and the drug *vellai parpam* treated group at the low dose level (200 mg/Kg BW) and at the high dose level (400 mg/Kg BW). The drug *vellai parpam* treated rats showed the more potent anti-inflammatory activity.

DISCUSSION

DISCUSSION

The drug *Vellai Parpam* was selected to study the anti urolithiasis, diuretics and anti inflammatory activity. The trial drug was selected from *Agathiyar Vaithiya Kaaviyam* 1500 which is indicated for *Kalladaippu*, *Mahodharam*, *Neerchiruppu*, *Kuttam*.

The preclinical study substantiated the literary evidence of *Vellai Parpam* in the management of renal calculi.

Chemical analysis:

Chemical analysis of the drug *Vellai Parpam* reveals that the presence of Magnesium, Chloride and Calcium.

Magnesium:

Magnesium is inhibit the crystals formation. Thus decreasing the risk for forming kidney stone.

Chloride:

It regulates the water balance by maintaining the osmotic pressure of the body fluids and helps maintain proper blood volume, blood pressure and pH of body fluids.

Calcium :

It maintains the water balance in the body and also to maintain the bone density.

Thus the Chemical constituents of *Vellai Parpam* help in controlling stone formation and maintain the electrolyte balance in the body.

Toxicological studies:

This study reveals that no significant toxic effect of the drug *Vellai Parpam* upto the higher dose level 2000mg/kg in acute oral toxicity and also sub acute toxicity and sub chronic toxicity has no toxic effects from the results. Therefore the *Vellai Parpam* can be classified under the category of drug with non-toxic.

Pharmacological studies:

In the Pharmacological study the experimental data showed that the *Vellai Parpam* has anti urolithiatic, diuretic, anti inflammatory activity and the results are as follows,

Anti urolithiatic activity:

- The trail drug *vellai parpam* significantly increased the excretion of calcium, oxalate, citrate, uric acid in urinary output of rats compared with the standard drug cystone at the dose level of 200 mg and 400 mg/ Kg B.W. The drug acts more potent at the high dose 400mg/Kg than the low dose level of 200 mg/Kg. The histopathological results of the trail drug were also confirmed that there is excretion of stone in ethylene glycol induced rats. Thus the drug *vellai parpam* exhibits the anti-urolithiatic activity.

Diuretic activity:

- At the dose level of 200mg and 400 mg/Kg B.W. the trail drug *vellai parpam* showed the results of there is an increase in the urinary output along with an increase in concentration of sodium, potassium and chloride ions in urine. Thus the trail drug *vellai parpam* exhibits the greater diuretic activity when compared with the standard drug Furosemide.

Anti inflammatory activity:

- The observed results exhibited the significant inhibition of paw edema in drug *indomethacin* treated group and the drug *vellai parpam* treated group at the low dose level (200 mg/Kg BW) and at the high dose level (400 mg/Kg BW). The drug *vellai parpam* treated rats showed the more potent anti-inflammatory activity.

SUMMARY

SUMMARY

- The literary evidence of the drug *Vellai Parpam* strongly support that it possesses anti-urolithiatic, diuretic and anti-inflammatory activity for that purpose it has been selected for this study.

- The qualitative chemical analysis was done at Biochemistry lab, NIS. Chemical analysis of the drug *Vellai Parpam* reveals that the presence of Calcium, Chloride and Magnesium.

- Preclinical evaluation (acute, sub-acute and sub-chronic toxicity study) of the drug was carried out as per OECD guideline. This study reveals no significant toxic effect of the *Vellai Parpam* upto the higher dose level 2000mg/kg used in this study.

- Pharmacological study (Anti urolithiatic activity, Diuretic and Anti inflammatory activity) of the drug was carried out as per OECD guideline in K, K College of Pharmacy, Gerugambakkam, Chennai. In the pharmacological studies, the drug *Vellai Parpam* exhibits significant anti urolithiatic, diuretic and anti inflammatory.

- From the results and the statistical analysis it is proved that the drug *Vellai Parpam* is significant of anti urolithiatic, diuretic and anti inflammatory activity in the management of renal calculi.

CONCLUSION

CONCLUSION

From the literature evidence, Physico chemical analysis, Chemical analysis, Toxicological evaluation and Pharmacological studies, the drug *Vellai Parpam* has anti-urolithiatic, diuretic and anti-inflammatory activity. It is concluded that the drug *Vellai Parpam* can be used in the management of renal calculi and the related urinary problems.

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ANNEXURE

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

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

This certificate is awarded to

Dr./~~Mr.~~/~~Ms.~~ G. UMA

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

"Research Methodology & Biostatistics"

for AYUSH Post Graduates & Researchers

Organised by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University from 5th to 9th May 2014.

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

Dr. JHANSI CHARLES, M.D.
Registrar

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

K.K. COLLEGE OF PHARMACY

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

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

Ref: 4529/KKCP/2015

Date: 10.08.2015

APPROVAL CERTIFICATE

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

Project Code	Name of the species	Breakup sexwise	Weight	Number proposed	Number approved
KKCP/2015/033	Wistar Albino rat	49 Male + 55 female	150–200gms	108	104
Wistar albino rats - One hundred and four only					

Chairman IAEC

(Prof. A. Meena)

CPCSEA Nominee

10/08/15

(Dr. C. Kathirvelan)

Veterinary Officer

(V. VAIDHYALINGAM)

Members

Dr K Sadasivam M.V.Sc.

10/8/15

CERTIFICATE

This is certify that the project title... ~~Safety~~ Profile of ... "Velki Parpam" (12 Male + 12 Female Wistar albino Rats) ... has been approved by the IAEC. (NO: NIS/IAEC-II/2016/07)

Name of ~~Chairman~~/Member Secretary IAEC: nominee:

Dr. B.R. SENTHILKUMAR

Signature with date

24, Feb 2016

~~Chairman~~/Member Secretary of IAEC:

Name of CPCSEA

K. NAGENDRAN

17/2/2016

CPCSEA nominee:

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

சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம், அரும்பாக்கம், சென்னை - 600106
सिद्ध केन्द्रीय अनुसंधान संस्थान, अरुम्बाक्कम, चेन्नै - 600106
Siddha Central Research Institute
(Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India)
Arumbakkam, Chennai – 600106
[Ph: 044-26214925, 26214809, Fax: 26214809, Email: crisiddha@gmail.com, Web: www.siddhacouncil.com]

15.3.2016

CERTIFICATE

Certified that the samples submitted for identification by Dr. G. Uma, III year MD Student, Department of Gunapadam, National Institute of Siddha, Sanatorium, Chennai-600 047 are identified as Vengaram – Borax and Karpoora silasathu – Hydrous calcium sulphate.

(R. Shakila)
Research Officer (Chemistry)

(Dr. P.Sathiyarajeswaran)
Assistant Director (Scientist 2)-I/c

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

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

Ficus glomerata Roxb. (Moraceae) Root

Gossypium herbaceum Linn. (Malvaceae), Root

MEDICINAL BOTANY CHENNAI 600 047 National Institute of Siddha

Certificate No: NISMB1992015

Date: 14-8-2015

Authorized Signatory

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

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

CERTIFICATE

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

[DR.R.MURUGESAN]

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