SAFETY AND PHARMACOLOGICAL PROFILE OF VELLAIPARPAM

The dissertation Submitted by
Dr. G.UMA
Under the Guidance of
Prof. Dr. M. RAJASEKARAN., M.D(S)
H.O.D, & Guide., Department of Gunapadam
National Institute Of Siddha, Chennai - 47.

Dissertation Submitted to
The Tamil Nadu Dr. M.G.R. Medical University, Chennai – 32

For the partial fulfillment of the requirements to the Degree of
DOCTOR OF MEDICINE (SIDDHA)
BRANCH II - DEPARTMENT OF GUNAPADAM
2013-2016
NATIONAL INSTITUTE OF SIDDHA
Chennai – 47
# CONTENTS

<table>
<thead>
<tr>
<th>S.NO</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1-2</td>
</tr>
<tr>
<td>2</td>
<td>AIM AND OBJECTIVES</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>MATERIALS AND METHODS</td>
<td>4-7</td>
</tr>
<tr>
<td>4</td>
<td>REVIEW OF LITERATURE</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.1 GUNAPADAM REVIEW</td>
<td>8-15</td>
</tr>
<tr>
<td></td>
<td>4.2 BOTANICAL REVIEW</td>
<td>16-17</td>
</tr>
<tr>
<td></td>
<td>4.3 MINERALOGICAL REVIEW</td>
<td>18-23</td>
</tr>
<tr>
<td>5</td>
<td>ANALYTICAL STUDY OF THE DRUG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.1 PHYSICOCHEMICAL EVALUATION</td>
<td>24-26</td>
</tr>
<tr>
<td></td>
<td>5.2 CHEMICAL ANALYSIS</td>
<td>27-31</td>
</tr>
<tr>
<td></td>
<td>5.3 ELEMETAL ANALYSIS</td>
<td>32-33</td>
</tr>
<tr>
<td></td>
<td>5.4 ANALYSIS OF PARTICLE SIZE</td>
<td>34-35</td>
</tr>
<tr>
<td>Chapter</td>
<td>Section</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>6</td>
<td>6.1</td>
<td>ACUTE TOXICITY STUDY</td>
</tr>
<tr>
<td></td>
<td>6.2</td>
<td>REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY</td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td>REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY</td>
</tr>
<tr>
<td>7</td>
<td>7.1</td>
<td>ANTI-UROLITHIATIC ACTIVITY</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>DIURETIC ACTIVITY</td>
</tr>
<tr>
<td></td>
<td>7.3</td>
<td>ANTI-INFLAMMATORY ACTIVITY</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>RESULTS</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>DISCUSSION</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>SUMMARY</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>CONCLUSION</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>BIBLIOGRAPHY</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>ACKNOWLEDGEMENT</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>ANNEXURE</td>
</tr>
</tbody>
</table>
INTRODUCTION
1. INTRODUCTION

In siddha system of medicine is profunded by Agathiya 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 Agathiya 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.

\[
\begin{align*}
\text{Low dose} & = \text{Human dose } 400 \text{ mg } \times 0.018 (200 \text{ gms of rat}) \\
& = 7.2 \text{ mg} \\
\text{Mid dose} & = \text{low dose } \times 5 \\
& = 7.2 \times 5 = 36 \text{ mg} \\
\text{High dose} & = \text{low dose } \times 10 \\
& = 7.2 \times 10 = 72 \text{ mg}.
\end{align*}
\]

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:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified <em>Karpoora silasathu</em> (Selenite)</td>
<td>1 palam (35 gm)</td>
</tr>
<tr>
<td>Purified <em>Porikaram</em> (Borax)</td>
<td>1 palam (35 gm)</td>
</tr>
</tbody>
</table>

Decoctions:

<table>
<thead>
<tr>
<th>Decoction</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Paruthi ver</em> (Gossypium herbaecum)</td>
<td>1 palam (35 gm)</td>
</tr>
<tr>
<td><em>Athiver thol</em> (Ficus racemosa)</td>
<td>1 palam (35 gm)</td>
</tr>
</tbody>
</table>

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

*Kalladaippu*,

Indication : *Mahotharam, Neer chiruppu.*

Date of expiry : 100 years
REVIEW OF LITERATURE
4. LITRATURE REVIEW

4.1. GUNAPADAM REVIEW

1. அக்கிவிம் - *Ficus glomerulata roxb*

கிளை வினாம் :
அக்கிவிம், என்பட்டு, ரோல்பிக், கார்கில், கார்பிக்கும்.

பலபூச்சி வினாம் :
தேசு.

கையா முறை :
தேசுப்பிட்டு

கறியம் :
தேசு

போற்று :
தேசுப்பிட்டு

கோப்பை :
தேசுப்பிட்டு

கலந்து :

"சின்ன கிளைப்பிட்டு கையாத்து காட்டியில்
நருவில கல்லறைகள் நிறைந்தவை-குழுக்கள்
அக்கிவிம் விளையாட்டு சுற்றுவலை! காய்ந்ததில்
அக்கிவிம் பெரும் கிறோ.

- ஆகிவிம் கொண்டாம்.

சின்னாள் கையா, கிளைப்பிட்டு, சுற்றுவலைகள், நிறைந்தவை பொழுது,
குழுக்கள் சுற்றுவலையை பெரும் கிறோ."
4.2. மரியா - *Gossypium herbaceum* Linn

சிவப்பு பொடி:

ஆட்சக, புது, கன்றி, வெண்டுமை, பல்கா.

பச்சை பொடி:

 காம்புப்பற.

பனி:

காம்பு.

கல்லான:

மிகுந்த பக்தங்கலி

சகைப்பாக்கம்

2. சார்பாக்கம்
மந்து:

> முடிவுகள், குறுகி முடிவுகள், குறிப்பிட்டு தொடர்ந்து, புயல், செய்கை சூட்டக்கு முடிவு.

> பாதுகாப்புக்கு, " கல்லால் " முழுமையான திருத்தலமாகிறது முயல் நோய்ப்போகும். முற்பக்கம் மேலும் செய்திகளும் விளக்கம் செய்ய, அதற்கு தான் நூற்றைகம் மையக்கொண்டு வருகிறது. அவ்விருப்பில் மேற்கத்து நூற்றைக் கொள்ளும் வழக்கமற்று, பாதுகாப்பு, நேர்வாயா நூற்றைப் பெருகவிய முடிவு.

பிறக்குப் பயர் வழங்கும்:

2-ம் வட்டம்:

புதுக்கோட்டை வழங்கும்:

இருப்பு விளக்கம்: அப்பு விளக்கம், கோட்டைப்.

முனைவைச்செல்ல வழங்கும்:

இருப்பு விளக்கம்: இருக்கும், முனைவை விளக்கம், 40 முதல் அப்பு விளக்கம்.
குறிப்பு: 

- பதிவு - 120 ஆண்டு. 

சிலையின் கல்வியிற்கு செலவுகள் மற்றும் கட்டுப்பாடுகளை நோக்கினால் தீமைகளை வழங்கும்.

நூற்றாண்டு குண்டு & விளக்கம்:

"அழிற்சின்னம் போக்குவரத்திற்கு தவறுக்குட்படுவது தன்னை நான் பொருளைப்படுத்தி வந்து கவலை நீங்கிய பல்லிய பொருளை நாங்கள் பார்த்து அது தூய வந்து பல்லிய பொருளை பார்த்து நான் சிலையை புதுக்கோள் ஏற்று சிலையை நாட்டு."

பொருள் 7000 -ஆம் காலனியம்.

- அழிற்சின்னம் போக்குவரத்திற்கு தவறு போக்குவரத்திற்கு பொருளைப்படுத்தி வந்து நான், தன்னை பொருளை போக்குவரத்திற்கு தவறு பிடி பொருள் கவலை நீங்கி சிலையை.

- தீமைகள் சிலையின் தவறுக்கு செலவுகளை வழங்கும்.

கலை, கலனை, & பிரிவு:

- கலை - குண்டு
- கலனை - குண்டு
- பிரிவு - குண்டு

சிலையும் & பிரிவுகளும் குறிப்பிட்டு:

யுவராஜ - நான்கு, காவல், செலுத்தல், துறவு
முற்பாடு - முற்பாடு
பதிப்பு பணியில் ஆராட்டல்:

பின்புறியில் கையெடுப்பு

நூற்று விளம்ப வகை:

1. ஈருவரின் பிற மாய்கால் பொழுதுதான் நிற்க ஒளித்தேன்.
2. அனைத்து குழுப் பின் ஒளித்தேன்.
3. காலைகளின் பண்பாட்டுகள் குறுநடவெட்டிய குமா பற்று ஒளித்தேன்.

வேதியியல்:

குருநிகையின் வசயம், ஆண்டவை முழும் நோக்காக வேதியியல்

புரோத்த வழக்கம்:

"காலைகளில் புச்சையும் வள்ளக்கை
நூற்றுவரிசை விளங்குகிறது விளங்குகிறது -செய்யும் படத்துக்காக
கவலாக்கு ஈருவின் புரோமசர் நடுவாக
கலைநிகழ்த்து எனக்கு வந்ததால் காலை".

கலைநிகழ்த்து முறை குரு, அதிறங்கர, குருநிகை, குருநிகைகளினர். சுருக்கியவற்றிற்கு

புரோத்த வழக்கம்:

1. புரோத்த வழக்கம்
2. புரோத்த வழக்கம்
3. புரோத்த வழக்கம்

புரோத்தம் வழக்கம்:

புரோத்தம் வழக்கம்: புரோத்த வழக்கம், புரோத்த வழக்கம், புரோத்த வழக்கம்

முன்னணுத் தளப்பு:

முன்னணுத் தளப்பு: முன்னணுத் தளப்பு, முன்னணுத் தளப்பு, முன்னணுத் தளப்பு

12
விளக்கத்து

விளக்கத்து காலந்தோட்டம் 25 ம் வருடம் உள்ளது.

விளக்கம்:

போக்கினங்கு, மலர்கள், மூன்றாண்டு, காற்று, மூன்றாண்டுக்குள் கொடிகளை, திருக்கத்தூர்களை நிறுவவும்.

பொடி: 1200 விளை, 

கோத்தி, குடி, குருவகிளி, வாசிக்கி.

சீர்கற்று குறி:

சீர்கற்றிப் பிரிவுகளுக்கான சிறந்த பெருந்தெரிச்சல். பொடி கலை சிறிய குறிகளும், 

சீர்கற்றிப் பிற பொடி, பொடி பிரிவு வழியான சிற்றியடம் 25 வருட பிறிகளின் மீது விளாதியான விளை சுருக்கத் தீவைகளா.

கணக்கள்:

1. வைத்தியங்காட்க தலை வெளிச்சக்கைகள் பிரிவியப்படுத்து குறிக.
2. சிறந்த குறிக.
3. தான் குறிக.
4. பொடி பிரிவுப் பிள்ளை விளை 
5. பொடி பிரிவு 
6. பொடி காலம் 
7. பொடி விளை 
8. பொடி பிரிவு 
9. பொடி காலம் 
10. பொடி விளை 

சீர்ப் குறி:

1. 64 குறிப்பிட்டு குறிக.
2. 120 புரி குறிப்பிட்டு குறிக.
3. குறிப்பிட்டு 
4. குறிப்பிட்டு 
5. குறிப்பிட்டு 
6. குறிப்பிட்டு
சிங்கல் சுருக்கம்:

திணக்கம், அலங்காரம், கலாச்சாரம், புகழ், குடி.

அடத்தியல் பக்கீட்டுணர்-1200

பிறக்கு சுருக்கம்:

பாராட்டம், பங்கும், புதுக்காட்டம்.

மைசுற்றில் கலம் முழும் நீர்நிலை:

கலம்:

தமிழ்ப், மருதிதுணர் குடை தமிழ்ப்.

மீதம்:

மீதம்

செயல்பாடு:

செயல்பாடு:

> தோற்றிய செயல்பாடு
> சிறுவர் செயல்பாடு
> மத்தியச் செயல்பாடு
> பிரிவுப் பாடல்

மீட்பாடு:

> செயல்பாடு
> செயல்பாடு
> மத்தியச் செயல்பாடு
> அதிகாரக் குடி
> சுருக்கம்
புஷ்கரிப்பு:

"பார்வையான பெரும் கிேிரி பெரும் பார்வையான
புத்தாயிரம் கல்வியால் பல்லினனால் - நிர்வாகத்துக்கு
வாழ்க்கைக் குறிப்பிட்டியது இளைஞர்
பின் காட்டும் அனைத்து பொய்யான்."

புஷ்கரிப்புகள்:

> பார்வையான பெரும் கிேிரி 5 முதல் 10 கிேிரி பெரும்
  பார்வையான புத்தாயிரம் குறிப்பிட்டியது.

> புறநோட்டம் இருவர்: குறிப்பிட்டிய குறிப்பிட்டிய
  அக்காலம் புத்தாயிரம் பெரும் கிேிரியால் புத்தாயிரம்

இந்து பயின்றார்கள்:

2 நாள் பயின்றார்

சிற்றியலிக்க குறிக்கள்:

தினம் வினாம்: இருக்களால், மனச்சால், அருகை வினாம்.

முக்கியமான வினாம்:

தினம் வினாம்: இருக்கமல், அருகைமல்.

சிற்றியலிக்க வினாம்:

தினம் வினாம்: இருக்கமல், மனச்சால், அருகை வினாம், குறிப்பிட்டு
  இருக்கமல்
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.  
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. *Gossypium 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, Gossycaulin, Gossyoin,
- Gossypetin, Gossypitrin, Herbacetin, Herbacin, 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 **uroolithiasis** 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

<table>
<thead>
<tr>
<th>Physical Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Formula</td>
<td>Na₂B₄O₇·10H₂O</td>
</tr>
<tr>
<td>Composition</td>
<td>Hydrous sodium borate</td>
</tr>
<tr>
<td>Colour</td>
<td>Colourless, white, light grey. Also in light tints of blue, green and yellow.</td>
</tr>
<tr>
<td>Streak</td>
<td>White</td>
</tr>
<tr>
<td>Hardness</td>
<td>2 - 2.5</td>
</tr>
<tr>
<td>Transparency</td>
<td>Transparent to opaque</td>
</tr>
<tr>
<td>Specific Gravity</td>
<td>1.7</td>
</tr>
<tr>
<td>Luster</td>
<td>Itryeous to dull</td>
</tr>
<tr>
<td>Other ID Marks</td>
<td>1. Has a sweetish, metallic taste</td>
</tr>
<tr>
<td></td>
<td>2. Dissolve in water</td>
</tr>
<tr>
<td>In Group</td>
<td>Borates; Hydrous Borates</td>
</tr>
</tbody>
</table>

### Borax 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 VELLAIPARPAM

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 particle 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°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°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°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°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°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 **VELLAII PARPAM**

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

<table>
<thead>
<tr>
<th>S.No</th>
<th>EXPERIMENT</th>
<th>OBSERVATION</th>
<th>INFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Physical Appearance of extract</td>
<td>Light white in colour</td>
<td></td>
</tr>
</tbody>
</table>
| 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.
<table>
<thead>
<tr>
<th>S.No</th>
<th>EXPERIMENT</th>
<th>OBSERVATION</th>
<th>INERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I. Test For Acid Radicals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Test For Sulphate:</td>
<td>Cloudy appearance Presence.</td>
<td>Presence of Sulphate</td>
</tr>
<tr>
<td></td>
<td>2 ml of the above prepared extract was taken</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>in a test tube to this added 2ml of 4% dil</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ammonium oxalate solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Test For Chloride:</td>
<td>Cloudy appearance present.</td>
<td>Presence of Chloride</td>
</tr>
<tr>
<td></td>
<td>2 ml of the above prepared extract was added</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>with 2ml of dil-HCl is added until the</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>effervescence ceases off.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Test For Phosphate:</td>
<td>No cloudy yellow appearance</td>
<td>Presence of Phosphate</td>
</tr>
<tr>
<td></td>
<td>2ml of the extract were treated with 2ml of</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>dil. ammoniummolybdate solution and 2ml of</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>con.Hno3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Test For Carbonate:</td>
<td>Cloudy appearance presence.</td>
<td>Presence of carbonate</td>
</tr>
<tr>
<td></td>
<td>2ml of the extract was treated with 2ml dil.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>magnesium sulphate solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Test For Nitrate:</td>
<td>No Brown gas is evolved</td>
<td>Absence of nitrate</td>
</tr>
<tr>
<td></td>
<td>1gm of the extract was heated with copper</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>turning and concentrated H₂SO₄ and viewed the</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>test tube vertically down.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Test For Sulphide:</td>
<td>No rotten egg smelling gas is</td>
<td>Absence of sulphide</td>
</tr>
<tr>
<td></td>
<td>1gm of the extract was treated with 2ml of</td>
<td>evolved</td>
<td></td>
</tr>
<tr>
<td></td>
<td>con. HCL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Test For Fluoride &amp; Oxalate:</td>
<td>No cloudy appearance.</td>
<td>Absence of fluoride and</td>
</tr>
<tr>
<td></td>
<td>2ml of extract was added with 2ml of dil.</td>
<td></td>
<td>oxalate</td>
</tr>
<tr>
<td></td>
<td>Acetic acid and 2ml dil.calcium chloride</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>solution and heated.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
8. **Test For Nitrite:**
   3 drops 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-luminous part of the flame.
   - No blue colour precipitate
   - Absence of copper

3. **Test For Aluminium:**
   To the 2ml of extract dil. sodium hydroxide was added in 5 drops.
   - 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
<table>
<thead>
<tr>
<th>No.</th>
<th>Test For Substance</th>
<th>Description</th>
<th>Result</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td><strong>Test For Calcium:</strong></td>
<td>2ml of the extract was added with 2ml of 4% dil. ammonium oxalate solution</td>
<td>Cloudy appearance and white precipitate is obtained</td>
<td><strong>Presence of calcium</strong></td>
</tr>
<tr>
<td>7.</td>
<td><strong>Test For Magnesium:</strong></td>
<td>To 2ml of extract dil. sodium hydroxide solution was added in drops to excess.</td>
<td>White precipitate is obtained</td>
<td><strong>Presence of magnesium</strong></td>
</tr>
<tr>
<td>8.</td>
<td><strong>Test For Ammonium:</strong></td>
<td>To 2ml of extract 1 ml of Nessler's reagent and excess of dil. sodium hydroxide solution were added.</td>
<td>No brown colour</td>
<td><strong>Absence of ammonium</strong></td>
</tr>
<tr>
<td>9.</td>
<td><strong>Test For Potassium:</strong></td>
<td>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.</td>
<td>No yellow precipitate is obtained</td>
<td><strong>Absence of potassium</strong></td>
</tr>
<tr>
<td>10.</td>
<td><strong>Test For Sodium:</strong></td>
<td>2 pinches (50mg) of the extract is made into paste by using HCl and introduced into the blue flame of Bunsen burner.</td>
<td>No yellow colour flame evolved.</td>
<td><strong>Absence of sodium</strong></td>
</tr>
<tr>
<td>11.</td>
<td><strong>Test For Mercury:</strong></td>
<td>2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.</td>
<td>No yellow precipitate is obtained</td>
<td><strong>Absence of Mercury</strong></td>
</tr>
<tr>
<td>12.</td>
<td><strong>Test For Arsenic:</strong></td>
<td>2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.</td>
<td>No brownish red precipitate is obtained</td>
<td><strong>Absence of arsenic</strong></td>
</tr>
</tbody>
</table>

**III. Miscellaneous**

<table>
<thead>
<tr>
<th>No.</th>
<th>Test For Substance</th>
<th>Description</th>
<th>Result</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><strong>Test For Starch:</strong></td>
<td>2ml of extract was treated with weak dil. iodine solution</td>
<td>No blue colour developed</td>
<td><strong>Absence of starch</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Test For Reducing Sugar:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-------------------------------</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>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.</td>
<td>No brick red colour is developed</td>
<td>Absence of reducing sugar</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Test For The Alkaloids:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>2ml of the extract was treated with 2ml of dil. picric acid</td>
<td>No yellow colour was developed</td>
<td>Absence of Alkaloid</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Test For Tannic Acid:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>2ml of extract was treated with 2ml of dil.ferric chloride solution</td>
<td>No Blue-black precipitate is obtained</td>
<td>Absence of Tannic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Test For Unsaturated Compound:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>To the 2ml of extract 2ml of dil.Potassium permanganate solution is added.</td>
<td>Potassium permanganate is not decolourised</td>
<td>Absence of unsaturated compound</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Test For Amino Acid:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>2 drops of the extract was placed on a filter paper and dried well. 20ml of Burette reagent is added.</td>
<td>No violet colour</td>
<td>Absence of amino acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Test For Type Of Compound:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>2ml of the extract was treated with 2 ml of dil.ferric chloride solution</td>
<td>No green colour was developed</td>
<td>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.</td>
<td></td>
</tr>
</tbody>
</table>
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 EMISSION 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 intensity of the individual wavelength can be measured. The number of photons 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

<table>
<thead>
<tr>
<th>Rf frequency</th>
<th>40 M Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>165 – 782 nm</td>
</tr>
<tr>
<td>Detection limit</td>
<td>Up to ppm level using SCD detector</td>
</tr>
</tbody>
</table>
ANALYSIS OF PARTICLE SIZE
5.5 ANALYSIS OF PARTICULAR SIZE

SCANNED ELECTRON MICROSCOPY (SEM)

The particular 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 8°C. 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 24°C ±1°C 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%,
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, triglylyceride, 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 22°C ±3°C.
Relative humidity : between 30% and 70%.
Air changes : 10 to 15 per hour
Dark and light cycle : 12:12 hours.
Duration of the study : 90 Days.

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 “I.” 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:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Control</td>
</tr>
<tr>
<td>Group II</td>
<td>Urolithiatic rats- 0.75% of ethylene glycol for 28 days</td>
</tr>
<tr>
<td>Group III</td>
<td>Urolithiatic rats + Vellai Parpam 200mg/kg for 28 days</td>
</tr>
<tr>
<td>Group V</td>
<td>Urolithiatic rats + Vellai Parpam 400mg/kg for 28 days</td>
</tr>
</tbody>
</table>

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, 3 and 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**

<table>
<thead>
<tr>
<th>Colour</th>
<th>White</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour</td>
<td>Pleasant</td>
</tr>
<tr>
<td>Taste</td>
<td>Characteristic taste</td>
</tr>
<tr>
<td>Texture</td>
<td>Fine powder</td>
</tr>
<tr>
<td>Particle size</td>
<td>Completely pass through sieve no 120</td>
</tr>
</tbody>
</table>

5.2. PHYSICOCHEMICAL ANALYSIS

**Determination of Ash Values**

**Table 4: Percentage of Total Ash**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash Value</td>
<td>90.94</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid insoluble Ash</td>
<td>20.78</td>
</tr>
</tbody>
</table>
Interpretation for ash values

Ash: Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter to 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying at 105°C</td>
<td>0.49</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Parameter</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Test for Sulphate</td>
<td>Cloudy appearance present</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>Test for Chloride</td>
<td>Cloudy appearance present</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>Test For Phosphate</td>
<td>-</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Test For Carbonate</td>
<td>Cloudy appearance present</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>Test For Nitrate</td>
<td>-</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Test for Sulphide</td>
<td>-</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>Test For Fluoride &amp; oxalate</td>
<td>-</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>Test For Nitrite</td>
<td>-</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>Test For Borax</td>
<td>-</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Interpretation**

The acidic radicals test shows the presence of **Sulphate, Chloride, Carbonate**.
Table 8: Results Of Basic Radicals Studies:

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

**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

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

* 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 PARTICLE SIZE

SCANNED ELECTRON MICROSCOPY

Determination of Particle 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 VELLAIPARPAM IN
WISTAR ALBINO RATS

6.1. Acute oral toxicity study of VELLAIPARPAM

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      | + | - | + | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |


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

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

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*

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

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.*

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

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*

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

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*

![TWBC Chart]

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

![Hb and RBC Chart]
Chart 6: The mean value of Platelet of control and treated groups of wistar albino rats exposed to Vellai Parpam

![Platelet Chart](chart6)

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

![Cell Counts Chart](chart7)
Chart 8: The mean value of Monocytes, Basophils and Eosinophils of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

![Chart 8]

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

![Chart 9]
Table 15: Biochemical Parameters of of Wistar albino rats group exposed *Vellai Parpam*

<table>
<thead>
<tr>
<th>BIOCHEMICAL PARAMETERS</th>
<th>control</th>
<th>Mid dose</th>
<th>High dose</th>
<th>P Value (p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUCOSE (R) (mg/dl)</td>
<td>110.83±0.98</td>
<td>111.83±0.40</td>
<td>112.06±0.16</td>
<td>N.S</td>
</tr>
<tr>
<td>T.CHOLESTROL (mg/dl)</td>
<td>92.45±1.36</td>
<td>92.38±1.68</td>
<td>89.42±4.12</td>
<td>N.S</td>
</tr>
<tr>
<td>TGL (mg/dl)</td>
<td>47.31±3.15</td>
<td>45.08±2.23</td>
<td>46.83±2.33</td>
<td>N.S</td>
</tr>
</tbody>
</table>

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 Wistar albino rats group exposed to Vellai Parpam

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CONTROL</th>
<th>Mid dose</th>
<th>High dose</th>
<th>P Value (p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>UREA (mg/dl)</td>
<td>15.30±0.47</td>
<td>14.00±0.45</td>
<td>13.9±1.258</td>
<td>N.S</td>
</tr>
<tr>
<td>CREATININE (mg/dl)</td>
<td>0.5890±0.079</td>
<td>0.63±0.04</td>
<td>0.684±0.11</td>
<td>N.S</td>
</tr>
<tr>
<td>URIC ACID (mg/dl)</td>
<td>1.2±0.01</td>
<td>1.2±0.02</td>
<td>1.2±0.02</td>
<td>N.S</td>
</tr>
</tbody>
</table>

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*

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

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 28th 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
**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 VELLAIPARPAM IN WISTAR ALBINO RATS.

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

<table>
<thead>
<tr>
<th>DAYS</th>
<th>Control</th>
<th>Low dose</th>
<th>Mid dose</th>
<th>High dose</th>
<th>P value (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>162.4±29.65</td>
<td>154.4±21.83</td>
<td>155.6±13.09</td>
<td>161.5±28.17</td>
<td>NS</td>
</tr>
<tr>
<td>15</td>
<td>170.1±28.49</td>
<td>162.8±23.54</td>
<td>171.7±29.83</td>
<td>179.8±32.13</td>
<td>NS</td>
</tr>
<tr>
<td>30</td>
<td>184.4±28.83</td>
<td>184.71±14.76</td>
<td>182.8±32.17</td>
<td>190.2±28.55</td>
<td>NS</td>
</tr>
<tr>
<td>45</td>
<td>202.7±27.81</td>
<td>205.6±29.12</td>
<td>203.1±19.07</td>
<td>207.9±22.05</td>
<td>NS</td>
</tr>
<tr>
<td>60</td>
<td>226.6±33.47</td>
<td>232.6±23.91</td>
<td>230.6±33.61</td>
<td>226.6±23.63</td>
<td>NS</td>
</tr>
<tr>
<td>75</td>
<td>240.8±26.76</td>
<td>244.8±28.08</td>
<td>242.0±28.70</td>
<td>240.8±26.87</td>
<td>NS</td>
</tr>
<tr>
<td>90</td>
<td>263.5±27.94</td>
<td>266.9±27.68</td>
<td>268.2±27.31</td>
<td>266.1±29.41</td>
<td>NS</td>
</tr>
</tbody>
</table>

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*.

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

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 14: T.WBC Chart]

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

![Chart 15: Hb and T.RBC Chart]
Chart 16: The mean value of Platelet of control and treated groups of wistar albino rats exposed to *Vellai Parpam*

![Platelet Chart](chart16)

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

![PCV, MCH, MCHC, MCV Chart](chart17)
Table 20: Biochemical Parameters of of Wistar albino rats group exposed to Vellai Parpam.

<table>
<thead>
<tr>
<th>BIOCHEMICAL PARAMETERS</th>
<th>CONTROL</th>
<th>LOW DOSE</th>
<th>MID DOSE</th>
<th>HIGH DOSE</th>
<th>P Value (p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUCOSE (R) (mg/dl)</td>
<td>121.67±5.6</td>
<td>122.37±10.1</td>
<td>121.33±10.8</td>
<td>120.97±11.8</td>
<td>N.S</td>
</tr>
<tr>
<td>T.CHOLESTEROL (mg/dl)</td>
<td>105.17±11.89</td>
<td>120.00±4.73</td>
<td>110.50±6.50</td>
<td>119.67±8.52</td>
<td>N.S</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>43.50±3.33</td>
<td>40.83±5.34</td>
<td>44.67±2.73</td>
<td>40.50±1.64</td>
<td>N.S</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>42±10.7</td>
<td>52.17±10.8</td>
<td>38.67±5.95</td>
<td>42±11.3</td>
<td>N.S</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>29.60±1.85</td>
<td>26.93±3.77</td>
<td>27.20±1.67</td>
<td>23.30±2.32</td>
<td>N.S</td>
</tr>
<tr>
<td>TRIGLY (mg/dl)</td>
<td>148.00±9.25</td>
<td>134.67±18.85</td>
<td>136±8.36</td>
<td>116.50±11.6</td>
<td>N.S</td>
</tr>
</tbody>
</table>

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*

![Blood sugar chart](chart19)

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

![HDL, LDL, VLDL chart](chart20)
Table 21: Renal function test of Wistar albino rats group exposed to *Vellai Parpam*

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CONTROL</th>
<th>LOW DOSE</th>
<th>MID DOSE</th>
<th>HIGH DOSE</th>
<th>P Value (p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>UREA (mg/dl)</td>
<td>28.33±5.2</td>
<td>31.50±4.23</td>
<td>29.17±4.30</td>
<td>31.67±5.82</td>
<td>N.S</td>
</tr>
<tr>
<td>CREATININE (mg/dl)</td>
<td>0.567±0.13</td>
<td>0.517±0.172</td>
<td>0.550±0.137</td>
<td>0.483±0.116</td>
<td>N.S</td>
</tr>
</tbody>
</table>

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*

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

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 90th day and the result is produced. The results revealed there are no significant changes in the values of different parameters with that of the control. 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

![Heart Control](image1)

![Heart High Dose](image2)

LIVER

![Liver Control](image3)

![Liver High Dose](image4)
showing histopathologic slides of repeated dose 90 days oral toxicity of *Vellai Parpam*
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

<table>
<thead>
<tr>
<th>Biochemical Assays</th>
<th>Gr-I</th>
<th>Gr-II</th>
<th>Gr-III</th>
<th>Gr-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>0.82±0.07</td>
<td>2.66±0.15(^a)</td>
<td>1.60±0.14(^b)(***)</td>
<td>1.14±0.08 (^b)(***)</td>
</tr>
<tr>
<td>Oxalate</td>
<td>0.62±0.08</td>
<td>4.50±0.64 (^a)</td>
<td>2.70±0.50 (^b)(*)</td>
<td>2.05±0.06 (^b)(**)</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.82±0.04</td>
<td>2.75±0.30 (^a)</td>
<td>2.20±0.37 (^a)*</td>
<td>1.42±0.25 (^b)(*)</td>
</tr>
<tr>
<td>Citrate</td>
<td>2.42±0.20</td>
<td>0.76±0.05 (^a)</td>
<td>1.27±0.14 (^a)(****)</td>
<td>0.97±0.08 (^a)(****)</td>
</tr>
</tbody>
</table>

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:
Group I- Normal saline
Group II- Ethylene glycol
Group III- Vellai Parpam 200 mg/kg
Group IV- Vellai Parpam 400 mg/kg
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

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

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 29: Estimation Of Urinary Electrolytes](image)

Chart 30: Estimation Of Urinary Electrolytes

![Chart 30: Estimation Of Urinary Electrolytes](image)
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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage inflammation after carrageenan injection at hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>30.10± 2.82</td>
</tr>
<tr>
<td><strong>Vellai Parpam</strong></td>
<td>29.00± 3.34</td>
</tr>
<tr>
<td>200mg/kg</td>
<td></td>
</tr>
<tr>
<td><strong>Vellai Parpam</strong></td>
<td>20.00± 2.16</td>
</tr>
<tr>
<td>400mg/kg</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>27.18± 1.28</td>
</tr>
<tr>
<td>10mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

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.
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 \textit{Vellai Parpam} has anti urolithiatic, diuretic, anti inflammatory activity and the results are as follows,

\textbf{Anti urolithiatic activity:}

- The trial drug \textit{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 \textit{vellai parpam} exhibits the anti-urolithiatic activity.

\textbf{Diuretic activity:}

- At the dose level of 200mg and 400 mg/Kg B.W. the trail drug \textit{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 \textit{vellai parpam} exhibits the greater diuretic activity when compared with the standard drug Furosemide.

\textbf{Anti inflammatory activity:}

- The observed results exhibited the significant inhibition of paw edema in drug \textit{indomethacin} treated group and the drug \textit{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 \textit{vellai parpam} treated rats showed the more potent anti-inflammatory activity.
SUMMARY
The literary evidence of the drug *Vellai Parpam* strongly support that it possesses anti-ureolithiatic, 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 ureolithiatic 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 ureolithiatic, diuretic and anti inflammatory.

From the results and the statistical analysis it is proved that the drug *Vellai Parpam* is significant of anti ureolithiatic, 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.
BIBLIOGRAPHY
BIBLIOGRAPHY

2. Vaithiya Rathinam K.S. Murugesu Mudhalayar, Department of Indian medicine and homeopathy, Gunapadam Mooligai vaguppu part I, 2nd revised edition 2006
5. Sarakugalin suthi sei muraigal, department of Indian medicine and homeopathy First edition.
7. Agathiyar Vaithiya Rathina Surukkam 360, 1887, pg no-46,47.
8. Veeramaa Munivar Nasaganda Venba, 1926, Published by Manikkavel Mudhalayar, Chennai.
10. Dr. Ambika shanmugam, Fundamendals of Bio chemistry, 7th edition


24. The Indian Materia Medica, Vol 2, Dr.Natkarani


ACKNOWLEDGEMENT
ACKNOWLEDGEMENT

This dissertation is one of the milestones in the journey of siddha drug research. It is the key program in acquiring my MD(S) degree. Thus I came across this task which kept on completed with the support and encouragement of numerous people. So I take great pleasure in thanking all the people who made this dissertation study a valuable and successful one, which I owe to treasure it.

I feel enormous wonder and colossal gratitude in my heart of hearts to GOD and SIDDHARS Almighty for making this dissertation have its present form.

I express my sincere thanks to the Vice-Chancellor, The Tamilnadu Dr.MGR medical University, chennai-32.

I express my profound sense of gratitude to Prof. Dr.V.Banumathi M.D(s), Director, National Institute of Siddha, Chennai-47.

I express my sincere thanks to Prof.Dr.M.Rajasekaran M.D(s), Head of the Department of Gunapadam & Guide, National Institute of Siddha, Tambaram sanatorium Chennai-47, for his valuable suggestions and guidance in this dissertation work.

I express my sincere thanks to Dr.P.Kumar M.D(s), Associate Prof. Department of Gunapadam, NIS.Chennai, for his hopeful support and encouragement of my whole study.

I express my sincere thanks to Dr.S.Visweswaran M.D(s), Lecturer, Department of Gunapadam, NIS, Chennai-47, for his suggestions, hopeful support and encouragement of my whole study.

I express my sincere thanks to Dr.S.Sivakkumar M.D(s), Lecturer, Department of Gunapadam, NIS, chennai-47 for his suggestions, hopeful support and encouragement of my whole study.

I express my sincere thanks to Dr.A.Mariappan M.D(s), Lecturer, Department, of Gunapadam NIS,Chennai-47, for his suggestions, hopeful support and encouragement of my whole study.
I express my sincere thanks to Dr.V.Suba M.Pharm, P.hD., Assistant Professor in Pharmacology, NIS, Chennai-47, for her suggestions in the pharmacological study.

I express my sincere thanks to Chairman and Members of Institutional Animal Ethical Committee (IAEC), National Institute Of Siddha, Chennai-47, for their valuable guidance.

I express my sincere thanks to Dr.R.Murugesan, Scientific officer, SAIF, IIT, Chennai.

I express my sincere thanks to Dr.D.Aravind M.D(s), M.Sc., Assistant Professor, Medicinal Botany, National Institute Of Siddha, Chennai-47.

I express my grateful thanks to Late Dr.J.Rani B.V.sc, veterinarian, NIS, for her healthy support to me and my test animals throughout my studies.

I express my grateful thanks to Dr.R.Prakash M.Pharm., Ph.D, K.K College of pharmacy, Gerugampakkam, Chennai., for his assistance in the pharmacological study.

I express my sincere thanks to Mr.M.Subramanian M.Sc., (statistics) Senior Research Officer, National Institute of Siddha, Chennai-47.

I wish to thank Library assistants, NIS, Chennai-47.

I express my thanks to Penguin Xerox, West Tambaram, Chennai-45.

I express my gratefulness to All My Colleagues and My friends for lending their helping hands whenever needed during the course of the study.

Last but not least, I would like to pay high regards to all my family members, for their sincere encouragement throughout my research work and lifting me uphill this phase of life. I owe everything to them. Besides this, several people have knowingly and unknowingly helped me in the successful completion of this project.
ANNEXURE
The Tamil Nadu Dr. M.G.R. Medical University

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

This certificate is awarded to

Dr./Mrs. G. Uma

for participating as a 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. Kablar, M.D., Siddha
Deputy, Dept. of Siddha

Dr. Jhansi Charies, M.D.
Prof. Dr. D. Shanitharam, M.D., D.Dipl.
Vice-Chancellor

Register
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

<table>
<thead>
<tr>
<th>Project Code</th>
<th>Name of the species</th>
<th>Breakup sexwise</th>
<th>Weight</th>
<th>Number proposed</th>
<th>Number approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>KKCP/2015/033</td>
<td>Wistar Albino rat</td>
<td>49 Male + 55 Female</td>
<td>150–200gms</td>
<td>108</td>
<td>104</td>
</tr>
</tbody>
</table>

Wistar albino rats - One hundred and four only

Chairman IAEC
(Prof A. Meena)

CPCSEA Nominee
(Dr. C. Kathirvelan)

Veterinary Officer
(V. Vaidhyanathan)

Members

Dr. K. Sadasivan Pillai

[Signatures]
CERTIFICATE

This is certify that the project title "Safety Profile of "Valkei Parpan" (12 Male 112 Female, Lower Alumago)"

has been approved by the IAEC. (NO: NIS/IAEC-3/2010/07)

Name of Chairman/Member Secretary IAEC: Dr. B.R. Senthilkumar

Name of CPCSEA:

Signature with date 24th Feb 2016

Chairman/Member Secretary of IAEC: CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)
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 Karpooa silasaihu – 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 Unaniadham, 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

Certificate No: NISMR1992015

Date: 14-8-2015

Authorized Signatory

Dr. D. ARAVIND, M.D(5), M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha
Chennai - 600 047, 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.

Re: [DR.R.MURUGESAN]

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