SAFETY AND PHARMACOLOGICAL PROFILE OF INDHUPPU BHAVANAI

The dissertation Submitted by
Dr. C. RUBIKA DEVI

Under the Guidance of
Prof. Dr. M. RAJASEKARAN, M.D(S)
H.O.D.,& Guide, Department of Gunapadam,
National Institute of Siddha, Ch-47

Dissertation submitted to
THE TAMILNADU DR. MGR MEDICAL UNIVERSITY
CHENNAI-600032

In partial fulfilment of the requirements
For the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)
BRANCH-II-GUNAPADAM

2013-2016

NATIONAL INSTITUTE OF SIDDHA
Chennai – 47
<table>
<thead>
<tr>
<th>S.NO</th>
<th>TITLE</th>
<th>P.NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1</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</td>
</tr>
<tr>
<td>4</td>
<td>REVIEW OF LITERATURE</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>4.1 GUNAPADAM REVIEW</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>4.2 BOTANICAL REVIEW</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>4.3 MINERALOGICAL REVIEW</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>ANALYTICAL STUDY OF THE TRIAL DRUG</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>5.1 ORGANOLEPTIC EVALUATION</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>5.2 PHYSICOCHEMICAL ANALYSIS</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>5.3 CHEMICAL ANALYSIS</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>5.4 TLC/HPTLC FINGER PRINT ANALYSIS</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>5.5 MICROBIAL ANALYSIS</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>5.6 ELEMENTAL ANALYSIS</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>5.7 PARTICLE SIZE ANALYSIS</td>
<td>40</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>6</td>
<td>TOXICOLOGICAL STUDY</td>
<td>41</td>
</tr>
<tr>
<td>6.1</td>
<td>ACUTE TOXICITY STUDY</td>
<td>42</td>
</tr>
<tr>
<td>6.2</td>
<td>REPEATED DOSE 28 DAYS ORAL TOXICITY</td>
<td>46</td>
</tr>
<tr>
<td>6.3</td>
<td>REPEATED DOSE 90 DAYS ORAL TOXICITY</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>PHARMACOLOGICAL STUDY</td>
<td>53</td>
</tr>
<tr>
<td>7.1</td>
<td>ANTI-ULCER ACTIVITY</td>
<td>53</td>
</tr>
<tr>
<td>7.2</td>
<td>ANTI-INFLAMMATORY ACTIVITY</td>
<td>56</td>
</tr>
<tr>
<td>7.3</td>
<td>ANALGESIC ACTIVITY</td>
<td>58</td>
</tr>
<tr>
<td>8</td>
<td>RESULTS</td>
<td>61</td>
</tr>
<tr>
<td>9</td>
<td>DISCUSSION</td>
<td>111</td>
</tr>
<tr>
<td>10</td>
<td>SUMMARY</td>
<td>115</td>
</tr>
<tr>
<td>11</td>
<td>CONCLUSION</td>
<td>116</td>
</tr>
<tr>
<td>12</td>
<td>ANNEXURE</td>
<td>117</td>
</tr>
<tr>
<td>13</td>
<td>BIBLIOGRAPHY</td>
<td>123</td>
</tr>
<tr>
<td>14</td>
<td>ACKNOWLEDGEMENT</td>
<td>126</td>
</tr>
</tbody>
</table>
INTRODUCTION
INTRODUCTION

The Siddha system dates back to 5000 B.C profounded by Saint Agathiyar and his clan numbering 18 such Siddhars. This system was an amalgam of Tamil literature, culture, tradition, health and many such living forms of 64 types.

The Department of Gunapadam in National Institute of Siddha, Chennai is functioning from 30/9/2004 and many such research works had been carried out successfully..

The current research was on Indhuppu bhavanai to evaluate the safety and pharmacological activities in animal models.

The drug Indhuppu bhavanai was indicated for Pitha Gunmam (Hyperacidity) which was selected from the Siddha literature “Kadukkai vallaraiin thani maanbu” third edition-1992, pg.no: 84, authored by Hakkim P. Mohammed Abdulla sayub.

All the ingredients were identified and authenticated by the experts and the test drug was prepared by the given procedure.

Review of literature in various categories were carried out. Siddha aspect, botanical aspect and mineralogical aspect disclosed about the drug and the disease, which strongly supports that it possesses anti-ulcer (Hyperacidity), anti-inflammatory and analgesic activities, for that purpose it has been selected for this study

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

Preclinical evaluation of acute and Repeated dose 28 day oral toxicity study was carried out in K.K College of Pharmacy, Gerugambakkam and Repeated dose 90 day oral toxicity study of the drug was carried out in the Animal house, NIS, Chennai.

Wistar albino rats of either sex of weight 150-200 gm were used for toxicity and pharmacological studies. The animals were kept under standard conditions 12:12(day/night cycles) at room temperature in polypropylene cages. The animals were fed on standard pelleted diet and potable water in polypropylene bottles ad libitum. The animals were housed for one week prior to the experiments to acclimate to animal house conditions.
In acute toxicity study, various dose levels of *Indhuppu bhavanai* 5, 50, 300 and 2000 mg/kg b.w. was administered to female wistar albino rats which showed no abnormalities in external observation and necropsy examination and all the vital organs were normal.

In Repeated dose 28 day oral toxicity study and Repeated dose 90 day oral toxicity studies, various doses level of *Indhuppu bhavanai* at 900mg/kg and 1800 mg/kg was administered orally, did not show any significant changes in hematological parameters and histopathological slides of various organs.

In Pharmacological studies, Anti-ulcer (Hyperacidity), anti-inflammatory and analgesic activities of the drug was carried out in wistar albino rats and mice as per OECD guideline in K.K College of Pharmacy, Gerugambakkam revealed that the drug *Indhuppu bhavanai* exhibited significant anti-ulcer, anti-inflammatory and analgesic activities.

The above studies showed that the drug *Indhuppu bhavanai* was safe in animal studies and may be carried for clinical trial.
AIM AND OBJECTIVES
2. AIM AND OBJECTIVES

AIM

The aim of the study was to evaluate the Safety and Pharmacological profile of the test drug “INDHUPTU BHAVANAI” in animal models.

OBJECTIVE

The following methodology was adopted to evaluate the Safety and Pharmacological activities of the test drug.

- Review of various information (Siddha and modern) relevant to the study.
- Preparation of the drug as per classical Siddha literature.
- Analytical study of the prepared drug
  - Physicochemical and phytochemical analysis
  - Chemical analysis to evaluate acidic and basic radicals.
  - Elemental analysis
  - Analysis of Particle size

- Screening the toxicity in animal models.
  - Acute oral toxicity study (OECD – 423 Guideline)
  - Repeated dose 28 day oral toxicity (OECD – 407 Guideline)
  - Repeated dose 90 day oral toxicity (OECD – 408 Guideline)

- Evaluation of pharmacological activities in animal models.
  - Anti-ulcer (Aspirin induced ulcer model)
  - Anti-inflammatory (Cotton pellet induced granuloma method)
  - Analgesic (Eddy’s hot plate method)
MATERIALS AND METHODS
3. MATERIALS AND METHODS

STANDARD OPERATIVE PROCEDURE OF INDHUPPU BHAVANAI

Drug selection

The drug *Indhuppu Bhavanai*, a herbo-mineral formulation was taken as a trial drug from Siddha literature *Kadukkai Vallaraiin Thani Maanbu*, third edition-1992, pg.no: 84 ,Author:*Hakkim P. Mohammed Abdulla sayub*

The Ingredients of *Indhuppu Bhavanai* are, (3)

1. *Purified Indhuppu* (Sodium chloride impura) – 5 palam (175 g)

2. *Vallarai leaf juice* (*Centella asiatica*)

3. *Lemon pulp juice* (*Citrus limon*)

4. *Purified fresh Ginger juice* (*Zingiber officinale*)

5. *Nellikai pulp juice* (*Phyllanthus emblica*)

Collection of the Plant materials

All plant materials were freshly collected from Tambaram sanatorium, Tamilnadu. *Indhuppu* was procured from a well reputed country shop in Parrys, Chennai. *Indhuppu* was purified and the medicine was prepared in the Gunapadam laboratory of National Institute of Siddha.

Identification and Authentication of the drug

Mineral drug was authenticated by the Chemist in Central Research Institute of Siddha, Arumbakkam, Chennai.

The Herbal drugs were Identified and authenticated by the Botanist, National Institute of Siddha.
**Bhavanai**\(^{(1)}\)

Bhavana was a process in which powders were soaked in various fluids, such as the expressed juice of herbs, decoctions etc., and then dried. For this purpose the quantity of juice added to the powder should be sufficient to cover it. The mixture is then allowed to dry in a shaded place. This process is repeated twice or thrice or as many times, as in necessary.

**Purification process**

**Indhuppu**\(^{(2)}\)

*Indhuppu* was dissolved in vinegar and kept for 3 days and dried in sunlight.

**Preparation of the drug**\(^{(3)}\)

- *Indhuppu* was placed in *Kalvam* and powdered. *Vallarai* juice was poured until *Indhuppu* was immersed.
- Then it was grinded slowly till it loses moisture content and becomes dry and the same process was repeated for 7 times.
- Then the above mentioned process was carried out with the following juices respectively,
  - Lemon juice
  - Ginger juice
  - Goose berry juice

Finally powder was dried & preserved.

**Labelling**

<table>
<thead>
<tr>
<th>Name of the preparation</th>
<th>:</th>
<th>Indhuppu Bhavanai</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date of preparation</td>
<td>:</td>
<td>15/6/2015</td>
</tr>
<tr>
<td>Dose</td>
<td>:</td>
<td>1 gm bd</td>
</tr>
<tr>
<td>Adjuvant/Vehicle</td>
<td>Honey</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Indications</td>
<td><em>Pitha gunnam</em> (hyperacidity), <em>Vaanthi</em> (Vomiting), <em>Vayitru eraichal</em> (Gastric irritation), <em>Seriyaaamai</em> (Indigestion), <em>Vaayu muthaliya irappai sampanthamana noigal</em> (Gastro intestinal disorders) <em>theerum.</em></td>
<td></td>
</tr>
<tr>
<td>Date of expiry</td>
<td>100 years from the date of manufacture</td>
<td></td>
</tr>
</tbody>
</table>

**INDHUPPU BHAVANAI**
INGREDIENTS OF **INDHUPPU BHAVANAI**

*Indhuppu*

**Before purification**

- VALLARAI (*Centella asiatica*)
- **INJI** (*Zingiber officinalae*)
- **ELUMICHAI** (*Citrus limon*)
- **Nellikai** (*Phyllanthus emblica*)

**After purification**
REVIEW OF LITERATURE
GUNAPADAM REVIEW
**GUNAPADAM REVIEW**

**இந்துப்பு - INDHUPPU**

**வரலாற்று வம்பக்கள்**

- காரணமாய், திருத்தமாய், சூழலிருந்தாய், விளைவாய், பரிபாதமாய், மிகவும் குறையாய்.
- திருமணம் முடியாய் தெற்காய்.
- திருள் பாடலக் குறுக்கு மான், புவிய தம்பாய்.

**வரலாற்று வம்பக்கள்**

*கொழும்பு மருந்தில் அச்சிக்கார்கள் விளையாடுவது வருமாறு முனைவிட்டு என்று வருமாறு சொல்லி வருமாறு காலமாக கல்பந்து சொல்லி வருமாறு மீன்புற விளையாடுவது வருமாறு*

*காலமாக மீன்புற விளையாடுவது வருமாறு மீன்புற விளையாடுவது வருமாறு*

*நிலம் விளையாடுவது வருமாறு*

*செல்வாற்றிகள் பதிலிட்டு செல்வாற்றிகள் பதிலிட்டு*

*செல்வாற்று செல்வாற்று தான்காரவுடையுள்ளிடையே செல்வாற்று தான்காரவுடையுள்ளிடையே செல்வாற்று தான்காரவுடையுள்ளிடையே*

*செல்வாற்று செல்வாற்று தான்காரவுடையுள்ளிடையே செல்வாற்று தான்காரவுடையுள்ளிடையே செல்வாற்று தான்காரவுடையுள்ளிடையே*

*செல்வாற்று செல்வாற்று தான்காரவுடையுள்ளிடையே செல்வாற்று தான்காரவுடையுள்ளிடையே செல்வாற்று தான்காரவுடையுள்ளிடையே*

*செல்வாற்று செல்வாற்று தான்காரவுடையுள்ளிடையே செல்வாற்று தான்காரவுடையுள்ளிடையே செல்வாற்று தான்காரவுடையுள்ளிடையே *

*செல்வாற்று செல்வாற்று தான்காரவுடையுள்ளிடையே செல்வாற்று தான்காரவுடையுள்ளிடையே செல்வாற்று தான்காரவுடையுள்ளிடையே*
சொற்றுரு பட்டியல்

- சொற்றுரு வடிவாக சிற்றடி வடிவாக பொருள் விளக்கம்.
- சொற்றுரு வழங்கும் வகை முக்கியமாக சுருக்கி விளக்கம்.

சொற்றுரு வழங்கப்படும் பட்டியல்

1) சொற்றுரு வரலாறு
2) சொல்ல விளக்கம்
3) அயனி மறுகலை
4) சிற்றடி வடிவாக
5) கொல வண்ணம்
6) கவிதை வண்ணம்
7) பாடல்லல் பொருள்
8) கூற்று வடிவாக சுருக்கி
VALLARAI

Botanical name: Centella asiatica (Linn.)

English name: Indian Pennywort

Family: Apiaceae

Organoleptic characters

Taste: Sweet, sour, bitter

Odour: Mild

Gastric

Centella

Vallarai

The herb is used in Ayurvedic medicine. It is an expectorant and astringent. It is also used in the treatment of skin diseases and as a urinary antiseptic.

(தமிழ்மொழித் தகவல்)
குறிப்பிட்டல் பணிகள்

- மாசைமுடியாத கால், பாலகாலம், வெளியேறும் நோய்கள், வாபு மட்டும், அனைத்தும் நொய்யல் செய்துள்ளாலே ஒருவன் விளக்கத்திற்கு.
- மத்தியஞ்சள்களைச் சேர்த்து கொண்டுள்ளால், மருந்துகள், மருத்துவம் கறியும் முறைகளைக் கூறுகின்ற அனைத்து முறையும் வேலு புரிந்து அறிமுகப்படுத்தும்.
- முறையே செய்யப்படும் விளக்கம் அதிகமான 1, 2 காலம் முதலில்கேற்ப பாலால், குறுக்கு, மராத்திய விளக்கங்கள் செய்யவும்.
- மாசையான கொடுக்கப்பட்டு 130-300 கி.மீ. வரை பொருட்களும் பொருட்களும் வெளியேறுவது, அல்லது குறுக்கு வழியாக வெளியேறுவது போன்றவண்டு காணப்படும்.
- மாசையான நொய்யல் வாய்ப்புகள் பெயர் துளை மற்றும் பொருட்கள் வண்டியோ கண்டுபிடிக்கும்.

வேலைத்தரும் புதிய வருகைகள்

1) வேலைத்தரும் நொய்யல்
2) கப்பலரும் செய்யும்
3) வேலைத்தரும் வையும்
4) வேலைத்தரும் மாசைமுடியாத
5) செய்யும் பயன்பாடு
6) குறிப்பிட்டல் பயன்பாடு
English name: Lime
Botanical name: Citrus limon (Linn) Burm.f.
Family: Rutaceae

Organoleptic characters

Taste: Sweet, sour, bitter
Texture: Smooth
Smell: Citrusy

Flavor:

Sweet, sour, bitter
Texture: Smooth
Smell: Citrusy

(Taste profile of Citrus limon)
புரிந்து தவிர்ப்பாமல் தத்துவமற்ற நூற்றாண்டுகளுக்கு மேம்படுத்தும் தீர்மானங்கள் முதலே உள்ளன. மேலுமே, குறிப்பிட்டிக்கொள்ள இலைநிலைக்குட்பட்டு அனைத்தும் மேம்படுத்தப்படும். (வலையம்)

முருக்கல் போர்க்கோயில்

- இப்போர்க்கோயிலிலுள்ள தலைவர் குறைந்திருப்பது போல இவர் விளக்கம் செய்கிறார்
- குறிப்பிட்டிக்கொள்ளும் குற்றுகளும் போர்க்கோயிலில் தீர்மானங்கள் விளக்கம் கொண்டு போர்க்கோயிலில் மேம்படுத்தும்
- வெளியில் போர்க்கோயில் இயங்கிய தலைவர்கள் சுருக்கானது, வெளியில் முழுவதும், ஆசிரியர் மற்றும் தீர்மானங்களுக்கு சான்றுப்படும்
- தீர்மானங்கள் பின் மேம்படுத்தும் போர்க்கோயிலிலுள்ள மேம்படுத்தும். இது போர்க்கோயிலை முனைவுள்ள போர்க்கோயில் விளக்கிறது

எழுதும் குறுக்கை போர்க்கோயில்

1) குறுக்கை போர்க்கோயில்
2) புதுக்கை போர்க்கோயில்
3) காப்பாண் போர்க்கோயில்
4) சிறியக்கை போர்க்கோயில்
5) குறுக்கை போர்க்கோயில்
6) சிறியக்கை போர்க்கோயில்
Green ginger fresh root

Zingiber officinale

Zingiberaceae

Organoleptic characters

Taste : Aromatic

Odor : Aromatic

Texture : Fibrous

Yellowish milky latex

Yellowish gum

Cooking

Culinary uses: Used in various types of food such as meat, fish dishes, and vegetables. It is also used as a seasoning for pickles and curries.

(குறிப்பிட்டு: வரலாற்றுரை, குறிப்பிட்டுரை, வணிகச் சுற்றுலasan, வசவத்துறை பல்வேறு வகை வசவத்துறைச் சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுலasan, வசவத்துறை பல்வேறு வகை சுற்றுлasan,
சுருக்கப்பட்டுள்ள கூற்று பன்முகம்: அறிக்கைகள் நோக்கியது

வலரும் காலம் காலத்துலப் பிறந்து வருக

இன்றைய விதையும் விளையும் வென்று வந்தவை

பின்புறத் தொடர்பு விளக்கம் விளக்கப்படும் ஐந்து வகை

புதற்கு

சுருக்கப்பட்டுள்ள அறிக்கைகள், அணிகள், வரலாற்று விளக்கங்கள் மற்றும் வரைப்படங்கள்

வரலாற்று வரைப்படங்கள்

- எண்ணறிகையில் புனிதப் பயிற்சி அறிக்கை, பயிற்சியில் விளக்கும் அறிக்கை, கட்டு தொடங்கி, புனிதத்தில் விளக்கும் வேறு அறிக்கைகள்
- வரலாற்று வரைப்படங்கள் அறிக்கை, வரலாற்று வரைப்படங்கள்
- வரலாற்று வரைப்படங்கள் வெவ்வேறு வகைகள் வழங்கும் வேறு வகைகள் வெவ்வேறு வகைகள்
- வரலாற்று வரைப்படங்கள் அறிக்கைகளுக்கு அதிகாரம் அளியும் மொத்தம், விளக்கம், மொத்தம்

சுருக்கப்பட்டுள்ள பின் வரலாற்று வரைப்படங்கள்

1) தொடர்ந்தும் சொல்லிகள்
2) தொடர்ந்தும் சொல்லிகள்
3) சிகருந்தும் சொல்லிகள்
4) புலியாதும் சொல்லிகள்
5) அறிக்கைகள் நோக்கியவை
6) தொடர்ந்தும் சொல்லிகள்
Phyllanthus emblica

Botanical name: Phyllanthus emblica, Linn

Family: Euphorbiaceae

Organoletic characters

Scent: Fragrant, sweet, and苾mber

Taste: Astringent

Pulp: Sweet

Kinds:

Indian gooseberry

Medicinal Uses:

It is used as a medicine for various ailments such as gastric problems, liver disorders, and as a tonic. (தெய்வநாவல் விளையாட்டுச் சான்று)
பத்ரகம்:

அறிக்கை, முக்கியமான முடிவை கொடுக்க குறிப்பிட்டுகிறது. இக்குறிப்பிட்டு முக்கியமான நூற்றணியால் விளக்கம் இருக்கும்.

சாத்தேன் பங்களிக்கல்

• பத்ரகம் கொண்டுள்ளது குறிப்பிட்டு முக்கியமான நூற்றணியால், குறிப்பிட்டு முக்கியமான நூற்றணியால் விளக்கம் இருக்கும்.
• பத்ரகம் கொண்டுள்ளது குறிப்பிட்டு முக்கியமான நூற்றணியால், குறிப்பிட்டு முக்கியமான நூற்றணியால் விளக்கம் இருக்கும்.
• திட்டம் கொண்டுள்ளது குறிப்பிட்டு முக்கியமான நூற்றணியால், குறிப்பிட்டு முக்கியமான நூற்றணியால் விளக்கம் இருக்கும்.

நூற்றணியுடன் பிரித்து முக்கியக் குறிப்பிட்டு

1) நூற்றணியில் பிரித்து குறிப்பிட்டு
2) நூற்றணியில் குறிப்பிட்டு
3) நூற்றணியில் குறிப்பிட்டு
4) நூற்றணியுடன் குறிப்பிட்டு
5) குறிப்பிட்டு குறிப்பிட்டு
BOTANICAL REVIEW
4.2 BOTANICAL ASPECT

VALLARAI - CENTELLA ASIATICA

Synonym\(^{(6)}\) : *Hydrocotyle asiatica* Linn

Family : Apiaceae

Vernacular names

Tamil : *Vallarai*, Chandaki

English : Indian pennywort

Hindi : Khulakudi

Parts used

Leaf

Organoleptic characters

Taste : Astringent, bitter, sweet

Nature : Neutral

Division : Sweet

Action

Alternative, Tonic, Diuretic, Stimulant, Emmenogogue.

Chemical constituents

Leaves : Essential oil, fatty oil, sitosterols, tannin and resinous substances

Plant : Triterpenoid asiatocoside – A and B

Major Chemical constituents\(^{(8)}\)

Madecassoside, Asiaticoside, Asiatic acid, Brahmic acid
Minor chemical constituents

Asiaticoside B, Brahminoside, Brahmoside, Centelloside, Indcentelloside, Thankuniside, Isothankuniside, Brahmic acid, Isobrahmic acid, Betulic acid, Centic acid, Centoic acid and flavanoid glycosides.

RESEARCH ARTICLE RELATED TO THE STUDY

Acute and sub acute toxicity study\textsuperscript{(9)}

Acute toxicity study of Centella asiatica revealed that the drug at the dose of 10gm/kg did not cause any toxic signs and death within the observation period of 14 days.

Sub chronic toxicity study of ECa\textsubscript{233} at the doses of 10, 100,1000 mg/kg/day showed no difference with regards to body weight, food consumption and health in comparison to the control group except that female rats receiving 1000mg/kg/day of ECa\textsubscript{233} had significantly higher white blood cell counts than the control group.

Pharmacological activities

Anti ulcer activity\textsuperscript{(10)}

The research article revealed that the Centella asiatica extract of dose 100mg/kg, 200mg/kg, 400mg/kg exhibited protection of gastric mucosal layer. Histopathological studies reveals reduction or absence of edema and leucocytes infiltration of submucosal layer. It also possess analgesic and anti-inflammatory activity\textsuperscript{(34)}

Medicinal uses\textsuperscript{(7)}

- The extract from the plant promotes epithelialisation and a very early decrease in the wound surface area.
- It cures haemorrhagic diseases and heart trouble, efficacious in skin affections, anorexia, subduces deranged pitta.
- The leaves of Centellia asiatica is fried in castor oil and applied over swellings in elephantiasis and scrotal swelling.
**Elumichai - Citrus limon**

**Synonym**: Citrus medica L.

**Family**: Rutaceae

**Vernacular names**

Tamil: Elumichchai, arasakani, Kesari

Hindi: Nimbu

English: Acid lime, limon

**Parts used**

Fruit

**Organoleptic Characters**

**Unripe fruit, fruit**

Taste: Sour

Nature: Hot

Division: Acrid

**Action**

Lemon juice: Refrigerent, antiscorbutic action

Fruit: Refrigerant

**Chemical constituents**

Lemons are a rich source of vitamin C. Lemons contain numerous phytochemicals, including polyphenols and terpenes. Abscisic acid, Gibberellic acid, Limonin, Linoleic acid, Ascorbic acid, Carotenoids, Pantothenic acid, pectin and vitamin B1. Fresh lemon juice contains minerals such as Zinc, Selenium, Manganese, Iron, Sodium, Chloride, Calcium. It also contains Vitamin A, Niacin, Vitamin C, Vitamin E and Vitamin K. As with other citrus fruits, they have significant concentrations of citric acid (about 47 g/l in juice).
RESEARCH ARTICLES RELATED TO THE STUDY

Anti-ulcer activity\(^{(33)}\)

The research article revealed that the healthy male albino wistar rats of weight 200-250 g were pretreated with the extract of *Citrus medica* at the dose level of 250 mg kg\(^{-1}\) and 500mg/kg\(^{-1}\) showed a significant reduction in ulcer formation. Histopathological sections showed significant decrease in mucosal ulceration, inflammatory mucosal changes and submucosal edema compared to ethanol treated group and ranitidine group.

It is concluded that the fruits of *Citrus medica* possesses significant anti-ulcer activity against ethanol-induced ulcers in rats and the anti-ulcer activity could be due to the presence of flavonoids as these compounds have well documented anti-ulcer activity.

Analgesic activity\(^{(11)}\)

The research article revealed that the fruit decotion of *Citrus medica* Linn. were found to be effective in a dose level of 4ml/kg which inhibited the pain produced by the hot plate. Thus it is concluded that this study validates the traditional use of *Citrus medica* Linn. as an analgesic.

Medicinal uses\(^{(7)}\)

- The fruit is efficacious in the treatment of vomiting and gastric stimulant.
- The ripe fruit is highly appetizing.
- It is highly recommended in rheumatism affections such as plerodynia, sciatica, lumbago, pain in the hip joints.
Zinziber officinale - Inji

Vernacular names\(^{(6)}\)

Tamil : Inchi, allam, ilaakottai, narumaruoou mathil
English : Ginger
Hindi : Adrakh

Parts used

Bulbous root or rhizome

Organoleptic characters

Taste : Acrid
Nature : Hot
Division : Acrid

Action

Stimulant, Carminative, Stomachic, Sialagogue, Digestive, Rubefacient

Chemical constituents\(^{(8)}\)

Major:

Gingerol, \(\alpha\) – Zingiberene, \(\beta\)-sesquiphellandrene and ar - curcumene, Lipids, Proteins and Starch.

Minor:

- Gingerdiols,
- Gingerdiacetates,
- Gingerdiones,
- Diterpenes, 6- gingersulfonic acid,
- Gingerglycolipids A, B and C.
RESEARCH ARTICLES RELATED TO THE STUDY

Toxicity study\(^{(12)}\)

The research article revealed that the aqueous extract of gingiber rhizome at the dose of 2g/kg b.w. for 14 days indicated that there were no lethal effect upto 1g/kg b.w. and no adverse effect on the major organs.

Pharmacological activity\(^{(13)}\)

The research article revealed that the aqueous extract of ginger root extract of dose 200mg/kg, 400mg/kg when administered to rats, the percentage inhibition of gastric ulcer was 40.91%, 57.58% respectively.

This shows that the ginger root extract significantly inhibited the gastric damage induced by indomethacin and its efficacy as a gastro protective agent was comparable to that of Omeprazole.

Another study reveals that the active components in ginger root could have an antisecretory activity and cytoprotection by increasing mucus wall thickness.

Ginger and its constituents act as digestive aids: possess antiulcer\(^{(18-24)}\), Cholagogic\(^{(25)}\) and antiemetic\(^{(26-28)}\) properties.

Medicinal uses\(^{(7)}\)

- Ginger decotion is giver for colic pain, tympanites and vomiting.
- It is aromatic and beneficial in dropsy, dyspepsia, digestive and stomachic.
- Ginger juice rubbed on and around navel cures all kinds of diarrhoea.
**Emblica officinalis - Nellikai**

**Synonyms**\(^{(6)}\) : *Phyllanthus emblica* Linn.; Cicca emblica Kurz

**Common name** : Emblic myrobalan, Indian Goose berry.

**Family** : Euphorbiaceae

**Vernacular names**

Tamil : Nelli

English : Gooseberry

Hindi : Amlika

**Parts used**

Fruit

**Organoleptic characters**

Taste : Astringent, sweet, sour

Nature : Neutral

Division : Sweet

**Action**

Fruit : Diuretic, refrigerant, laxative

**Chemical Constituents**\(^{(8)}\)

**Fruit**

- Trigalloyliglucose, terchebin, covilagin, ellagic acid, phylembic acid
- Vitamin C, Gallic acid, Ellagic acid, Phylemblic acid, emblicol
- Phyllantidine, Phyllantine, Pectin, Minerals
RESEARCH ARTICLES RELATED TO THE STUDY

Acute toxicity study\textsuperscript{(14)}

The acute and chronic toxicity study revealed that the hydroalcoholic extract of amla (HAE) was prepared and was administered at the dose of 250, 500 and 1000 mg/kg body orally does not cause any mortality up to 48 hr. Hence the LD\textsubscript{50} of \textit{Emblica officinalis} is more than 1000 mg/kg and there was no untoward effect and no gross observational effects were observed at any dose of \textit{Emblica officinalis}.

Pharmacological activity\textsuperscript{(16)}

The research article revealed that the aqueous extract of \textit{Phyllanthus emblica} (PE-AqE 47) when administered to rats inhibited the \textit{H.pylori} growth while its antisecretory, anti-ulcer and gastroprotective effects may be due to its potent antioxidant characteristics. Its significant ulcer protective and healing effects and this might be due to its effects both on offensive and defensive mucosal factors.

Medicinal uses\textsuperscript{(7)}

- It is effective in the treatment of peptic ulcer\textsuperscript{(25-27)} and dyspepsia\textsuperscript{(28)}.
- The leaves of \textit{Phyllanthus emblica} is boiled in water and goggled for mouth ulcers. Decoction of goose berry will remove excessive thirst, giddiness and vomiting sensation.
- Fruits are good liver tonic. The fruit juice is useful in indigestion, jaundice, anaemia, heart complaints. It is a rich source of vitamin C.
MINERALOGICAL REVIEW
4.3 MINERALOGICAL ASPECT

Sodium Chloride Impura – Rock salt

Vernacular names

Tamil : Indhuppu
Eng : Rock salt
Hindi : Sendhalon

**Halite** commonly known as **rock salt**, is the mineral form of sodium chloride (NaCl). Halite forms isometric crystals.

**Source**

Found in nature in extensive beds mostly associated with clay and calcium sulphate.

**Characters**

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

**Physicochemical properties**

- Category : Halide mineral
- Chemical formula : NaCl
- Crystal symmetry : Isometrical hexoctahedral
- Molar mass : 58.433g/mol
- Crystal system : Cubic
- Luster : Vitreous
- Streak : White
- Optical properties : Isotropic
- Refractive index : 1.544
- Solubility : Water soluble
- Other characters : Salty flavour, Fluorescent
Action

- Carminative
- Stomachic
- Digestive

Medicinal uses

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

INDHUPPU BHAVANAI
5. ANALYTICAL STUDY OF THE DRUG *INDHUPPU BHAVANAI*

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

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

Analytical study of the drug includes:

- **Organoleptic characters**
  - Colour
  - Odour
  - Taste
  - Texture
- **Physicochemical analysis**
  - Determination of Ash Values
  - Physical characterization
- **Phytochemical analysis**
  - HPTLC and TLC finger print analysis
- **Chemical analysis**
  - Preliminary Basic and Acidic radical studies
- **Elemental analysis**
  - Inductively Coupled Plasma Optical Emissions Spectrometry (ICP- OES)
- **Analysis of particle size**
  - Scanned Electron Emission (SEM)
PHYSICO CHEMICAL ANALYSIS
5.1 Organoleptic characterization of *Indhuppu bhavanai*

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

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

**Colour**

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

**Odour**

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

The results of Organoleptic character were showed in table - 1

5.2 PHYSICOCHEMICAL ANALYSIS

Physicochemical studies of the trial drug have been done according to the WHO guidelines

**Physical properties of *Indhuppu bhavanai***

The physical properties of *Indhuppu bhavanai* was analyzed at Captain Srinivasamurti Research Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Ch-106.

1. **Determination of pH:**

Five grams of *Indhuppu bhavanai* was weighed accurately and placed in clear 100 ml beaker. Then 50 ml of distilled water was added to it and dissolved well. After 30 minutes it was then applied in to pH meter at standard buffer solution of 4.0, 7.0, and 9.2. Repeated the test four times and average was recorded.
2. Loss on drying of the sample at 105°C -

4g of test drug was weighed in a previously weighed 100ml beaker and heated in an oven at 105°C for 5 hours. Cooled in a dessicator and weighed. Repeated the procedure till constant weight was obtained. The percentage loss in weight of the test drug was calculated by the following formula.

Calculation:

\[
\frac{\text{Loss in weight of test drug}}{\text{Weight of test drug taken}} \times 100
\]

3. Ash content

Total ash content

4g of test drug was weighed accurately in a previously ignited and tared silica dish. The material was evenly spread and ignited in a muffle furnace at 600°C until it became white indicating the absence of carbon. The dish was cooled in a dessicator and weighed. As carbon free ash cannot be obtained in this manner, the dish was cooled and the residue moistened with 2 sufficient quantity of water. Dried on a water bath and then ignited in the electric furnace to get the constant weight. Cooled the dish in a dessicator and then weighed. The percentage of total ash of air-dried materials was calculated as per the formula given below.

Calculation:

\[
\frac{\text{Weight of the ash}}{\text{Weight of test drug taken}} \times 100
\]

4. Acid-insoluble ash

The total ash of the test drug was found out as described above. To the dish containing the total ash was added 45 ml of 1:5 hydrochloric acid in three portions of 13 ml each time. Boiled gently for 5 minutes and filtered. Collected the insoluble matter on an ashless filter paper (Whatman No.41) and washed with distilled water until the residue was free from acid. Transfer the filter paper containing the insoluble matter to the original dish. Dried and ignited to the constant weight. Cooled the dish in a dessicator, and then weighed. Calculation was made by given formula.
Calculation:

\[
\text{Percentage of acid-insoluble ash} = \frac{\text{Weight of the acid-insoluble residue}}{\text{Weight of test drug taken}} \times 100
\]

5. Water-soluble extractive of the test drug

4 g of the test drug was weighed accurately in a glass stoppered flask. Added 100 ml of distilled water and shaken occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre weighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105°C for 6 hours. Cooled in a dessicator and weighed. Repeated the experiment twice, and taken the average value. The percentage of water soluble extractive was calculated by the formula given below.

Calculation:

\[
\text{Percentage of water soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100
\]

6. Alcohol-soluble extractive of the sample

4 g of the sample was weighed accurately in a glass stoppered flask. Added 100 ml of distilled alcohol (approximately 95%) and shaken occasionally for 6 hours and then allowed to stand for 18 hours. Filtered rapidly taking care not to lose any solvent and pipetted out 25 ml of the filtrate in a pre weighed 100 ml beaker and evaporated to dryness on a water bath. Kept in an air oven at 105°C for 6 hours and cooled in a dessicator and weighed. Repeated the experiment twice, and taken the average value. The percentage of alcohol soluble extractive was calculated by the formula given below.

Calculation:

\[
\text{Percentage of alcohol soluble extract} = \frac{\text{Weight of the extract}}{\text{Weight of sample taken}} \times \frac{100}{25} \times 100
\]

RESULTS

The results of physico chemical properties were represented in table 2.
CHEMICAL ANALYSIS
The chemical analysis of *Indhuppu Bhavanai* 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>INFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Physical Appearance of extract</td>
<td>Dark brown in colour</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><strong>Test for Silicate</strong>&lt;br&gt;a. A 500 mg of the sample was shaken well with distilled water.</td>
<td>Completely soluble</td>
<td>Absence of Silicate</td>
</tr>
<tr>
<td>3.</td>
<td><strong>Action of Heat:</strong>&lt;br&gt;A 500 mg of the sample was taken in a dry test tube and heated gently at first and then strong.</td>
<td>No White fumes evolved.&lt;br&gt;No brown fumes evolved.</td>
<td>Absence of Carbonate&lt;br&gt;Absence of Nitrate.</td>
</tr>
<tr>
<td>4.</td>
<td><strong>Flame Test:</strong>&lt;br&gt;A 500 mg 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.</td>
<td>No bluish green flame appeared</td>
<td>Absence of copper</td>
</tr>
<tr>
<td>5.</td>
<td><strong>Ash Test:</strong>&lt;br&gt;A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.</td>
<td>No yellow colour flame appeared</td>
<td>Absence of sodium</td>
</tr>
</tbody>
</table>

**Preparation of Extract:**

5 gm of *Indhuppu bhavanai* (IB) was taken in a 250 ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 10 minutes. Then it is cooled and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation is used for the qualitative analysis of acidic/basic radicals and chemical constituents in it.
<table>
<thead>
<tr>
<th>S. No</th>
<th>EXPERIMENT</th>
<th>OBSERVATION</th>
<th>INFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I. Test For Acid Radicals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Test For Sulphate:</td>
<td>a. 2 ml of the above prepared extract was taken in a test tube to this add 2 ml of 4% dil ammonium oxalate solution</td>
<td>Cloudy appearance present</td>
<td>Presence of Sulphate</td>
</tr>
<tr>
<td></td>
<td>b. 2 ml of the above prepared extract is added with 2 ml of diluted HCL is added until the effervescence ceases off. Then 2 ml of Barium Chloride solution is added.</td>
<td>A white precipitate insoluble in con.HCL was obtained.</td>
<td>Sulphate was confirmed</td>
</tr>
<tr>
<td>2. Test For Chloride:</td>
<td>2 ml of the above prepared extracts was added with 2 ml of dil- HCl is added until the effervescence ceases off.</td>
<td>Cloudy appearance present.</td>
<td>Presence of Chloride</td>
</tr>
<tr>
<td>3. Test For Phosphate:</td>
<td>2 ml of the extract were treated with 2 ml of dil.ammonium molybdate solution and 2 ml of con.HNO3</td>
<td>No Cloudy yellow appearance formed</td>
<td>Absence of Phosphate</td>
</tr>
<tr>
<td>4. Test For Carbonate:</td>
<td>2 ml of the extract was treated with 2 ml diluted magnesium sulphate solution</td>
<td>No cloudy appearance</td>
<td>Absence of carbonate</td>
</tr>
<tr>
<td>5. Test For Nitrate:</td>
<td>1 gm of the extract was heated with copper turning and concentrated H2So4 and viewed the test tube vertically down.</td>
<td>No Brown gas was evolved</td>
<td>Absence of nitrate</td>
</tr>
<tr>
<td>6. Test For Sulphide:</td>
<td>1 gm of the extract was treated with 2 ml of con. HCL</td>
<td>No rotten egg smelling gas evolved</td>
<td>Absence of sulphide</td>
</tr>
<tr>
<td>7. Test For Fluoride &amp; Oxalate:</td>
<td>2 ml of extract was added with 2 ml of dil. Acetic acid and 2 ml dil.calcium chloride solution and heated.</td>
<td>No cloudy appearance.</td>
<td>Absence of fluoride and oxalate</td>
</tr>
<tr>
<td>8. Test For Nitrite:</td>
<td>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.</td>
<td>No characteristic changes</td>
<td>Absence of nitrite</td>
</tr>
</tbody>
</table>
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. 

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>OBSERVATION</th>
<th>INFERENGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluish green colour flame not appeared</td>
<td>Absence of borate</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S.No</th>
<th>EXPERIMENT</th>
<th>OBSERVATION</th>
<th>INFERENGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>II. Test For Basic Radicals</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| 1.  | **Test For Lead:**
2ml of the extract was added with 2ml of dil. potassium iodine solution. | No Yellow precipitate appeared | Absence of lead |
| 2.  | **Test For Copper:**
a. One pinch (25 mg) of extract was made into paste with con. HCl in a watch glass and introduced into the non-luminuous part of the flame. | No blue colour precipitate appeared | Absence of copper |
| 3.  | **Test For Aluminium:**
To the 2 ml of extract dil.sodium hydroxide was added in 5 drops to excess. | No characteristic changes | Absence of Aluminium. |
| 4.  | **Test For Iron:**
a. To the 2 ml of extract add 2 ml of dil.ammonium solution
b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added | Mild Red colour appeared | Presence of Iron |
| 5.  | **Test For Zinc:**
To 2 ml of the extract dil.sodium hydroxide solution was added in 5 drops to excess and dil.ammonium chloride is added. | No White precipitate was formed | Absence of Zinc |
| 6.  | **Test For Calcium:**
2 ml of the extract was added with 2 ml of 4% dil.ammonium oxalate solution | Cloudy appearance and white precipitate was obtained | Presence of calcium |
| 7.  | **Test For Magnesium:**
To 2 ml of extract dil.sodium hydroxide solution was added in drops to excess. | No White precipitate was obtained | Absence of magnesium |
8. **Test For Ammonium:**
   To 2 ml of extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution are added.
   
   **Observation:** No Brown colour appeared
   **Inference:** Absence of ammonium

9. **Test For Potassium:**
   A pinch (25 mg) of extract was treated of with 2 ml of dil.sodium nitrite solution and then treated with 2 ml of dil.cobalt nitrate in 30% dil.glacial acetic acid.
   
   **Observation:** No Yellow precipitate was obtained
   **Inference:** Absence of potassium

10. **Test For Sodium:**
    2 pinches (50 mg) of the extract is made into paste by using HCl and introduced into the blue flame of Bunsen burner.
    
    **Observation:** No yellow colour flame evolved
    **Inference:** Absence of sodium

11. **Test For Mercury:**
    2 ml of the extract was treated with 2 ml of dil.sodium hydroxide solution.
    
    **Observation:** No Yellow precipitate obtained
    **Inference:** Absence of Mercury

12. **Test For Arsenic:**
    2 ml of the extract was treated with 2 ml of dil.sodium hydroxide solution.
    
    **Observation:** No Brownish red precipitate obtained
    **Inference:** Absence of arsenic

<table>
<thead>
<tr>
<th>S.No</th>
<th>EXPERIMENT</th>
<th>OBSERVATION</th>
<th>INFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><strong>Test For Starch:</strong></td>
<td>No Blue colour developed</td>
<td>Absence of starch</td>
</tr>
<tr>
<td></td>
<td>2 ml of extract was treated with weak dil.Iodine solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><strong>Test For Reducing Sugar:</strong></td>
<td>No Brick red colour developed</td>
<td>Absence of reducing sugar</td>
</tr>
<tr>
<td></td>
<td>5 ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes are noted.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td><strong>Test For The Alkaloids:</strong></td>
<td>Yellow colour developed</td>
<td>Presence of Alkaloid</td>
</tr>
<tr>
<td></td>
<td>a) 2 ml of the extract was treated with 2ml</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
of dil. potassium iodide solution.
b) 2 ml of the extract was treated with 2 ml of dil. picric acid.
c) 2 ml of the extract was treated with 2 ml of dil. phosphotungstic acid.

<table>
<thead>
<tr>
<th></th>
<th>Test For Tannic Acid:</th>
<th>Black precipitate obtained</th>
<th>Presence of Tannic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2 ml of extract was treated with 2 ml of dil. ferric chloride solution</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Test For Unsaturated Compound:</th>
<th>Potassium permanganate was not decolourised</th>
<th>Absence of unsaturated compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>To the 2 ml of extract 2 ml of dil. potassium permanganate solution is added.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Test For Amino Acid:</th>
<th>No violet colour</th>
<th>Absence of amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>2 drops of the extract was placed on a filter paper and dried well. 20 ml of Burette reagent is added.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Test For Type Of Compound:</th>
<th>No green and red colour</th>
<th>Absence of quinolepinephrine pyrocatechol Anti pyrine.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>2 ml of the extract was treated with 2 ml of dil. ferric chloride solution.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

The results of acid and basic radicals were showed in table – 3 and table 4
TLC/HPTLC FINGER PRINT ANALYSIS
5.4 TLC/HPTLC FINGER PRINT ANALYSIS

Thin layer chromatography (TLC) was a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound.

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

TLC and HPTLC Methodology

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

Retention Factor

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

$$ R_f = \frac{\text{distance traveled by sample}}{\text{distance traveled by solvent}} $$

The Rf value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions. The compound with the larger Rf value was less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower Rf value.
ELEMENTAL ANALYSIS
5.5 DETERMINATION OF MICROBIAL LOAD

Microbial analysis was carried for determination of microbial contamination as per procedures of Quality Control Methods for Medicinal plant Materials, WHO Geneva 1998 Guideline.

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

RESULTS

The results of microbial load were represented in table 6.

5.6 HEAVY METAL ANALYSIS – ICP-OES

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 EMISSIONS SPECTROMETRY

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 photos emitted is directly proportional to the concentration of the element. The photos may be detected either sequentially or simultaneously.

Quantitative analysis is achieved by measuring the intensity of these specific wavelength and after performing the calibration using known standards.

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

Perkin Elmer Optima 5300DV was used for standard ICP-OES analysis.

**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 upto 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.

**RESULT**

The analytical results of heavy metals and trace elements in *Indhuppu bhavanai* using ICP-OES were showed in table 7.
PARTICLE SIZE ANALYSIS
5.7 SCANNED ELECTRON MICROSCOPY (SEM)

The particle size of the *Indhuppa bhavanai* 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.

**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.

The results were represented in figure 1.
TOXICOLOGICAL STUDIES
6. TOXICOLOGICAL EVALUATION OF INDHUPPU BHAVANAIIN RODENTS

Introduction:

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

Scope of work:

Monitoring Programme, while there are regulatory and cultural differences in the preparation and use of different types of medicines, they are all equally important from a pharmacovigilance assurance of safety, quality and efficacy of Indian System of Medicines (ISM) was the key issue that needs to be addressed while conducting toxicity studies. It was an essential step, which will strengthen the acceptance of Siddha medicines by scientific community. Information of toxicity and adverse effects of these formulations were lacking. Some of the formulations were proved to be effective in various animal studies and many more are yet to be tested.

Hence, the present study was carried out to evaluate the Preclinical animal toxicity studies of Indhuppu bhavana in rodents.

Plan of work:

The following studies were carried out on Indhuppu bhavanai.

- Acute Oral toxicity - OECD 423
- Repeated dose 28 days oral toxicity Study - OECD 407
- Repeated dose 90 days oral toxicity study - OECD 408
ACUTE TOXICITY STUDY
6.1 ACUTE ORAL TOXICITY STUDY OF INDHUPPU BHAVANAI

(OECD GUIDELINE – 423)

Acute oral toxicity-experiment procedure:

Acute toxicity study was carried out according to the OECD (Organization of Economic Co-operation and Development) guidelines 423. Healthy female rats, weighing 150–200g, were selected and oral administration of the single doses of Indhuppu Bhavanai was done aseptically by suspending in 1ml honey.

Experimental animals:

Albino rats (wistar rats) of either sex, weighing (150-200 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 ± 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 (IAEC approval no - KKCP/2015/037). Animal experimentation protocols are approved by Institutional Animal Ethical Committee.

Administration of doses:

Indhuppu Bhavanai was mixed with honey and it was administered as a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for further 3 to 4 hours. The principle of laboratory animal care was followed.

Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

An oral (p.o) dose of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg was administered step by step according to the guidelines. The general behaviors of the rats
were continuously monitored for 1 h after dosing, periodically during the first 24 h (with special attention given during the first 4 hours and then daily thereafter, for a total of 14 days. Changes in the normal psychomotor activity and external morphology and their body weights were monitored periodically before dosing and the time at which signs of toxicity or mortality were recorded.

The visual observations included skin changes, morbidity, aggressiveness, sensitivity to sound and pain, as well as respiratory movements were recorded. They were deprived of food, but not water 12 h prior to the administration of the test substance. Finally, the number of survivors were noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>: Indhuppu Bhavanai</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Source</td>
<td>: Animal house of King Institute of Preventive Medicine</td>
</tr>
<tr>
<td>Animals</td>
<td>: Male and Female Wistar Albino Rats</td>
</tr>
<tr>
<td>Age</td>
<td>: More than 8 weeks</td>
</tr>
<tr>
<td>Acclimatization</td>
<td>: Seven days prior to dosing.</td>
</tr>
<tr>
<td>Veterinary examination</td>
<td>: Prior to and at the end of the acclimatization period.</td>
</tr>
<tr>
<td>Identification of animals</td>
<td>: By cage number, animal number and individual marking on fur.</td>
</tr>
<tr>
<td>Diet</td>
<td>: Pelleted feed supplied by Godrej foods Pvt Ltd, Bangalore</td>
</tr>
<tr>
<td>Water</td>
<td>: Potable water in polypropylene bottles ad libitum.</td>
</tr>
</tbody>
</table>
Housing & Environment: The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature: Between 20 & 24°C,

Relative humidity: Between 30% and 70%,

Dark and light cycle: Each of 12 hours.

Duration of the study: 14 days

Number of animals and dose levels:

Three animals were used for each step. The dose level used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level was most likely to produce mortality in some of the dosed animals. The available information suggests that mortality is likely at the highest starting dose level 2000mg/kg body weight, so the trial or limit test was conducted. The time interval between treatment groups is determined by the onset, duration and severity of toxic signs.

Observations

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

All observations were systematically recorded with individual records being maintained for each animal. Observations include changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavior pattern.
Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Humane End points Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed. When animals are killed for humane reasons or found dead, the time of death should was recorded. From the maximum dose 1/5th or 1/10th of the dose was considered as therapeutic dose for further study.

Results:

All data were summarized in table 8.
REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY
6.2 REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF

**INDHUPPU BHAVANAI (OECD GUIDELINE - 407)**

Sub-acute toxicity study was carried out according to OECD 407 and rats were divided into 3 groups of 10 animals (5 male and 5 female). *Indhuppu Bhavanai* with honey was administered to rats at the dose of 900mg /kg/day and 1800 mg/kg/day continuously for 28 days. The animals were observed daily for gross behavioural changes and other sign of sub acute toxicity. The weight of the each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per rat was calculated. At the end of 28 days they were fasted overnight, each animal were anaesthetized with diethyl ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of 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 : *Indhuppu Bhavanai*

Animal Source : Animal house of King Institute of Preventive Medicine

Animals : Male and Female Wistar Albino Rats

Age : More than 8 weeks

Acclimatization : Seven days prior to dosing.

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

Identification of animals : By cage number, animal number and individual marking on fur.

Diet : Pelleted feed supplied by Godrej foods Pvt Ltd, Bangalore

Water : Portable water in polypropylene bottles ad libitum.
Housing & Environment: The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature: Between 20 & 24°C,

Relative humidity: Between 30% and 70%,

Dark and light cycle: Each of 12 hours.

Duration of study: 28 days

Justification for Dose Selection:

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

Preparation and administration of dose:

Indhuppu Bhavanai at two doses level 900mg/kg and 1800 mg/kg respectively were prepared. The test substance was prepared every day for 7 days once 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 consists of 10 rats (5 male and 5 female) dosing up to 28 days. Animals acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were 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 percentage of body weight gain were calculated (table -9 and table 17)

ii) Food Consumption:

The quantity of food consumed by groups consisting of ten animals for different doses was recorded at weekly interval. Food consumed per animal was calculated for control and the treated dose groups. (table 11)

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 h, 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.

vi) Haematological Investigations:

Blood samples of control and experimental rats were analyzed for hemoglobin, 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.
vii) Biochemical Investigations:

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

viii) Necropsy:

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

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

ix) Histopathology:

Histopathological investigation of the vital organs were done. The organ piece (3-5µm thick) of the highest dose level of 4500mg /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, stomach 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, hematology and blood chemistry were subjected to One-way Anova Followed by Dunnet’s test using a computer software programme. (Graph Pad Prism 5.0). All data were summarized in tabular form(Table 9 – 17)
REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY
6.3 REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY OF

**INDHUPPU BHAVANAI (OECD GUIDELINE - 408)**

<table>
<thead>
<tr>
<th>Test Substance</th>
<th><strong>INDHUPPU BHAVANAI</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Source</td>
<td>Animal house of King Institute of Preventive Medicine</td>
</tr>
<tr>
<td>Animals</td>
<td>Wister Albino Rats (Male -3, and Female-3)</td>
</tr>
<tr>
<td>Age</td>
<td>6-8 weeks</td>
</tr>
<tr>
<td>Body Weight</td>
<td>150-200gm.</td>
</tr>
<tr>
<td>Acclimatization</td>
<td>Seven days prior to dosing.</td>
</tr>
<tr>
<td>Veterinary examination</td>
<td>Prior and at the end of the acclimatization period.</td>
</tr>
<tr>
<td>Identification of animals</td>
<td>By cage number, animal number and individual marking by using Picric acid.</td>
</tr>
<tr>
<td>Diet</td>
<td>Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore</td>
</tr>
<tr>
<td>Water</td>
<td>Aqua guard portable water in polypropylene bottles.</td>
</tr>
<tr>
<td>Housing &amp; Environment</td>
<td>The animals were housed in Polypropylene cages provided with bedding of husk.</td>
</tr>
<tr>
<td>Housing temperature</td>
<td>between 22°C + 3°C.</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>between 30% and 70%,</td>
</tr>
<tr>
<td>Air changes</td>
<td>10 to 15 per hour</td>
</tr>
<tr>
<td>Dark and light cycle</td>
<td>12:12 hours.</td>
</tr>
<tr>
<td>Duration of the study</td>
<td>90 Days.</td>
</tr>
</tbody>
</table>
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) IAEC approval no NIS/IAEC – I/2016/05. Ist group treated as a control and other three group were treated with test drug (low, mid, high) for 90 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Justification for Dose Selection:

As per OECD guideline three dose levels were selected for the study. They were low dose (X), mid dose dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose (5gm) and the body surface area of the rat (0.018). i.e X dose is 180 mg/kg, 5X dose is 900mg/kg and 10 X 1800 mg/kg of animal.

Preparation and Administration of Dose:

*Indhuppu bhavanai* 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:

Body Weight:

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study (Table 18)

Clinical signs:

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

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

Laboratory Investigations:

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

Haematological Investigations:

Haematological parameters were determined using Haematology analyzer.

Biochemical Investigations:

Biochemical parameters were determined using auto-analyzer.

Histopathology:

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

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, haematology and blood chemistry were subjected to One-way ANOVA followed by dunnet’t test using a computer software programme -INSTAT-V3 version. All data were summarized in tabular form(Table 18–23).
PHARMACOLOGICAL STUDY
ANTI-ULCER ACTIVITY
7.1 ANTIULCER ACTIVITY OF *INDHUPPU BHAVANAION* ON WISTAR ALBINO RATS

**Aim:**

To evaluate the antiulcer activity of *Indhuppu bhavanai* in Wistar albino rats by *Aspirin induced ulcer model*.

**Selection of Experimental animals:**

Healthy Wistar albino rats of either sex weighing (150-200 gms) were used for this study. The animals were obtained from animal house, K.K college of pharmacy, Gerugambakkam, Chennai. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70% at 12:12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet. All the experimental procedures and protocols used in this study were reviewed by (IAEC) Institutional Animal Ethics Committee (IAEC approval No KKCP/2015/037) of K.K college of Pharmacy and were in accordance with the guidelines of the IAEC.

**Aspirin Induced Gastric Ulcers**

**Principle:**

Aspirin was a NSAID which inhibit the synthesis of prostaglandins. Prostaglandins protect the gastric mucosa by producing leukotrienes and bicarbonate ions. Aspirin also inhibit the gastric peroxidase and may increase mucosal hydrogen peroxide and hydroxyl ions level to cause oxidative mucosal damage.

**Procedure:**

Albino rats of either sex weighing between 150-250 gms were divided into five groups and each consisting of six rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control (2ml/kg p.o.)</td>
</tr>
<tr>
<td>II</td>
<td>Negative control received only Aspirin (500mg/kg)</td>
</tr>
<tr>
<td>III</td>
<td>Standard drug Ranitidine(20mg/kg)+ Aspirin (500 mg/kg)</td>
</tr>
<tr>
<td>IV</td>
<td>Received <em>Indhuppu bhavanai</em> 200mg/kg + Aspirin (500 mg/kg)</td>
</tr>
<tr>
<td>V</td>
<td>Received <em>Indhuppu bhavanai</em> 400mg/kg + Aspirin (500 mg/kg)</td>
</tr>
</tbody>
</table>
The animals were fasted for 24 hours. The test drug in varying concentration based on the
design of the experiment is administered orally 30 minute prior to aspirin at dose of 500
mg/kg. 4hours later the rats were scarified by using anaesthetic ether and their stomachs
were dissected and they were opened along the greater curvature for the determination of
gastric lesions. Ulcer index was calculated by the number of ulcers per animal and
severity scored by observing the ulcers microscopically with the help of 10X lens.

**Evaluation of parameters:**

**Effect of Free Acidity and Total Acidity :**

The free acidity and total acidity was determined based on the titre values. One ml
of gastric juice was pipetted into 100 ml of conical flask and titrates with 0.01N NaOH
using topers reagent as an indicator (It is Dimethyl –amino-azo-benzene with
phenolphthalein and used for the detection and estimation of hydrochloric acid and total
acidity in gastric fluids) titrate to end point, when the solution turns to orange colour, note
the volume of NaOH which corresponds to free acidity. Titrate further till the solution
regains its pink colour. Note the volume of NaOH which corresponds to the free acidity.
Acidity (mEq/L/100g) can be expressed as

\[
\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times 100
\]

**Ulcer index :**

The ulcer index was calculated by taking the mean ulcer score of each groups.
Then the mean ulcer score graph was plotted with groups on x-axis and ulcer index on y-axis. The histograms of different groups were then interpolated by comparing the ulcer index of groups

**Collection of Gastric Juice**

The stomach was excised carefully opened along the greater curvature and the
gastric contents were removed. The gastric contents were collected in plain tubes and
centrifuged at 3000 rpm for 5 min, the volume of the supernatant was expressed as ml /100
gm body weight. The mucosa was flushed with saline and observed for gastric lesions using a dissecting microscope, ulcer score was determined.

**Ulcer Scoring**

After sacrificing the rat, stomach was removed and opened along the greater curvature, and washed it slowly under running tap water. Put it on the glass slide and observe under 10X magnification for ulcer. Score the ulcers as below.

0 = normal coloured stomach
0.5 = red colouration
1 = spot ulcers
1.5 = haemorrhagic streaks
2 = Ulcers ≥ 3 but ≤ 5
3 = Ulcers >5

Mean ulcer score for each animal is expressed as Ulcer Index.

The results of anti-ulcer activity by Aspirin induced ulcer model showed in table - 26
ANTI-INFLAMMATORY ACTIVITY
7.2 ANTI-INFLAMMATORY ACTIVITY OF *INDHUPPU BHAVANAI* ON WISTAR ALBINO RATS

**AIM:**

To evaluate the anti-inflammatory activity of *Indhuppu bhavanai* in Wistar albino rats by Cotton pellet granuloma method.

**Selection of Experimental animals:**

The experimental protocol was submitted and approved by institutional Ethical Committee, *(IAEC approval No KKCP/2015/037)*. Wistar albino rats (150-180 gm) of approximate same age were employed in this investigation. The animals were obtained from animal house, K.K college of pharmacy, gerugambakkam, Chennai. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70% at 12:12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet.

**Experimental Design for Cotton pellet granuloma model**

The animals were divided into four groups each group consists of 6 animals.

Group-I : Control - Vehicle control received distilled water (dose: 10 ml/kg).

Group-II : Standard drug - Animals treated with Dexamethasone (dose: 0.5 mg/kg).

Group-III : Animals treated with *Indhuppu Bhavanai* (200 mg/kg).

Group-IV : Animals treated with *Indhuppu Bhavanai* (400 mg/kg)

**Experimental procedure**

Inflammation was induced by cotton pellet granuloma model. This method was carried out by using sterilized cotton pellet implantation method in rats. Under light ether anesthesia by using blunted forceps, subcutaneous tunnel was made and sterilized cotton pellets (10 ± 1 mg) were implanted in the axilla and groin region of the rat. After recovering from anaesthesia, animals were treated orally with vehicle control (Distilled water 10 ml / kg), Dexamethasone 0.5 mg/kg, low dose (200mg/kg) and high dose (400mg/kg) of *Indhuppu Bhavanai* for consecutive 7 days, once per day. They were
sacrificed on day 8th by cervical dislocation and the pellets were removed and immediately the wet weight was taken, freed from extraneous tissue and dried at 600C for 24 hrs. The percentage inhibition of wet weight and dry weight of the granuloma were calculated and compared.

\[
\text{Control - Treated} \\
\text{Percentage inhibition (\%)} = \frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100
\]

The results of anti-inflammatory activity by Cotton pellet granuloma method showed in table - 27.
ANALGESIC ACTIVITY
7.3 ANALGESIC ACTIVITY OF INDHUPPU BHAVANAI ON SWISS ALBINO MICE

AIM:

To evaluate the Analgesic activity of Indhuppu bhavanai in Wistar albino rats by Eddy’s Hot plate method.

Selection of Experimental animals:

The experimental protocol was submitted and approved by institutional Ethical Committee, (IAEC approval No KKCP/2015/037). Swiss albino mice (20-25 gm) of approximate same age were employed in this investigation. The animals were obtained from animal house, K.K College of Pharmacy, Gerugambakkam, Chennai. Animals were housed at a temperature of 24±2c and relative humidity of 30-70% at 12:12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet.

Evaluation of Analgesic activity

Pain is the part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus. The ascending pathway of pain includes the contra lateral spinothalamic tract, lateral pons, mid brain to thalamus and ultimately through the somatosensory cortex of the brain that determines the locations, intensity and depth of pain

Eddy’s Hot plate method:

Principle:

Painful reactions can be produced in experimental animals by applying noxious stimuli such as thermal – using radiant heat as a source of pain, chemical – using irritants such as acetic acid and bradykinin and physical pressure – using tail compression.

The hot plate test was a test of the pain response in animals. It was used in basic pain research and in testing the effectiveness of analgesics by observing the reaction to pain caused by heat.
They used a behavioral model of nociception where behaviors such as jumping and hind paw-licking are elicited following a noxious thermal stimulus. Licking was a rapid response to painful thermal stimuli that was a direct indicator of nociceptive threshold. Jumping represents a more elaborated response, with a latency and encompasses an emotional component of escaping.

**Animals**

Mice 20-25 g were grouped in four groups, six animals in each group.

**Grouping:**

- **Group I**: Control - distilled water (10ml/kg, p.o.),
- **Group II**: Standard drug - Pentazocine (5mg/kg, p.o.)
- **Group III**: *Indhuppu Bhavannai* (200mg/kg)
- **Group IV**: *Indhuppu Bhavannai* (400mg/kg)

**Equipment:**

- Eddy’s Hot plate

**Procedure:**

Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at 55±1°C. Jumping response was seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals 10 seconds was kept as a cut off time. The time obtained was considered the basal / normal reaction time in all the untreated groups of animals. Increase in the basal reaction time was the index of analgesia.

All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. A final reading of the basal reaction time was recorded for the included animals. After selecting the animals, the drugs were administered to all the groups at the stipulated doses. The reaction times of the animals were then noted at 0, 30, 60, 90, 120 and 150 mins interval after drug administration.
Statistical analysis

Results were expressed as mean ± SEM and analyzed using Graph Pad Prism software. One way analysis of variance (ANOVA) test was applied.

P value less than 0.05 (P<0.05) was considered as statistically significant.

The results of analgesic activity by Eddy’s Hot plate method was represented in table 28.
RESULTS
8 RESULTS

5.1 ORGANOLEPTIC CHARACTER*

Table 1. Organoleptic characters of Indhuppu bhavanai

<table>
<thead>
<tr>
<th>Colour</th>
<th>Dark Brown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour</td>
<td>Characteristic odour</td>
</tr>
<tr>
<td>Taste</td>
<td>Characteristic taste</td>
</tr>
<tr>
<td>Texture</td>
<td>Fine powder</td>
</tr>
</tbody>
</table>

5.2 PHYSICOCHEMICAL ANALYSIS*

Table 2 Physicochemical properties of Indhuppu bhavanai

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>pH at 10% of aqueous solution</td>
<td>3.91</td>
</tr>
<tr>
<td>2.</td>
<td>Total Ash</td>
<td>62.12%</td>
</tr>
<tr>
<td>3.</td>
<td>Water soluble extractive</td>
<td>45.52%</td>
</tr>
<tr>
<td>4.</td>
<td>Acid insoluble ash</td>
<td>0.1%</td>
</tr>
<tr>
<td>5.</td>
<td>Loss on drying @ 105ºC (w/w)</td>
<td>6.04%</td>
</tr>
<tr>
<td>6</td>
<td>Alcohol soluble extractive</td>
<td>8.92</td>
</tr>
</tbody>
</table>

*The experimental procedure was analyzed at Captain Srinivasamurti Reseach Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Ch-106.
5.3 CHEMICAL ANALYSIS *

Table 3:  Results of acid radicals studies of Indhuppu bhavanai

<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</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>present</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Test for Chloride</td>
<td>Cloudy appearance</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>present</td>
<td></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></td>
<td>Negative</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 Fluride &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 Chloride and sulphate.

*The chemical analysis was carried out in Bio chemistry Lab, NIS, Ch – 47
Table: 4  Results of basic radicals studies of *Indhuppu bhavanai*

<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 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>Blood red colour</td>
<td>Positive</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>-</td>
<td>Negative</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>-</td>
<td>Negative</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 **Iron, Calcium** and absence of heavy metals such as lead, arsenic and mercury.

*The chemical analysis was carried out in Bio chemistry Lab, National Institute Of Siddha, Ch- 47*
5.4 TLC and HPTLC analysis of *Indhuppu bhavanai*

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

**Table 5:**

<table>
<thead>
<tr>
<th>S.No</th>
<th>254nm Colour</th>
<th>254nm Rf</th>
<th>366nm Colour</th>
<th>366nm Rf</th>
<th>Dipped in Vanillin-Sulphuric Acid Colour</th>
<th>Dipped in Vanillin-Sulphuric Acid Rf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Green</td>
<td>0.08</td>
<td>Red</td>
<td>0.54</td>
<td>Grey</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>Green</td>
<td>0.34</td>
<td>Blue</td>
<td>0.86</td>
<td>Grey</td>
<td>0.23</td>
</tr>
<tr>
<td>3</td>
<td>Green</td>
<td>0.97</td>
<td>Blue</td>
<td>0.91</td>
<td>Red orange</td>
<td>0.27</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>Red</td>
<td>0.98</td>
<td>Grey</td>
<td>0.47</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grey</td>
<td>0.81</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Grey</td>
<td>0.86</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brown</td>
<td>0.97</td>
</tr>
</tbody>
</table>

*The experimental procedure was analyzed at Captain Srinivasamurti Research Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Ch-106.*
TLC Photo documentation of DTL 1510354 Sample (Ethanol extract)

254nm

366nm

After dipping vanillin sulphuric acid

Solvent system : Toluene: Ethyl acetate: formic acid (4:6:0.1)

Track 1-5 µl; Track 2-10µl; Track 3-15µl
HPTLC fingerprint profile of DTL 1510354 sample (Ethanol extract)-5µl -254nm

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Position</th>
<th>Start Height</th>
<th>Max Position</th>
<th>Max Height</th>
<th>Max %</th>
<th>End Position</th>
<th>End Height</th>
<th>Area</th>
<th>Area %</th>
<th>Assigned Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00 Rf</td>
<td>2.9 AU</td>
<td>0.02 Rf</td>
<td>102.4 AU</td>
<td>8.34%</td>
<td>0.03 Rf</td>
<td>38.4 AU</td>
<td>1567.6 AU</td>
<td>4.07%</td>
<td>unknown *</td>
</tr>
<tr>
<td>2</td>
<td>0.03 Rf</td>
<td>88.8 AU</td>
<td>0.04 Rf</td>
<td>97.1 AU</td>
<td>7.91%</td>
<td>0.06 Rf</td>
<td>9.8 AU</td>
<td>1240.0 AU</td>
<td>3.18%</td>
<td>unknown *</td>
</tr>
<tr>
<td>3</td>
<td>0.07 Rf</td>
<td>0.5 AU</td>
<td>0.11 Rf</td>
<td>85.2 AU</td>
<td>6.94%</td>
<td>0.15 Rf</td>
<td>0.3 AU</td>
<td>2632.0 AU</td>
<td>6.74%</td>
<td>unknown *</td>
</tr>
<tr>
<td>4</td>
<td>0.17 Rf</td>
<td>0.3 AU</td>
<td>0.28 Rf</td>
<td>571.1 AU</td>
<td>46.52%</td>
<td>0.33 Rf</td>
<td>4.2 AU</td>
<td>26074.7 AU</td>
<td>68.78%</td>
<td>unknown *</td>
</tr>
<tr>
<td>5</td>
<td>0.39 Rf</td>
<td>0.6 AU</td>
<td>0.47 Rf</td>
<td>118.2 AU</td>
<td>9.63%</td>
<td>0.48 Rf</td>
<td>9.0 AU</td>
<td>3144.7 AU</td>
<td>8.05%</td>
<td>unknown *</td>
</tr>
<tr>
<td>6</td>
<td>0.48 Rf</td>
<td>99.9 AU</td>
<td>0.49 Rf</td>
<td>131.3 AU</td>
<td>10.69%</td>
<td>0.51 Rf</td>
<td>25.3 AU</td>
<td>1757.5 AU</td>
<td>4.60%</td>
<td>unknown *</td>
</tr>
<tr>
<td>7</td>
<td>0.55 Rf</td>
<td>22.4 AU</td>
<td>0.56 Rf</td>
<td>25.1 AU</td>
<td>2.04%</td>
<td>0.59 Rf</td>
<td>0.2 AU</td>
<td>345.5 AU</td>
<td>0.88%</td>
<td>unknown *</td>
</tr>
<tr>
<td>8</td>
<td>0.61 Rf</td>
<td>6.0 AU</td>
<td>0.64 Rf</td>
<td>43.5 AU</td>
<td>3.54%</td>
<td>0.67 Rf</td>
<td>2.8 AU</td>
<td>1072.7 AU</td>
<td>2.75%</td>
<td>unknown *</td>
</tr>
<tr>
<td>9</td>
<td>0.85 Rf</td>
<td>1.8 AU</td>
<td>0.88 Rf</td>
<td>22.7 AU</td>
<td>1.85%</td>
<td>0.91 Rf</td>
<td>1.8 AU</td>
<td>528.3 AU</td>
<td>1.35%</td>
<td>unknown *</td>
</tr>
<tr>
<td>10</td>
<td>0.94 Rf</td>
<td>0.2 AU</td>
<td>0.97 Rf</td>
<td>31.1 AU</td>
<td>2.54%</td>
<td>0.99 Rf</td>
<td>4.6 AU</td>
<td>822.3 AU</td>
<td>1.58%</td>
<td>unknown *</td>
</tr>
</tbody>
</table>
HPTLC finger print profile of DTL 1510354 sample (Ethanol extract)-10µl-254nm
HPTLC finger print profile of DTL 1510354 sample (Ethanol extract)- 15µl -254nm
### Table: 6 Screening for Micro-organisms in *Indhuppu bhavanai* *

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Results</th>
<th>Permissible Limit for Internal use</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Bacterial Count (TBC)</td>
<td>&lt;10³ cfu/g</td>
<td>10⁵ CFU/g</td>
</tr>
<tr>
<td>2</td>
<td>Total Fungal Count (TBC)</td>
<td>&lt;10³ cfu/g</td>
<td>10³ CFU/g</td>
</tr>
<tr>
<td>3</td>
<td>Enterobacteriaceae</td>
<td>Absent</td>
<td>10³ CFU/g</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia coli</em></td>
<td>Absent</td>
<td>10 CFU/g</td>
</tr>
<tr>
<td>5</td>
<td><em>Salmonella spp</em></td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>6</td>
<td><em>Staphylococcus aureus</em></td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

**RESULTS**

The microbial load analysis confirms *Indhuppu bhavanai* was free from microorganisms and fungal infections.

---

*The experimental procedure was analyzed at Captain Srinivasamurti Research Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Ch-106.*
Table 7 ICP-OES study results of *Indhuppu bhavanai*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Elements</th>
<th>Wavelength in nm</th>
<th>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>12.460</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>Mercury</td>
<td>Hg 253.652</td>
<td>BDL</td>
</tr>
<tr>
<td>6</td>
<td>Potassium</td>
<td>K 766.491</td>
<td>13.801</td>
</tr>
<tr>
<td>7</td>
<td>Sodium</td>
<td>Na 589.592</td>
<td>124.310</td>
</tr>
<tr>
<td>8</td>
<td>Nickel</td>
<td>Ni 231.604</td>
<td>BDL</td>
</tr>
<tr>
<td>9</td>
<td>Lead</td>
<td>220.353</td>
<td>BDL</td>
</tr>
<tr>
<td>10</td>
<td>Phosphorus</td>
<td>P 213.617</td>
<td>06.741</td>
</tr>
</tbody>
</table>

**BDL** - Below Detection Limit, ppm – Parts per million

Table 7 shows the quantitative analysis of the elements present in *Indhuppu bhavanai*. The heavy metals were found to be within normal limits. The presence of other elements shows the therapeutic value of *Indhuppu bhavanai*.

* The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.
5.7 PARTICLE SIZE ANALYSIS *

SCANNED ELECTRON MICROSCOPY

Determination Of Particle size of Indhuppu bhavanai

Figure 1

The picture shows that the particles are stabilize, have irregular morphology and distributed in near nano range. *Indhuppu bhavanai* has particle size of 0.06 to 0.15µ.

*The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.*
6 TOXICITY STUDY ON **INDHUPPU BHAVANAI**

6.1 Acute oral toxicity study of *Indhuppu bhavanai* *

**Table 8:** Dose finding experiment and its behavioural Signs of Toxicity in wistar albino rats

<table>
<thead>
<tr>
<th>No</th>
<th>Dose mg/kg</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>2000</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>


**+ Presence of Activity**

**- Absence of Activity**

**RESULT**

All the data were summarized in the form of table (8) revealed no abnormal signs and behavioral changes in rats upto the dose level of 2000 mg/kg body weight administered orally

* Acute oral toxicity was done at K.K college of Pharmacy, Gerugambakkam, Chennai
Table 9: Body wt gram of wistar albino rats exposed to Indhuppu Bhavanai for 28 days

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>Days</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Control</td>
<td>157±4.37</td>
<td>157.42±1.17</td>
<td>156.92±1.21</td>
<td>157.36±1.41</td>
<td>158.01±1.03</td>
</tr>
<tr>
<td>MD dose</td>
<td>154.13±0.82</td>
<td>154.72±0.55</td>
<td>155.24±0.98</td>
<td>156.1±1.17</td>
<td>159.12±0.52</td>
</tr>
<tr>
<td>HD dose</td>
<td>155.8±1.03</td>
<td>156.12±0.75</td>
<td>156.69±0.89</td>
<td>157.22±0.75</td>
<td>158.11±1.52</td>
</tr>
</tbody>
</table>

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

Table 10: Water (ml/day) intake of albino rats exposed to Indhuppu Bhavanai for 28 days

<table>
<thead>
<tr>
<th>Dose(mg/kg/day)</th>
<th>Days</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Control</td>
<td>32.0±0.92</td>
<td>33.36±0.23</td>
<td>33.23±0.23</td>
<td>33.18±0.16</td>
<td>33.64±0.38</td>
</tr>
<tr>
<td>MD dose</td>
<td>35.83±0.49</td>
<td>34.5±1.52</td>
<td>34.33±1.51</td>
<td>35.5±1.05</td>
<td>35.83±1.72</td>
</tr>
<tr>
<td>HD dose</td>
<td>34.72±1.51</td>
<td>35.19±1.79</td>
<td>36.17±1.51</td>
<td>36.42±1.33</td>
<td>36.59±1.52</td>
</tr>
</tbody>
</table>

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

Table 11: Food (g/day) intake of albino rats exposed to Indhuppu Bhavanai for 28 days

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>Days (gms/rats)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Control</td>
<td>33.5±0.84</td>
<td>34.72±1.17</td>
<td>35±0.75</td>
<td>35.32±1.97</td>
<td>35.86±1.37</td>
</tr>
<tr>
<td>MD dose</td>
<td>35.82±0.82</td>
<td>38.16±0.75</td>
<td>38.02±0.98</td>
<td>39.83±1.60</td>
<td>39.33±1.21</td>
</tr>
<tr>
<td>HD dose</td>
<td>34.75±2.02</td>
<td>37.13±1.33</td>
<td>37.93±1.10</td>
<td>39.33±1.51</td>
<td>39.83±1.17</td>
</tr>
</tbody>
</table>

Values are mean of 10 animals ± S.E.M (Dunnet’s test) * p<0.05 ;**p<0.01.N=10
Table 12: Hematological parameters after 28 days treatment with *Indhuppu Bhavanai* in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>MD dose</th>
<th>HD dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RBC (10^6 mm^-3)</td>
<td>9.08±0.03</td>
<td>9.09±0.02</td>
<td>9.11±0.02</td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>14.40±0.18</td>
<td>14.60±0.32</td>
<td>14.63±0.29</td>
</tr>
<tr>
<td>Leukocyte (10^3 mm^-3)</td>
<td>12.33±0.22</td>
<td>12.13±0.12</td>
<td>12.16±0.15</td>
</tr>
<tr>
<td>Platelets lakhs/µl</td>
<td>315.81±4.43</td>
<td>320.83±0.98</td>
<td>325.30±3.18</td>
</tr>
<tr>
<td>MCV%ft</td>
<td>51.01±0.76</td>
<td>52.62±1.22</td>
<td>52.21±1.18</td>
</tr>
<tr>
<td>PCV%</td>
<td>39.13±0.12</td>
<td>39.24±0.37</td>
<td>39.45±1.22</td>
</tr>
</tbody>
</table>

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

Chart 1: The mean value of HB, Total RBC of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days
Chart 2: The mean value of Platelet of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days

Chart 3: The mean value of Leucocyte, MCV and PCV of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days
Table 13: Effect of treatment with *Indhuppu Bhavanai* on biochemical parameters

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Control</th>
<th>MD dose</th>
<th>HD dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bilirubin (mg/dl)</td>
<td>0.3013±0.01</td>
<td>0.3015±0.01</td>
<td>0.27±0.01</td>
</tr>
<tr>
<td>Bilirubin direct (mg/dl)</td>
<td>0.2±0.02</td>
<td>0.16±0.02</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td>Bilirubin indirect (mg/dl)</td>
<td>0.1±0.02</td>
<td>0.14±0.01</td>
<td>0.1±0.01</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>80.0±0.89</td>
<td>82.0±1.17</td>
<td>83.0±1.51</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>52.16±0.75</td>
<td>54.24±0.84</td>
<td>54.07±0.63</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>25.01±0.16</td>
<td>23.41±0.29</td>
<td>24.26±1.60</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.24±0.10</td>
<td>6.30±0.10</td>
<td>6.24±0.10</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.04±0.04</td>
<td>3.04±0.04</td>
<td>3.06±0.04</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>5.023±0.03</td>
<td>5.29±0.01</td>
<td>5.15±0.04</td>
</tr>
</tbody>
</table>

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

Chart 4: The mean value of Total Bilirubin, Direct bilirubin and indirect bilirubin of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai*
Chart 5  The mean value of ALP, SGOT and SGPT of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days

Chart 6  The mean value of Albumin, globulin and total protein of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days
Table 14 Effect of *Indhuppu bhavanai* on Renal function test

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Control</th>
<th>MD dose</th>
<th>HD dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>17.42±0.10</td>
<td>17.23±0.11</td>
<td>17.71±0.11</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.67±0.01</td>
<td>0.70±0.02</td>
<td>0.72±0.02</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.4±0.02</td>
<td>1.4±0.02</td>
<td>1.47±0.04</td>
</tr>
<tr>
<td>Na m.mol</td>
<td>145.91±0.55</td>
<td>146.50±0.55</td>
<td>146.66±0.52</td>
</tr>
<tr>
<td>K m.mol</td>
<td>6.11±0.01</td>
<td>6.16±0.02</td>
<td>6.46±0.20</td>
</tr>
<tr>
<td>Cl m.mol</td>
<td>98.20±0.10</td>
<td>100.20±0.10</td>
<td>99.68±1.10</td>
</tr>
</tbody>
</table>

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

Chart 7 The mean value of Urea, Creatinine and uric acid of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days
Chart 8  The mean value of sodium, potassium and chloride levels of control and treated groups of wistar albino rats exposed to *Indhuppu bhavana* for 28 days

Table 15: Lipid Profile

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>MD dose</th>
<th>HD dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/kg)</td>
<td>34.86±0.39</td>
<td>34.50±0.38</td>
<td>35.01±0.35</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>12.05±0.02</td>
<td>12.2±0.05</td>
<td>12.17±0.09</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>35.83±0.42</td>
<td>36.73±0.36</td>
<td>37.18±0.70</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>15.80±0.03</td>
<td>15.81±0.02</td>
<td>15.75±0.04</td>
</tr>
<tr>
<td>Triglycerides (mg/kg)</td>
<td>78.33±0.52</td>
<td>79.0±1.26</td>
<td>81 ±0.82</td>
</tr>
<tr>
<td>TC/HDL ratio (g/dl)</td>
<td>3.41±0.04</td>
<td>3.36±0.04</td>
<td>3.22±0.03</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>121.90±0.66</td>
<td>121.84±0.82</td>
<td>121.98±0.36</td>
</tr>
</tbody>
</table>

Values are mean of 10 animals ± S.E.M (Dunnet’s test) *p<0.05 ;**p<0.01.N=10
Chart 9  The mean value of Sr.TC, Sr.TG, HDL, LDL, VLDL of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 28 days

Chart 10 The mean value of Blood sugar of control and treated groups of wistar albino rats exposed *Indhuppu bhavanai* for 28 days
Table 16: Urine Analysis of 28 Days Repeated oral toxicity study of *Indhuppu bhavanai* in wistar albino rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>MD dose</th>
<th>HD dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transparency</td>
<td>Clear</td>
<td>Slightly turbid</td>
<td>Slightly turbid</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.010</td>
<td>1.010</td>
<td>1.010</td>
</tr>
<tr>
<td>PH</td>
<td>&gt;7.2</td>
<td>&gt;7.2</td>
<td>&gt;7.2</td>
</tr>
<tr>
<td>Protein</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Glucose</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Ketones</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Blood</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>Normal</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Pus cells</td>
<td>0-cells/HPF</td>
<td>0-cells/HPF</td>
<td>1-cells/HPF</td>
</tr>
<tr>
<td>RBC</td>
<td>Nil</td>
<td>Nil</td>
<td>1-cells/HPF</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>Nil</td>
<td>1-cells/HPF</td>
<td>Nil</td>
</tr>
<tr>
<td>Crystals</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Casts</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Others</td>
<td>Bacteria seen</td>
<td>Bacteria seen</td>
<td>Bacteria seen</td>
</tr>
<tr>
<td>Colour</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
</tbody>
</table>
Table 17: Effect of oral administration of *Indhuppu Bhavanai* on organ weight

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Control</th>
<th>MD dose</th>
<th>HD dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver (g)</strong></td>
<td>4.39±0.07</td>
<td>4.51±0.01</td>
<td>4.54±0.03</td>
</tr>
<tr>
<td><strong>Heart (g)</strong></td>
<td>0.39±0.04</td>
<td>0.39±0.03</td>
<td>0.39±0.02</td>
</tr>
<tr>
<td><strong>Lung (g)</strong></td>
<td>1.30±0.24</td>
<td>1.30±0.20</td>
<td>1.30±0.20</td>
</tr>
<tr>
<td><strong>Spleen (g)</strong></td>
<td>0.48±0.16</td>
<td>0.50±0.18</td>
<td>0.50±0.22</td>
</tr>
<tr>
<td><strong>Ovary (g)</strong></td>
<td>1.47±0.01</td>
<td>1.49±0.02</td>
<td>1.49±0.02</td>
</tr>
<tr>
<td><strong>Testes (g)</strong></td>
<td>1.20±0.10</td>
<td>1.22±0.22</td>
<td>1.22±0.28</td>
</tr>
<tr>
<td><strong>Brain (g)</strong></td>
<td>1.34±0.02</td>
<td>1.36±0.02</td>
<td>1.37±0.01</td>
</tr>
<tr>
<td><strong>Kidney (g)</strong></td>
<td>0.60±0.04</td>
<td>0.62±0.04</td>
<td>0.62±0.04</td>
</tr>
<tr>
<td><strong>Stomach (g)</strong></td>
<td>1.36±0.03</td>
<td>1.38±0.02</td>
<td>1.38±0.02</td>
</tr>
</tbody>
</table>

Values are mean of 10 animals ± S.E.M (Dunnet’s test) *p<0.05 ;**p<0.01.N=10
HISTOPATHOLOGICAL OF VARIOUS ORGANS AFTER THE REPEATED DOSE 28 DAY ORAL TOXICITY STUDY OF \textit{INDHUPPU BHAVANAI} IN WISTAR ALBINO RATS

Heart

\begin{itemize}
  \item [a.] Control
  \item [b.] Mid dose group
  \item [c.] High dose group
\end{itemize}

Observations

\begin{itemize}
  \item a. Section of the heart showed normal muscle fibres with acidophilic cytoplasm and centrally located nuclei.
  
  \item b. Section of the heart showed normal muscle fibres with acidophilic cytoplasm and centrally located nuclei
  
  \item c. Section of the heart showed normal muscle fibres with acidophilic cytoplasm and centrally located nuclei.
\end{itemize}
Observations

a. Section of kidney from control animals showed normal size of glomeruli with normal tubules.

b. Section of kidney showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney.

c. Section of kidney showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney
Observations

a. Section of liver from control animals showed no degeneration of hepatocytes, focal steatosis, congestion of central vein and inflammation of portal tract.

b. Section of liver showed no degeneration of hepatocytes, focal steatosis, congestion of central vein and inflammation of portal tract.

c. Section of liver showed no degeneration of hepatocytes, focal steatosis, congestion of central vein and inflammation of portal tract.
Spleen

a. Control

b. Mid dose

c. High dose

Observations

a. Section of spleen from control animal showed normal granular hemosiderin pigment predominantly within macrophages in the red pulp.

b. Section of spleen showed normal granular hemosiderin pigment predominantly within macrophages in the red pulp with normal structure.

c. Section of spleen showed normal granular hemosiderin pigment predominantly within macrophages in the red pulp with normal structure.
Stomach

a. Control

b. Mid dose

c. High dose

Observation

a. Section of stomach shows gastric mucosa lined by tall columnar cells that detected no abnormality.

b. Section of stomach shows gastric mucosa lined by tall columnar cells that detected no abnormality

c. Section of stomach shows gastric mucosa lined by tall columnar cells that detected no abnormality.
RESULTS OF REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY

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

**Clinical signs:** No abnormal behavioural signs were observed during the study period.

**Mortality**

The test drug “*Indhuppu bhavanai*” did not cause any mortality in mid and high dose levels and were considered as safe dose levels.

**Body weight:**

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

**Haematological investigation interpretation**

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.

**Biochemical investigation interpretation**

The biochemical investigations were conducted on 28th day and the result was produced. The results revealed there was no significant changes in the values of different parameters with that of the control. All the values were within the normal biological and laboratory limits.

**Histopathology interpretation**

Histopathological study of the organ such as heart, kidney,liver,spleen and stomach were normal in control and all test groups.
Table 18: Body wt of wistar albino rats exposed to *Indhuppu bhavanai* for 90 days

<table>
<thead>
<tr>
<th>DAYS</th>
<th>Weight(gms)/Days</th>
<th>P value (p)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Low dose</td>
</tr>
<tr>
<td>1</td>
<td>161.6±33.68</td>
<td>148.1 ± 21.11</td>
</tr>
<tr>
<td>15</td>
<td>172.8±28.87</td>
<td>162.5 ± 21.71</td>
</tr>
<tr>
<td>30</td>
<td>181.8 ±28.31</td>
<td>175.71 ± 14.88</td>
</tr>
<tr>
<td>45</td>
<td>204.5± 27.73</td>
<td>194.5± 29.76</td>
</tr>
<tr>
<td>60</td>
<td>218.6±33.68</td>
<td>214.6±23.68</td>
</tr>
<tr>
<td>75</td>
<td>236.8±26.85</td>
<td>226.8±28.87</td>
</tr>
<tr>
<td>90</td>
<td>248.5±27.32</td>
<td>239.5±27.68</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 *Indhuppu bhavanai* for 90 days

<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>Haemoglobin(g/dl)</td>
<td>12.53±0.31</td>
<td>12.7±0.59</td>
<td>12.63±0.45</td>
<td>12.78±0.67</td>
<td>N.S</td>
</tr>
<tr>
<td>Total WBC (cells/cu.mm)</td>
<td>11.2±4.02</td>
<td>11±0.54</td>
<td>11.15±5.12</td>
<td>11.4±6.37</td>
<td>N.S</td>
</tr>
<tr>
<td>Platelets lkhs/μl</td>
<td>351±21</td>
<td>367±25</td>
<td>387.05±26</td>
<td>36±33</td>
<td>N.S</td>
</tr>
<tr>
<td>Total RBC (cells/cu.mm)</td>
<td>7.25±0.3</td>
<td>7.08±0.3</td>
<td>7.13±0.2</td>
<td>7.16±0.3</td>
<td>N.S</td>
</tr>
<tr>
<td>PCV%</td>
<td>37.59±0.95</td>
<td>38.29±0.18</td>
<td>37.91±1.34</td>
<td>38.36±2.05</td>
<td>N.S</td>
</tr>
<tr>
<td>MCHC g/dl</td>
<td>30±3.03</td>
<td>33.66±1.86</td>
<td>31±1.22</td>
<td>30.5±0.07</td>
<td>N.S</td>
</tr>
<tr>
<td>MCV%ft</td>
<td>92.16±2.62</td>
<td>91.3±3.75</td>
<td>92.3±1.98</td>
<td>91.5±1.89</td>
<td>N.S</td>
</tr>
<tr>
<td>MCH pg</td>
<td>30±3.03</td>
<td>32.75±1.86</td>
<td>31±1.22</td>
<td>30.5±0.07</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 11: The mean value of HB, Total RBC, Total WBC of control and treated groups of wistar albino rats exposed *Indhuppu bhavanai* for 90 days

![Bar chart](chart11.png)

Chart 12: The mean value of Platelets of control and treated groups of wistar albino rats exposed *Indhuppu bhavanai* for 90 days

![Bar chart](chart12.png)
Chart 13: The mean value of PCV, MCHC, MCV, MCH of control and treated
groups of Wistar albino rats exposed *Indhuppu bhavanai* for 90 days

<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>Bl.sugar (mg/dl)</td>
<td>118.30±8</td>
<td>126.5±6.28</td>
<td>112.6±16.1</td>
<td>121±20.9</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 Blood sugar of control and treated groups of wistar albino rats *Indhuppu bhavanai* for 90 days

Table 21: Lipid profile test of Wistar albino rats group exposed to *Indhuppu bhavanai* for 90 days

<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>Sr.TC(mg/dl)</td>
<td>108.±8.3</td>
<td>114.83±12.93</td>
<td>115.6±8.28</td>
<td>112.83±6.76</td>
<td>N.S</td>
</tr>
<tr>
<td>Sr.TG(mg/dl)</td>
<td>129.83±2.4</td>
<td>136.55±9.35</td>
<td>4132.5±9.73</td>
<td>136.5±9.31</td>
<td>N.S</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>38.5±1.64</td>
<td>41.16±4.40</td>
<td>42.69±2.04</td>
<td>42.83±4.02</td>
<td>N.S</td>
</tr>
<tr>
<td>LDL(mg/dl)</td>
<td>44.66±9.30</td>
<td>50.83±12.54</td>
<td>44.16±8.68</td>
<td>43.66±7.33</td>
<td>N.S</td>
</tr>
<tr>
<td>VLDL(mg/dl)</td>
<td>25.66±1.25</td>
<td>27.3±1.87</td>
<td>26.4±2.01</td>
<td>27.3±1.86</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 15: The mean value of Sr.TC, Sr.TG of control and treated groups of wistar albino rats exposed *Indhuppu bhavana* for 90 days

Chart 16: The mean value of HDL, LDL, VLDL of control and treated groups of wistar albino rats exposed *Indhuppu bhavanai* for 90 days
Table 22: Liver Function Test of Wistar albino rats group *Indhuppu bhavanai* for 90 days

<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>0.86±0.04</td>
<td>0.79±0.1</td>
<td>0.75±0.1</td>
<td>0.80±0.13</td>
<td>N.S</td>
</tr>
<tr>
<td>SGOT(IU/L)</td>
<td>21.6±5.08</td>
<td>22.5±9.97</td>
<td>27.6±9.09</td>
<td>26.15±2.04</td>
<td>N.S</td>
</tr>
<tr>
<td>SGPT(IU/L)</td>
<td>27±5.9</td>
<td>30.8±5.45</td>
<td>28.5±3.72</td>
<td>31.57±10.9</td>
<td>N.S</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>82.83±17.56</td>
<td>79.8±12.09</td>
<td>79±15.7</td>
<td>75.3±17.84</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 17: - The mean value of Bilirubin of control and treated groups of wistar albino rats *Indhuppu bhavanai* for 90 days
Chart 18: The mean value of SGOT, SGPT, ALP of control and treated groups of wistar albino ras exposed to Indhuppu bhavanai for 90 days

Table 23 Renal Function Test of Wistar albino rats group Indhuppu bhavanai for 90 days

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Low dose</th>
<th>Mid dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>32</td>
<td>30.16</td>
<td>28.3</td>
<td>26.5</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.48</td>
<td>0.5</td>
<td>0.51</td>
<td>0.53</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 19: The mean value of Urea of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 90 days

![Urea Chart](chart19)

Chart 20: The mean value of Creatinine of control and treated groups of wistar albino rats exposed to *Indhuppu bhavanai* for 90 days

![Creatinine Chart](chart20)
HISTOPATHOLOGICAL STUDIES OF VARIOUS ORGANS AFTER THE REPEATED DOSE 90 DAY ORAL TOXICITY STUDY OF INDHUPPU BHAVANAI IN WISTAR ALBINO RATS

Control - Heart

High dose - Heart

Control - Kidney

High dose - Kidney

Control - Liver

High dose - Liver
INTERPRETATION FOR HISTOPATHOLOGICAL SLIDES OF INDHUPPU BHAVANAI FOR 90 DAYS

Kidney

- Appearance of glomerular architecture was normal in control and glomeruli showing striking lobular architecture in sample high dose. Cortex and medulla appears normal opening of lumen appears normal
- Increased glomerular permeability was observed.

Heart

- Cardiac muscle appears continuous with intact nucleus
- Myocardial tissue appears normal with orderly striated heart muscle fibers and a clear nuclear and muscle bands.
Liver

- Central vein appears prominent with no signs of cellular infiltration
- Hepatocellular architecture, including hepatic sinusoid and hepatic cord was normal

Spleen

- Marginal vascular zone radiated in between red and white pulp
- No signs of immunological activities

Stomach

- Mucosal epithelium appears normal with no signs of ulceration
- Microscopic analysis of stomach of both the sample reveals anatomy of muscular stomach with epithelial layer keratinized stratified squamous epithelium, Lamina propria and Sub-mucosa
- Gastric wall architecture appears normal.

RESULTS OF REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY

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

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

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

Histopathological study of the organ such as heart, kidney, liver, spleen and stomach were normal in control and test groups.
PHARmacological activity of Indhuppu Bhavanai for Anti-UlcER activity

Table 24 Effect of Indhuppu bhavanai (IB) on volume of gastric juice

<table>
<thead>
<tr>
<th>Group no</th>
<th>Body wt.gms</th>
<th>Treatment</th>
<th>Vol.of gastric juice</th>
<th>Ph</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>170.8±0.75</td>
<td>Control (distilled water 2ml/kg)</td>
<td>5.28±0.20</td>
<td>2.27±0.19</td>
</tr>
<tr>
<td>II</td>
<td>172.13±0.33</td>
<td>Negative control (Aspirin 500mg/kg)</td>
<td>6.63±0.18</td>
<td>1.74±0.06</td>
</tr>
<tr>
<td>III</td>
<td>171.5±1.38</td>
<td>Standard drug ranitidine (20mg/kg) + Aspirin</td>
<td>1.91±0.38**</td>
<td>5.26±0.11**</td>
</tr>
<tr>
<td>IV</td>
<td>171.5±1.4</td>
<td>IB - 200mg/kg + Aspirin</td>
<td>4.33±0.24*</td>
<td>2.90±0.48</td>
</tr>
<tr>
<td>V</td>
<td>172.2±0.41</td>
<td>IB - 400mg/kg + Aspirin</td>
<td>2.46±0.24**</td>
<td>4.11±0.10**</td>
</tr>
</tbody>
</table>

Effects are statistically significant. *P<0.05;**P<0.01 (in comparison with standard).
Values are expressed in terms of mean ± SEM of 6 rats (ANOVA).

Chart 21 Effect of Indhuppu bhavanai on volume of gastric juice and pH

![Chart showing the effect of Indhuppu bhavanai on gastric juice volume and pH](chart.png)
Table 25  Effect of *Indhuppu bhavanai* on Free acidity and Total acidity

<table>
<thead>
<tr>
<th>Group no</th>
<th>Body wt. gms</th>
<th>Treatment</th>
<th>Free acidity</th>
<th>Total acidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>170.8 ± 0.75</td>
<td>Control (distilled water 2ml/kg)</td>
<td>20.22 ± 0.97</td>
<td>31.84 ± 2.04</td>
</tr>
<tr>
<td>II</td>
<td>172.13 ± 0.33</td>
<td>Negative control (Aspirin 500mg/kg)</td>
<td>23.87 ± 0.86</td>
<td>46.67 ± 1.36</td>
</tr>
<tr>
<td>III</td>
<td>171.5 ± 1.38</td>
<td>Standard drug (Ranitidine- 20 mg/kg) + Aspirin</td>
<td>16.14 ± 0.19**</td>
<td>22.66 ± 1.01**</td>
</tr>
<tr>
<td>IV</td>
<td>171.5 ± 1.4</td>
<td>IB -200mg/kg + Aspirin</td>
<td>18.17 ± 0.09*</td>
<td>29.33 ± 0.34*</td>
</tr>
<tr>
<td>V</td>
<td>172.2 ± 0.41</td>
<td>IB – 400mg/kg + Aspirin</td>
<td>17.18 ± 0.14**</td>
<td>27.13 ± 0.17**</td>
</tr>
</tbody>
</table>

Values are expressed in terms of mean ± SEM of 6 rats (ANOVA). Effects are statistically significant *P<0.05;**P<0.01 (in comparison with standard)

Chart :22  Effect of *Indhuppu bhavanai* on Free acidity and Total acidity
### Table 26  Effect of Indhuppu bhavanai on Ulcer index and Percentage of ulcer protection

<table>
<thead>
<tr>
<th>Group no</th>
<th>Body wt.gms</th>
<th>Treatment</th>
<th>Ulcer Index</th>
<th>Percentage of ulcer protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>181.3 ± 1.5</td>
<td>Control distilled water 2 ml/kg</td>
<td>6.6 ± 0.26</td>
<td>–</td>
</tr>
<tr>
<td>II</td>
<td>182.1 ± 2.0</td>
<td>Negative control (Aspirin 500mg/kg)</td>
<td>7.4 ± 0.50</td>
<td>23.5</td>
</tr>
<tr>
<td>III</td>
<td>181.5 ± 0.84</td>
<td>Standard drug- Ranitidine (20mg/kg)+Aspirin</td>
<td>1.25 ± 0.17**</td>
<td>91.5**</td>
</tr>
<tr>
<td>IV</td>
<td>181.83 ± 1.6</td>
<td>IB-200mg/kg+Aspirin</td>
<td>3.45 ± 0.37*</td>
<td>54.5</td>
</tr>
<tr>
<td>V</td>
<td>181.3 ± 1.63</td>
<td>IB – 400 mg/kg+ Aspirin</td>
<td>2.21 ± 0.24**</td>
<td>75.5*</td>
</tr>
</tbody>
</table>

Values are expresses in terms of mean ± SEM of 6 rats (ANOVA).

Percentage protection = (Contol mean ulcer index – Test mean ulcer index )/Control mean ulcer index X 100.

Effects are statistically significant. *P<0.05; **P<0.01 (in comparison with Standard)

- **Group I** - Control
- **Group II** - Negative control received aspirin (500mg/kg)
- **Group III** - Standard drug ranitidine + Aspirin (500mg//kg)
- **Group IV** - Indhuppu bhavanai 200mg/kg + Aspirin (500mg//kg)
- **Group V** - Indhuppu bhavanai 400mg/kg + Aspirin (500mg//kg)
Chart 23  Effect of Indhuppu bhavanai on Ulcer index

Chart 24  Effect of *Indhuppu bhavanai* on percentage of ulcer protection
HISTOPATHOLOGICAL SLIDES OF OPEN EXCISED STOMACH IN ASPIRIN INDUCED GASTRIC LESIONS MODEL

a. Control

b. Negative control

c. Standard drug – Ranitidine

d. IB 200mg/dl

e. IB- 400mg/dl

a. Inhibition of gastric lesions at control
b. Gastric lesions induced by Aspirin (500mg/dl)
c. Absence of gastric lesions in Ranitidine (20mg/kg)
d. Fractional inhibition of gastric lesions at Indhuppu Bhavanai (200mg/kg)
e. Fractional inhibition of gastric lesions at Indhuppu Bhavanai (400mg/kg)
Results of Anti-ulcer (Hyperacidity) activity of *Indhuppu bhavanai (IB)* in wistar albino rats

**Gastric volume**

The gastric volume was increased in aspirin induced group when compared to control group. Administration of ranitidine and IB showed a significant (p<0.01) decrease in gastric volume level, when compared to negative control.

The pH level was decreased (p<0.01) in the aspirin induced method, when compared to control group. Administration of Ranitidine and IB showed a significant (p<0.01) increase in pH level, when compared to negative group animals. Results were showing Table 24.

**Free and total acidity**

The free acidity (mEq/L/100g) was increased (p<0.01) in the aspirin induced animals, when compared to control group. Administration of IB and ranitidine showed a significant (p<0.01) decrease in free acidity, when compared to negative control.

Total acidity (mEq/L/100g) was increased (p<0.01) in aspirin induced model, when compared to control group. Administration of ranitidine and IB showed a significant (p<0.01) decrease in total acidity, when compared to negative control.

**Conclusion**

*Indhuppu bhavanai* exhibited anti ulcer activity in aspirin induced ulcer model for screening anti ulcer drugs. The percentage of inhibition of ulcer was 91.5%, 54.5%, 75.5% produced by the treatment of standard drug Ranitidine, *Indhuppu bhavanai* (IB) at the dose level of 200mg/kg and 400mg/kg respectively. In case of vehicle control, aspirin induced rats showed increase in acid secretion, which in turn caused increase in gastric volume, low pH, increased free and total acidity resulting in increase of ulcer index.

*Indhuppu bhavanai* (IB) when administered at dose level of (200mg/kg and 400mg/kg) produced a reduction in the gastric fluid volume, free acidity, total acidity and ulcer index significantly in comparison with control group.

Hence, based on the results, it can be concluded that the *Indhuppu bhavanai*, significantly p<0.05 and p<0.01, decreased the ulceration in Aspirin induced ulcer model in rats which suggest a direct ulcer protective effect on the gastric mucosa at the dose level of 200mg/kg and 400mg/kg respectively. The result was represented in table 26.
Table 27: Effect of Indhuppu bhavanaion cotton pellet induced granuloma model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Mean wet weight of pellet (mg)</th>
<th>Percentage inhibition</th>
<th>Mean dry weight of pellet (mg)</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>191.16±1.60</td>
<td>0</td>
<td>40.60±0.12</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>Dexamethasone (0.5 mg/kg)</td>
<td>92.5±3.39*</td>
<td>51</td>
<td>22.4±1.64*</td>
<td>44.2</td>
</tr>
<tr>
<td>III</td>
<td>Indhuppu bhavanai (200mg/kg)</td>
<td>154.66±3.78</td>
<td>20.15</td>
<td>35.4±0.26*</td>
<td>11.94</td>
</tr>
<tr>
<td>IV</td>
<td>Indhuppu bhavanai (400mg/kg)</td>
<td>152.50±3.45*</td>
<td>19.37</td>
<td>30.2±1.20*</td>
<td>24.87</td>
</tr>
</tbody>
</table>

N= 6, values are expressed as mean± SEM P<0.05 when compared with control. The results were analyzed by ANOVA followed by Dunnet’s test (P value less than 0.05 was considered as statistically significant).

Table 25: Effect of Indhuppu bhavanaion the mean wet of cotton pellet induced granuloma model

![Bar chart showing the mean wet weight of pellet in different groups](chart.png)
Table 26: Effect of Indhuppu bhavanaion percentage inhibition on wet cotton pellet induced granuloma model

Table 27: Effect of Indhuppu bhavanaion mean dry of cotton pellet induced granuloma model
Table 28: Effect of Indhuppu bhavanaion percentage inhibition on dry cotton pellet induced granuloma model

RESULTS

The results indicate that Indhuppu bhavana at the dose level of 200mg/kg and 400mg/kg produced a decrease in wet granuloma weight 154.66±3.78 (20.15% inhibition) and 152.50±3.45*(19.37% inhibition) respectively when compared to control. Similarly there was a significant decrease in dry granuloma weight 35.4±0.26*(11.94% inhibition) and 30.2±1.20*(24.87% inhibition) respectively compared to control. Among the two doses 400 mg/kg showed slightly lower reduced weight of granuloma than standard drug which was showed in table 27. Thus it was concluded that administration of Indhuppu bhavana at the dose of 400 mg/kg exhibited significant (p<0.05) anti-inflammatory activity in Cotton pellet granuloma model of inflammation in rats.
Table 28: Pharmacological analysis – Analgesic activity of *Indhuppu bhavana*i in Swiss albinomice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Reaction time in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0min</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>2.32±0.11</td>
</tr>
<tr>
<td>II</td>
<td>Pentazocine (5mg/kg)</td>
<td>2.04±0.18</td>
</tr>
<tr>
<td>III</td>
<td>Low dose (200mg/kg)</td>
<td>2.18±0.46</td>
</tr>
<tr>
<td>IV</td>
<td>High dose (400mg/kg)</td>
<td>2.24±0.21</td>
</tr>
</tbody>
</table>

N= 6, values are expressed as mean± SEM P<0.05 ,P<0.01 when compared with control.

The results were analyzed by ANOVA followed by Dunnet’s test (P value less than 0.01 was considered as statistically significant)

Table 29: Analgesic activity of *Indhuppu bhavana*i by Eddy’s hot plate method
Result of Analgesic activity of *Indhuppu bhavanai* in swiss albino mice

Analgesic activity was carried out by Eddy’s Hot plate method.*Indhuppu bhavanai* at the two doses 200 mg/kg showed significant (p<0.05) analgesic activity at reaction time 90 min (4.80±1.11*) and 400 mg/kg showed significant (p<0.01) analgesic activity at 120 min (8.22±0.24**) was slightly lower than the standard drug Pentazocine 11.63±0.39**.

From these results it was obvious that *Indhuppu bhavanai* has significant analgesic activity.
DISCUSSION
9. Discussion

The drug *Indhuppu bhavanai* was selected from the Siddha literature “Kadukkai vallaraïin thani maanbu” to validate the safety and its pharmacological activities (Anti ulcer, anti-inflammatory and analgesic) in animal models.

The ingredients of the test drug was identified and authenticated by Siddha experts. The drug was prepared as per the procedure and subjected to various studies such as qualitative, quantitative, toxicity and pharmacological activities. Qualitative analysis includes Chemical analysis and Physicochemical properties of *Indhuppu bhavanai*.

Quantitative analysis included ICP-OES and HR SEM analysis to reveal its potency and effectiveness against the disease.

From the above analysis we came to know the presence of active ingredients responsible for its activity.

Literary collections:

Literary collections include drug review, which consist of both Botanical aspect, Gunapadam aspect, Pharmacological review that supported the study.

Chemical analysis:

Chemical analysis of the drug *Indhuppu bhavanai* revealed the presence of Iron, Sulphate, Chloride, Calcium, tannic acid and alkaloid.

**Sulphate:**

Sulphate gives strength to the mucous membrane of the stomach, intestine and also promote the appetite.

**Chloride:**

It regulates the water balance by maintaining the osmotic pressure of the body fluids and helps in the formation of Hcl in gastric juice which reduces the gastric problems.
Calcium:

Calcium in the form of calcium carbonate is an antacid. It is an absorbable antacid, which neutralizes the gastric acid medium.

Tannic acid:

The properties of tannic acid was hardening the mucous membrane to exert a protective effect and it has anti-ulcer (Hyperacidity) property.

These chemical elements present in Indhuppu bhavanai enhances the anti-ulcer activity of the drug.

Physico chemical analysis

In physico chemical analysis, the pH of Indhuppu bhavanai was found to be in the range of 3.91. This shows the acidic nature of the medicine. Thus it can be easily absorbed from the gastrointestinal tract on oral administration.

The acid insoluble ash value denotes the drug quality. The drug possess within normal value (0.1%) of acid insoluble ash indicating that the preparation did not contain any sand, dust and stones.

The loss on drying value of Indhuppu bhavanai was found to be 6.01%w/w, hence the drug will not lose much of its volume on exposure to the atmospheric air at room temperature.

The microbial load analysis confirms Indhuppu bhavanai was free from microbial organisms and fungal infections.

In ICP-OES study, heavy metals were found below detection limit in Indhuppu bhavanai. Calcium, Potassium, Sodium, Phosphorous were present.

In HR SEM analysis, the particle size of Indhuppu bhavanai showed irregular morphology and distributed in near nano range. Indhuppu bhavanai has particle size of 0.06 to 0.15µ. This ensures the absorption of the drug was more active and the drug have increased bio-availability.
Toxicological studies:

In acute oral toxicity study, there were no abnormal signs and behavioral changes in rats up to the dose level of 2000 mg/kg body weight administered orally. No mortality was observed in all groups. All the vital organs were normal. In repeated dose 28 day oral toxicity and repeated dose 90 day oral toxicity study, the experimental animals were sacrificed by excessive anesthesia and blood samples were collected and sent for investigation. There were no significant changes in body weight, food and water intake, hematological parameters, renal parameters, Liver function test, Lipid profile and blood glucose level. The organs were collected and sent for histopathology study. It revealed the organs such as heart, kidney, liver, spleen and stomach was normal in Control, Mid dose and High dose. Thus the toxicological study of the test drug greatly establishes the safety and gives the justification for long time administration.

Pharmacological studies:

The pharmacological study was carried out in the animal model Wistar albino rats and mice. Three activities were seen in the drug Indhuppu bhavanai. The activities were:
- Anti-ulcer (Hyperacidity)
- Anti-inflammatory
- Analgesic

**Anti-Ulcer activity (Hyperacidity)**

*Indhuppu bhavanai* (IB) when administered at dose level of (200mg/kg and 400 mg/kg) produced a reduction in the gastric fluid volume, free acidity, total acidity and ulcer index significantly in comparison with control group. Hence, based on the results, it can be concluded that the *Indhuppu bhavanai*, significantly decreased the ulceration in Aspirin induced ulcer in rats which suggest a direct ulcer protective effect on the gastric mucosa at the dose level of 200mg/kg and 400mg/kg.

**Analgesic activity**

Analgesic activity was carried out by Eddy’s Hot plate method. Analgesic effect lasted for a period of 120 min was found to be possess significant (p<0.01) analgesic activity at the dose level of 400mg/kg by increase in reaction time (Increase threshold potential of pain). From these results it was obvious that *Indhuppu bhavanai* has significant analgesic activity.
Anti inflammatory activity

Administration of Indhuppu bhavanai at the dose of 200 mg/kg and 400 mg/kg exhibited significant p <0.05 anti inflammatory activity in Cotton pellet granuloma model of inflammation in rats.

From the discussion, it was concluded that the test drug *Indhuppu bhavanai* was a safe and a potent drug for *Pitha Gunmam* (Hyperacidity).
10. SUMMARY

- The test drug *Indhuppu bhavanai* was selected from the siddha literature “*Kadukai vallarain thani maanbu*” for its anti-ulcer (Hyperacidity), anti-inflammatory and analgesic activities.

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

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

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

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

- Pharmacological study revealed that the drug *Indhuppu bhavanai* exhibited significant anti–ulcer (Hyperacidity), anti inflammatory and analgesic activity in animal models.

- From the results and the statistical analysis it was proved that the drug *Indhuppu bhavanai* has,
  1. Anti-ulcer
  2. Anti inflammatory
  3. Analgesic activity
  4. No side effect
  5. No adverse effect

Hence the trial drug *Indhuppu bhavanai* was safe for clinical trial.
CONCLUSION
11. CONCLUSION

From the literature evidence, Physico chemical analysis, chemical analysis, Toxicological evaluation and Pharmacological studies, the drug *Indhuppu bhavanai* have anti-ulcer, anti-inflammatory and analgesic activity. It was concluded that the *Indhuppu bhavanai* can be used in the management of *Pitha gunnam* (Hyperacidity).
ANNEXURE
This certificate is awarded to
Dr. M. C. Rubika Devi
for participating as Resource Person / Delegate in the Fourteenth Workshop on
"Research Methodology & Biostatistics"
for AYUSH Post Graduates & Researchers

Organized by the Department of Siddha

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

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

Prof. Dr. D. Shantharam, M.D., D.Dipl.,
Vice-Chancellor

Dr. J. K. Charles, M.D.
Ref: 4533/KKCP/2015

Date: 10.08.2015

APPROVAL CERTIFICATE

This is to certify that the project titled "Safety and pharmacological profile of Indhuppu Bhavanai" 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/2015/037</td>
<td>Wistar Albino rat</td>
<td>40 male + 46 female</td>
<td>150–200gms</td>
<td>90</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Swiss albino Mice</td>
<td>12 male + 12 female</td>
<td>20 – 25gms</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>

Wistar Albino rat – Eight six only; Swiss albino Mice – Twenty four only

Chairman IAEC
(Prof. A. Meena)

CPCSEA Nominee
(Dr. C. Kathirvelan)

Veterinary Officer
(V. Vaidyalingam)

Members

118
CERTIFICATE

This is certify that the project title "SAFETY PROFILE OF 

THDHPPLP...BHAYANAI" (L.M.15.12.Ponnal Kum. Alwar) 

has been approved by the IAEC. (No: NIS/IAEC-I/2016/05)

Name of Chairman/Member Secretary IAEC: nominee: 

Dr. B. R. Senthil Kumar

Signature with date

Chairman/Member Secretary of IAEC:

24, Feb 2016

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

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

Centella asiatica (Apiaceae), Whole plant
Citrus limon (Linn.) Burm. f. (Rutaceae), Fruit
Zingiber officinale Rosc. (Zingiberaceae), Rhizome
Phyllanthus emblica Linn. (Euphorbiaceae), Fruit

Certificate No: NISMB1862015

Date: 14-8-2015

Authorized Signatory
Dr. D. Aravind, M.D.(S), M.Sc.,
Associate Professor
-Departmental Medical Botany
National Institute of Siddha
Chennai - 600 007, Pudh
CERTIFICATE

Certified that the samples submitted for identification by Dr. C. Rubika Devi, III year MD Student, Department of Gunapadam, National Institute of Siddha, Sanatorium, Chennai-600 047 is identified as Indhuppu – Sodium chloride (impura)

(R. Shakila)
Research Officer (Chemistry)

(Dr. P. Elankani) 15/04/16
Research Officer (Scientist 2)-I/c
CERTIFICATE

This is to certify that Herbal/Mineral Drug Indhuppu Bhavanai formulated by Dr. C. Rubikadevi, 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.

[R. MURUGESAN]

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

1. Nadkarni K.M, Indian materia medica, prakashan Pvt Ltd, Bombay, (vol I) 1976
8. INDIAN HERBAL PHARMACOPOEIA, published by Indian drug manufactures Association members, Revised New edition 2002
13. Sameer Uz Zaman 1, Mrutyunjay M. Mirje 2 and S. Ramabhimaiah 3, Evaluation of the anti-ulcerogenic effect of Zingiber officinale (Ginger) root in rats, International


33. 1 B. Nagaraju, 1 S.C.Anand, 1 NazeerAhmwd, 2 J.N.Narendra Sharath Chandra, 3 Faiyaz Ahmed and 4 G.V.Padmavathi, Antiulcer Activity of Aqueous Extract of Citrus medica Linn. Fruit Against Ethanol-Induced Ulcer in Rats, Advances in Biological Research 6 (1): 24-29, 2012
35. Dr. Ambika shanmugam, Fundamentals of Biochemistry, 7th edition
36. Treatise of Indian medicinal plants, Volume – 4,5,6
40. www.phytochemicals.info/phytochemicals tanic acid-php
14. ACKNOWLEDGEMENT

This dissertation is one of the milestones in the journey of my professional carrier as it is the key program in acquiring my MD(S) degree. 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 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.R.Rajasekaran, M.D(s), Head of the Department of Gunapadam & Guide, National Institute of Siddha, Chennai-47, for his valuable suggestions and guidance in this dissertation work.

❖ I express my sincere thanks to Dr.Kumar, M.D(s) Associate Prof., for his suggestions.

❖ I express my sincere thanks to Dr.S.Visweswaran, M.D(s),, Lecturer, Gunapadam department, NIS, Chennai-47, for his suggestions.

❖ I express my sincere thanks to Dr.S.Sivakumar, M.D(s),, Lecturer, Gunapadam department, NIS, Chennai-47, for his suggestions.

❖ I express my sincere thanks to Dr.A.Mariappan ,M.D.(s)., Lecturer, Gunapadam department, NIS,Chennai-47, for his suggestions.

❖ I express my sincere thanks to Dr.V.Suba, M.Pharm, Ph.D.,Assistant Professor in Pharmacology, NIS, and Chennai-47.
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.D.Aravind M.D(s) M.Sc., Assistant Professor, Medicinal Botany, NIS, and Chennai-47.

I express my sincere thanks to Late Dr.J.Rani, veterinarian, NIS, Chennai-47.

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

I express my grateful thanks to Mrs.C.Senthil kumari, Associate professor, K.K college of Pharmacy, Gerugambakkam, Chennai for her assistance in Acute, Sub acute and pharmacological study.

I express my sincere thanks to Dr.R.Murugesan Scientific officer, SAIF, IIT, Chennai - 36

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

Last but not least, I would like to pay high regards to all my family members, my Father Mr.R.Chinnasami M.Com, M.phill and my mother Mrs.C.Tamil selvi B.Com and my brother C.Ram nivas for their sincere encouragement and inspiration 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.