SAFETY AND PHARMACOLOGICAL PROFILE OF SEENTHIL CHOORANAM

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Chennai – 47
## CONTENTS

<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>01</td>
</tr>
<tr>
<td>2</td>
<td>AIM AND OBJECTIVE</td>
<td>02</td>
</tr>
<tr>
<td>3</td>
<td>REVIEW OF LITERATURE</td>
<td>03</td>
</tr>
<tr>
<td>3.1</td>
<td>GUNAPADAM REVIEW</td>
<td>03</td>
</tr>
<tr>
<td>3.2</td>
<td>BOTANICAL REVIEW</td>
<td>09</td>
</tr>
<tr>
<td>3.3</td>
<td>LATERAL RESEARCH</td>
<td>14</td>
</tr>
<tr>
<td>3.4</td>
<td>PHARMACEUTICAL REVIEW</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>MATERIALS AND METHODS</td>
<td>20</td>
</tr>
<tr>
<td>4.1</td>
<td>SOP OF TRIAL DRUG</td>
<td>21</td>
</tr>
<tr>
<td>4.2</td>
<td>ANALYTICAL STUDY OF TRIAL DRUG</td>
<td>25</td>
</tr>
<tr>
<td>4.2.1</td>
<td>PHARMACOGNOSTICAL STUDY</td>
<td>26</td>
</tr>
<tr>
<td>4.2.1.1</td>
<td>ORGANOLEPTIC EVALUATION</td>
<td>26</td>
</tr>
<tr>
<td>4.2.1.2</td>
<td>MICROSCOPIC STUDY</td>
<td>26</td>
</tr>
<tr>
<td>4.2.2</td>
<td>PHYSICOCHEMICAL ANALYSIS</td>
<td>27</td>
</tr>
<tr>
<td>4.2.3</td>
<td>CHEMICAL ANALYSIS</td>
<td>30</td>
</tr>
<tr>
<td>4.2.4</td>
<td>TLC/HPTLC FINGER PRINT ANALYSIS</td>
<td>35</td>
</tr>
<tr>
<td>4.2.5</td>
<td>ESTIMATION OF HEAVY METALS</td>
<td>35</td>
</tr>
<tr>
<td>4.2.6</td>
<td>MICROBIAL LOAD ANALYSIS</td>
<td>36</td>
</tr>
<tr>
<td>4.2.7</td>
<td>ELEMENTAL ANALYSIS</td>
<td>36</td>
</tr>
<tr>
<td>4.2.8</td>
<td>ANALYSIS OF PARTICLE SIZE</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>TOXICOLOGICAL STUDY</td>
<td>38</td>
</tr>
<tr>
<td>----</td>
<td>---------------------</td>
<td>----</td>
</tr>
<tr>
<td>5.1</td>
<td>ACUTE TOXICITY STUDY</td>
<td>38</td>
</tr>
<tr>
<td>5.2</td>
<td>REPEATED DOSE 28 DAYS ORAL TOXICITY</td>
<td>40</td>
</tr>
<tr>
<td>5.3</td>
<td>REPEATED DOSE 90 DAYS ORAL TOXICITY</td>
<td>44</td>
</tr>
<tr>
<td>6</td>
<td>PHARMACOLOGICAL STUDIES</td>
<td>47</td>
</tr>
<tr>
<td>6.1</td>
<td>ANTIDIABETIC ACTIVITY</td>
<td>47</td>
</tr>
<tr>
<td>6.2</td>
<td>HEPATOPROTECTIVE ACTIVITY</td>
<td>50</td>
</tr>
<tr>
<td>6.3</td>
<td>ANTI-INFLAMMATORY ACTIVITY</td>
<td>53</td>
</tr>
<tr>
<td>7</td>
<td>RESULTS</td>
<td>55</td>
</tr>
<tr>
<td>8</td>
<td>DISCUSSION</td>
<td>124</td>
</tr>
<tr>
<td>9</td>
<td>SUMMARY</td>
<td>128</td>
</tr>
<tr>
<td>10</td>
<td>CONCLUSION</td>
<td>130</td>
</tr>
<tr>
<td>11</td>
<td>ANNEXURE</td>
<td>I - VI</td>
</tr>
<tr>
<td>12</td>
<td>BIBLIOGRAPHY</td>
<td>131</td>
</tr>
<tr>
<td>13</td>
<td>ACKNOWLEDGEMENT</td>
<td></td>
</tr>
</tbody>
</table>
Introduction
1. INTRODUCTION

The National Institute of Siddha was started on 30\textsuperscript{th} October 2004 and Department of Gunapadam is also functioning from that date and doing continuous drug research. This study is about the toxicological and pharmacological evaluation of *Seenthil chooranam* on Wistar albino rat models. Standard operative procedure (SOP) was followed. All the ingredients were reviewed for its importance in this formula towards eliciting pharmacological actions.

The *Seenthil chooranam* was selected from the authentic book list *Agasthiyar paripuranam* 400 and it was subjected into analysis (physiochemical and chemical), it fulfills all the standardization parameters of *chooranam* as mentioned in AYUSH guidelines.

Review of literature in various categories was carried out. Siddha aspect, botanical aspect, lateral research and pharmaceutical aspect revealed about the drug and the disease, which strongly support that it possesses Antidiabetic and Hepatoprotective, for that purpose it has been selected for this study.

The human clinical dose of Seenthil chooranam was converted into animal dose by using standard method suggested by Pharmacologist. Based on OECD 423 the trial drug *Seenthil Chooranam* was studied and it is non-toxic up to the dose of 2000mg/kg. Gross and histopathological examination of organs of animals administered with highest dose showed normal histological architecture. This could be confirmed as no-observed-adverse-effect level (NOAEL) on acute, 28 days repeated oral and 90 days repeated oral toxicity studies.

The *Seenthil chooranam* is being used for various therapeutic conditions in siddha system of medicines. This study also proved the following pharmacological activities.

**Antidiabetic, Hepatoprotective and Anti-Inflammatory activity** of *SEENTHIL CHOORANAM* was scientifically validated.

So, this medicine can be included in the therapeutic intervention.
Aim & Objective
2. AIM AND OBJECTIVES

AIM

To evaluate the safety and pharmacological profile of the test drug “SEENTHIL CHOORANAM” in animal models.

OBJECTIVE:

- Review of various information relevant to the study from Siddha and modern literature.
- Preparation of the drug according to the classical Siddha literature.
- Pharmacognostical study of the test drug.
- Physicochemical, Chemical and phytochemical analysis of test drug.

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

- **Pharmacological activities in Wister albino rats**
  - Antidiabetic activity by Steptozotocin induced method.
  - Hepatoprotective activity by CCl₄ induced hepatotoxicity method.
  - Anti-inflammatory activity by Carrageenan induced paw edema method.
Review of Literature
3. REVIEW OF LITERATURE

3.1 GUNAPADAM ASPECT

निद्राकृति - Seenthil

| बotanical name | तिनोस्पोरा कोर्डिफ़ोलिया (विल्ड.) |
| English name  | Heart leaved moon seed               |
| Family name   | Menispermaceae                        |

निद्राकृति - Seenthil

<table>
<thead>
<tr>
<th>रूपांतरण</th>
<th>वानस्पतिक</th>
<th>मूलनकल्पना</th>
<th>व्यापारिक</th>
<th>उपक्रमण</th>
</tr>
</thead>
</table>

"अनुप्रयोग: बुधमात्र निजी खेती विज्ञान संस्थान में लगभग 4000 मीटर की ऊंचाई पर लॉकडाउन के दौरान अनियमित नियम अनुसरण करने वाले सामान्य मजदूरों को निद्राकृति 4000 मीटर की ऊंचाई पर लगभग 4000 मीटर की ऊंचाई पर निद्राकृति का सहायता करता है।"
பாடல்

வரவு, சேரிய, வாசத், எழுஷ், சேய், தரிசனம் உள்ளிட்டு கொண்டிய.

்பாடல்

அதைத் பிள்ளையாருக்கு பாடல் பயன்படுத்தி

சுருக்கு விளக்கம் மறைத் படுத்தி (தமிழ். வலையமைப்பு)

ேத்தியா

சுருக்கு விளக்கமான உரையின் தொடர்பில் கொண்டிய, வலையமைப்பு கூறுதல்

சொல்லால் பாடல்வை பயன்படுத்தி விளக்கியதைக் குறிப்பிட்டு

சுருக்கு விளக்கமான உரையின் தொடர்பில் கொண்டிய, வலையமைப்பு கூறுதல்

சொல்லால் பாடல்வை பயன்படுத்தி விளக்கியதைக் குறிப்பிட்டு

சுருக்கு விளக்கமான உரையின் தொடர்பில் கொண்டிய, வலையமைப்பு கூறுதல்

ேத்தியா

சுருக்கு விளக்கமான உரையின் தொடர்பில் கொண்டிய, வலையமைப்பு கூறுதல்

சொல்லால் பாடல்வை பயன்படுத்தி விளக்கியதைக் குறிப்பிட்டு
Botanical name : *Eclipta Prostrata*, Linn.

English name : false daisy

Family name : Asteraceae
நூற்றாண்டு கொண்டாட்டம், தேசிய, காரணம், குட்டி, மின்கைய, பாசடி, மலரிகள் அறிவியல் செயல். இரண்டு பார்வேரியம் அளிக்கலாம் போன்று ஒவ்வொன்று.

முன்னாள் பக்தங்கள்

❖ அறிவியல் ஊராட்சியச் செயலர் 1 பாண்டிய அம்மன் தண்பிய பாண்டிய காலத்துக்கு அரங்ககாரர் பொலிக்க பாண்டிய விளக்கம் பொருள். பதிக்கிய விஷயங்கள் உரையாக, தொலையே வேறு விளக்க வேண்டும். தொலையே வேறு 15 வருடங்கள் போன்ற 30 வருடங்கள் வரை அமைப்பு வல்லுணர் ஆளவுச் செய்திகள், மின்கைய, காரணம், குட்டி விளக்கத் தொடர்ந்து.

❖ முன்னாள் குற்றங்கள் அமாதிக்குவதற்கு அவர்கள் செய்திகளான பாண்டிய, தொலையே, காரணம் பார்வேரியம் செயல்.

❖ புதுதொலையே 2 அமர் விளக்கம். இரண்டு வரலாறு செய்திகள், காரணம் செய்திகள் வரையாக (விளக்கம்) மூலம்.

❖ தொலையே பார்வேரியம் ஆளவுச் செய்திகளான பாண்டிய விளக்கம் புரோம்ப. பதிக்கிய விளக்கம் வரையாக, தொலையே வேறு விளக்கம் வல்லுணர்த் தொடர்ந்து செய்திகள் பொருள்.

❖ முன்னாள் குற்றங்கள் அமாதிக்குவதற்கு விளக்கத்தைக் குறிப்பிட்டு, மொத்த விளக்கம் முன்னாள் வேறு விளக்க வேண்டும். ஆளவுச் செய்திகளான ஆளவுச் செய்திகள் தொடர்ந்து விளக்கத் தொடர்ந்து.

❖ வேறுபார்வேரியத்துக்கு வல்லுணர்த் தொடர்ந்து பாண்டிய விளக்கத்தை மூலமாக இளங்ககாரையை வெளியீட்டு.
**Earthworm**

**Definition**: A burrowing marine worm that lives in the ocean floor.

**Characteristics**
- **Body**: Tubular and flexible, with segments visible along the length.
- **Color**: Usually dark brown or black.
- **Feeding**: They feed on organic material by burrowing into the sediment.
- **Movement**: They move by waving their segments, creating a wriggling motion.

**Behavior**
- **Predation**: Some species are preyed upon by larger marine animals.
- **Reproduction**: They reproduce both sexually and asexually.

**Importance**
- **Ecosystem**: Essential in the ocean's ecosystem, helping in the breakdown of organic matter.
- **Aquaculture**: Used in some cultures for food and fertilizer production.

---

“*In the vast depths of the ocean, earthworms thrive.*

*Earthworms are a variety of marine worms that exist on the ocean floor.*

---

**Further Reading**

- [Earthworms in Aquaculture](https://www.aquaculture.com/earthworms)

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**Notes**

- Earthworms are a species of marine worms that live in the ocean floor.
- They are important for the decomposition of organic material.
- They are used in aquaculture for food and fertilizer production.
2.பிறப்பு

- பாகம்  புதிதமாக கருதி இரண்டு லைஸ்டில் சார்ந்த தொன்மைகள் பாதுகாப்பு துறையாகக் (12.6 கிளி) வரையறைகள் நோக்கம் வாய்ந்த தன்மையம் நிறுவுகிறது. இலட்சும் தொன்மையானது.

- இச்சூழல் மாற்றங்களின் விளக்கத் துறையாக பாதுகாப்பு துறையாக பாதுகாப்பு விளக்கத் துறையாக நிறுவுகிறது.

- பாகம் பிறப்பு அல்லது வணிகப் பாதுகாப்பு துறையாக வணிகப் பாதுகாப்பு துறையாக நிறுவுகிறது.

- இச்சூழல் பரப்பகை அல்லது வணிகப் பாதுகாப்பு துறையாக வணிகப் பாதுகாப்பு துறையாக நிறுவுகிறது. அவற்றின் மூலம் காலத்திற்கு குறைந்தது.
3.2 BOTANICAL ASPECT

*Tinospora cordifolia - Seenthil*

**Botanical Name** : *Tinospora cordifolia* (Miers.)

**Family** : Menispermaceae

**Vernacular names**

- Tamil : Shindil-kodi
- English : Moonseed, Heartleaf,
- Singala : Rasakinda
- Sans : Guduchi
- Hind : Gurach

**Parts used** : Stem³

**Organoleptic character**

- Taste : Bitter
- Nature : Hot
- Division : Hot

**Actions** :

- Hepatoprotective
- Antidiabetic
- Diuretic
- Stomachic
- Stimulates bile secretion⁴
Phytochemistry

Tinospora cordifolia have different classes of chemical constituents such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides

Stem & Root : Alkaloids

Berberine, Choline, Tembetarine, Magnoflorine, Tinosporin, Palmetine, Isocolumnin, Aporphine alkaloids, Jatrorrhizine, Tetrahydropalmatine,

Stem : Glycosides

18-norclerodane glucoside, Furanoid diterpene glucoside, Tinocordiside, Tinocordifolioside, Cordioside, Cordifolioside Syringin, Syringin- apiosylglycoside, Pregnane glycoside, Palmatosides, Cordifolioside A, B, C, D and E

Stem & Aerial plant : Steroids

β–sitosterol, δ-sitosterol, 20 β-hydroxyecdydysone, Ecdysterone, Makisterone A, Giloinsterol

Medicinal Uses

- The stem juice is useful in diabetes,
- The stem is bitter, stomachic, diuretic, stimulates bile secretion, causes constipation, allays thirst, burning sensation, vomiting, enriches the blood and cures jaundice.
- The stem is widely used in diabetes, liver disorders and jaundice.
**Eclipta alba - Karisalankanni**

<table>
<thead>
<tr>
<th><strong>Botanical Name</strong></th>
<th>: <em>Eclipta alba</em>.Hassk</th>
</tr>
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<tbody>
<tr>
<td><strong>Family</strong></td>
<td>: Asteraceae</td>
</tr>
<tr>
<td><strong>Vernacular names</strong></td>
<td></td>
</tr>
<tr>
<td>Tamil</td>
<td>: Kaikeshi; Karisha-langanni</td>
</tr>
<tr>
<td>English</td>
<td>: False daisy</td>
</tr>
<tr>
<td>Singala</td>
<td>: Keekirindiya</td>
</tr>
<tr>
<td>Sans</td>
<td>: Kesharaja</td>
</tr>
<tr>
<td>Hind</td>
<td>: Mochkand</td>
</tr>
<tr>
<td><strong>Parts used</strong></td>
<td>: Whole plant</td>
</tr>
</tbody>
</table>

**Organoleptic character**

- **Taste** : Bitter
- **Nature** : Hot
- **Division** : Hot

**Actions**

- Tonic,
- Cholagoge
- Alterative
- Hepato- tonic
Phytochemistry

Leaves
Wedelolactone, desmethywedelolactone, desmethylwedlalactone-7-glucosidde, stigmasterol

Roots
Henriciacontanol, heptacosanol & stigmasterol, ecliptal,eclalbatin.

Aerial parts
B–amyrin&luteolin-7-0-glucoside, apigenin, cinnaroside, sulphur compounds,eclabasaponins I-VI

Stems
wedelolactone

Seeds
Sterols, ecliptalbine(alkaloid)

Whole plant
Resin, ecliptine, reducing sugar, nicotine, stigmasterol, triterpenesaponin, eclabatin, ursolic acid,oleanolic acid⁷.
Earthworm

Scientific classification

Kingdom : Animalia
Phylum : Annelida
Class : Clitellata
Subclass : Oligochaeta
Order : haplotaxida
Family : Eudrilidae
Genus : Eudrilus
Species : E.eugeniae

General information

Earthworms are primary animal biomass in the soil. The importance of earthworms in ecology and medicine has been increasingly felt amongst researchers. More than 200 varieties of earthworms found in India, only 3 species are popular one among are Eudrilus eugeniae. Earthworms have been used in medicine for various remedies since 1340 AD. Extracting medicinal compounds from the earthworm has traditionally been practiced by indigenous people throughout the world, more particularly in Asia. Earthworm has been recognized in oriental medicine as anti-inflammatory, analgesic and antipyretic agent. It shows anticancer effect by preventing excess glucose uptake. Earthworm surface excreta were found to have potent antimicrobial activity. It is also having anticoagulatory or fibrinolytic activity which results in the facilitation of blood circulation the earthworm has been suspected to contain proteases which dissolve the fibrin clots or anticoagulants which selectively interfere with the intrinsic pathway of blood coagulation cascade.

Traditional uses

It posses copper element and also produce heat and dryness to the body. It is known to have Thirst, Fever, Backache nausea and Respiratory disorder
3.3 LATERAL RESEARCH

*Tinospora cordifolia*

**Acute toxicity study**

The aqueous extract of *T. cordifolia* was administrated orally in increasing dose up to 800 mg/kg. The rats were observed continuously for 2 h for behavioral, neurological, and autonomic profiles and after 24 and 72 h for any lethality. Acute toxicity studies revealed that the nontoxic nature of the aqueous extract of *T. cordifolia*. There was no lethality or toxic reaction found at any doses selected until the end of the study period\(^{16}\). (Kumar et al., 2013)

**Pharmacological studies**

**Anti diabetic activity:**

Oral administration of an aqueous *T. cordifolia* root extract to alloxan diabetic rats caused a significant reduction in blood glucose and brain lipids. Though the aqueous extract at a dose of 400 mg/kg could elicit significant anti-hyperglycemic effect in different animal models, its effect was equivalent to only one unit/kg of insulin\(^{17}\).

Ethanolic extracts of *Tinospora cordifolia* leaves in different dosages (200 and 400 mg/kg)Administered orally for 10 days and 30 days in streptozotocin diabetic albino rats. Present study clearly showed that TC has significant antidiabetic activity in diabetic animals and has an efficacy of 50% to 70% compared to insulin\(^{18}\). (Shekhar Singh et. Al)

**Hepato protective**

The study reported the Different extracts of *Tinospora cordifolia* against carbon tetrachloride (CCl4) induced liver damage in rats. It shows significant hepatoprotective activity\(^ {19}\). (Kavitha B.T. et al., 2011)

**Anti-inflammatory Activity**

The aqueous extract of *T. cordifolia* exerted a significant anti-inflammatory effect on cotton pellet granuloma and formalin induced arthritis models. The dried stem of *T. cordifolia* produced significant anti-inflammatory effect in both acute and sub acute models of inflammation\(^{20}\).
Another study shows that the water extract of the stem of *Tinospora cordifolia* has been checked for anti-inflammatory activity in albino rats. It has significantly inhibited acute inflammatory response evoked by carrageenin when administered orally and intraperitoneally\(^\text{21}\).

**Eclipta prostrata**

**Toxicity report**

**Acute toxicity study**

The observation of acute toxicity study indicated that there was no death in 2000mg/kg dose after 72hr\(^\text{22}\). (Mishra et al)

**Pharmacological activity**

**Anti-inflammatory**

The methanolic extract of leaves of *Eclipta prostrata* Linn was reported for anti-inflammatory activity in albino Wistar rats. The results lend support to the traditional use of *E. prostrata* in the treatment of inflammatory diseases\(^\text{23}\). (Arunachalam et al., 2009)

The anti-inflammatory effect of the plant of Eclipta alba was evaluated on acute and chronic phase inflammation models in rats. The results indicate the potent anti-inflammatory effect and therapeutic efficacy of *Eclipta alba* extract on animal models, which is compared with Indomethacin\(^\text{24}\).

**Antihyperglycemic Activity**

This study found that Oral administration of leaf suspension of E. alba (2 and 4 g/kg body weight) for 60 days resulted in significant reduction in blood glucose it possess potent antihyperglycemic activity\(^\text{25}\).

**Hypoglycemic activity**

The methanolic extract of Eclipta alba in alloxan induced diabetic model in albino rats was reported significant reduction in serum glucose\(^\text{26}\). (Hemalakshmi et.al., 2012)
**Hepatoprotective study**

The effect of Eclipta alba (EA) extract was studied on paracetamol induced hepatic damage in mice treatment with 50% ethanol extract of E.alba was found to protect the mice from hepato-toxic action of paracetamol as evidenced by significant reduction in the elevated serum transaminase levels. Histopathological studies showed marked reduction in fatty degeneration.

Another study was carried out to investigate the possible protective effect of leaf extract of Eclipta alba on paracetamol induced toxicity in liver. This study elucidated that eclipta alba has Significant hepatoprotective activity against paracetmol induced rat. (K. Prabu et al., 2011)
Earthworm

Toxicity evaluation

The acute toxicity effects of earthworm powder (EWP) obtained from Eudrillus euginae on wistar male rats. The animals are treated orally with EWP at the doses of 100 mg/kg, 200mg/kg and 300mg/kg daily for 12 days. The EWP did not show any significant effect in clinical signs, behavioral changes and histopathological studies. (Jaganathan Anitha et al., 2012)

Pharmacological activity

Anti-inflammatory activity

Various solvent extracts of an earthworm, Eudrilus eugeniae were reported for anti-inflammatory activity. (Mathur et al., 2011)

Haemostatic activity

Homeostasis represents the balance between the process of coagulation and fibrinolysis. In this study the earthworm powder (glycolipoprotein mixture) from Eudrilus eugeniae was extracted and tested for its haemostatic effect in Wistar rat model. It indicating a decrease (p< 0.05) in clotting parameter compared to its control. (Anjana.J.C1, Sruthy.P.B)

Anticancer Potentials

The coelomic fluids of Eudrilus eugeniae reportedto have the Cytotoxic effect so which can be useful in the treatment of cancer. (Dinesh et al., 2013)

Antimicrobial Activity

The paste prepared from earthworm, Eudrilus eugeniae was tested for the antimicrobial screening the study clearly found that the paste contain a good antibacterial potential and the bioactive compounds to inhibit the growth of bacteria and fungi. (Vasanthi et al., 2013)

Another study of earthworm, Eudrilus eugeniae was reported. It was found that 95% ethanol extract of earthworm was potent antibacterial agent against Streptococcus pyogens and antifungal agent against Candida albicans. (Abhishek Mathur et al., 2010)
3.4 PHARMACEUTICAL REVIEW

CHOORANAM

Definition

Chooranams are fine dry powders of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being, mixed to homogeneity. The chooranam should be fine and should be never damp. The finesses of the sieve should be 100 mesh or still finer. Chooranams retain their potency for 3 months. (Siddha Pharmacopoeia of India)

Purification of the chooranam

The prepared chooranam is first mixed with the milk, and then taken a pot fill with half quantity milk and half quantity water. The mouth of the pot is covered with a thin cloth material. Over the cloth the placed mixed chooranam. The pot is placed over the stove and heated.

Storage

The prepared chooranam should be allowed to cool by spreading and mixing, prior to packing. They should be stored in tightly stoppered glass, polythene or tin containers, or in polythene or cellophane bags and sealed. These bags should in turn be enclosed in cardboard boxes.

The chooranam to facilitate easy handling and to assure exact dosage administration could be pressed into tablets with the addition of a suitable binder. These tablets could be
packed in bottles or tubes made either of glass or plastic or packed in strip of metal foil or plastic sheets.

Then chooranam is said to retain its potency for 2 months and then gradually deteriorate. However if properly packed & stored they keep good for an year\textsuperscript{36}. (Formulary of Siddha Medicines, 1993)

According to AYUSH guidelines shelf life of chooranam is one year\textsuperscript{37}.
Material & Methods
4. MATERIALS AND METHODS

PREPARATION OF THE SEENTHIL CHOORANAM:

MATERIALS AND METHODS

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PREPARATION OF THE SEENTHIL CHOORANAM:
4.1. SOP for preparation of Seenthil chooranam

The test drug Seenthil Chooranam (Chooranam = one of the 32 types of internal medicine), mentioned in classical siddha text Agasthiyar Paripuranam – 400, has been used for Megam (Diabetic mellitus), Eelai (Tuberculosis), Kasam (Cough), Elai pu (Bronchial asthma), Eranda vayu (Scrotal swelling). The ingredients of this formulation are

- Seenthil (*Tinospora cordifolia*) - 10 palam (350gm)
- Karisalai (*Eclipta Alba*) - 10 palam (350gm)
- Earthworm (*Eudrilus eugeniae*) - 3 palam (105mg)

Procurement of Raw Drugs

The plant materials were collected from Tambaram sanatorium and Earth worm was collected at Thiruthani Agriculture farm Tamilnadu.

Identification and Authentication of Raw Drugs

The Herbs were Identified and authenticated by competent authority department of Gunapadam, National Institute Of Siddha and the Earth worm was identified & authenticated by Head, P.G. & Research Department of Zoology Govt. Arts College, C. Mutlur, Chidambaram.

Purification process

1. *Seenthil*:

   Peel off the outer skin of the stem and powdered, then washed it for 21 times in pure water and dried then sprinkled cow’s milk allow drying it

2. *Karisalai*:

   Wash the whole plant and dry it in sunshade& powdered

3. *Poonagam*:

   Soaked Earth worm Butter milk for a while to spit out mud then sprinkled lime water over it to kill then dry and powdered
Method of Preparation:

The above purified three ingredients were powdered individually and mixed together and stored & preserved in an air tight container.

Labelling

Name of the preparation : Seenthal Chooranam

Dose : 1 gm bd

Adjuvant/Vehicle : Ghee

Indications : Megam (Diabetic mellitus), Eelai (Tuberculosis),
Kasam (Cough), Elaipu (Bronchial asthma), Eranda vayu (Scrotal swelling)

Date of expiry : 1 year from the date of manufacture

Therapeutic administration of drug

Form of medicine - Chooranam

Route of administration - Oral

Dose - 1gm twice daily

Vehicle - Ghee
Ingredients of the Drug

Figure 1.1
SEENTHIL
(Before purification)

Figure 1.2
SEENTHIL
(After purification)

Figure 1.3
KARISALAI
(Before purification)

Figure 1.4
KARISALAI
(After purification)

Figure 1.5
EARTHWORM
(Before purification)

Figure 1.6
EARTHWORM
(After purification)
Seenthil Chooranam (SC)

Figure no: 1.6
4.2 ANALYTICAL STUDIES

ANALYTICAL STUDIES OF SEENTHIL CHOORANAM BY AYUSH GUIDELINES

Standardization of the drug brings the validation to be used as a medicine by subjecting the drug into many analysis and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic character, physico chemical characteristics studies and determination of phytochemical properties inorder to assess the active principles and elements present in the drug. Thus standardization brings the efficacy and potency of the drug.

Standardization of the drug includes:

- Pharmacognostic studies
- Physicochemical analysis
- Chemical analysis
- Heavy metal analysis
- Elemental analysis
- Particle size analysis
4.2.1 PHARMACOGNOSTIC STUDIES OF SEENTHIL CHOORANAM

The pharmacognostical study was done at Captain Srinivasamurti Research Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Chennai-106.

4.2.1.1 Organoleptic characterization - (The results expressed in Table – 01)

Colour

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

Odour

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

Taste

Small amount of Seenthil Chooranam was kept over the tip of the tongue

4.2.1.2 Microscopic

The drug sample passed through sieve 80 and used for microscopic studies. It was carried out as per the procedure. Microscopic slides were prepared either by soaking a pinch of fine powder in distilled water for 1hr. and staining with saffranin for 2-4 minutes or treating with solution of chloral hydrate for 1 hr and staining with phloroglucinol followed by addition of 1-2 drops of conc.HCl. The mounting medium is glycerine 50%.39,40,41

The images of pharmacognostical studies were shown in figures 2-4
4.2.2. PHYSICOCHEMICAL ANALYSIS

Physicochemical properties of *Seenthil Chooranam* was analyzed at Captain Srinivasamurti Research Institute for Ayurveda and Siddha Drug Development, Arumbakkam, Chennai-106.

Physico-chemical studies of the plant drugs are necessary for standardization, as it helps in understanding the significance of physical and chemical properties of the substance being analyzed in terms of their observed activities and especially for the determination of their purity and quality. The analysis include the determination of ash value, Loss on drying of the sample at 105°C, pH value, Extractive value. These were carried out as per guidelines 42.

i. **Determination of pH:**

Five grams of *Seenthil Chooranam* 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.

ii. **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 5hours. 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:**

\[
\text{Percentage of loss on drying at 105°C} = \frac{\text{Loss in weight of test drug}}{\text{Weight of test drug taken}} \times 100
\]
iii. Ash content

a. 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 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:

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

b. 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:

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

a. **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 shacked 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 preweighed 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:**

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

b. **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 preweighed 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:**

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

(The results were tabulated in Table – 02)
4.2.3 CHEMICAL ANALYSIS OF SEENTHIL CHOORANAM

The chemical analysis of *SEENTHIL CHOORANAM* was carried out in Bio chemistry Lab, NATIONAL INSTITUTE OF SIDDHA.

### Table : 1

<table>
<thead>
<tr>
<th>S.No</th>
<th>EXPERIMENT</th>
<th>OBSERVATION</th>
<th>INERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Physical Appearance of extract</td>
<td>Yellowish brown</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><strong>Test for Silicate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. A 500mg of the sample was shaken well with distilled water.</td>
<td>Sparingly soluble</td>
<td>Presence of Silicate</td>
</tr>
<tr>
<td>3.</td>
<td><strong>Action of Heat:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 500mg of the sample was taken in a dry test tube and heated gently at first and then strong.</td>
<td>No White fumes evolved. No brown fumes evolved.</td>
<td>Absence of Carbonate Absence of Nitrate.</td>
</tr>
<tr>
<td>4.</td>
<td><strong>Flame Test:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 500mg of the sample was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.</td>
<td>bluish green flame present</td>
<td>Present of copper</td>
</tr>
<tr>
<td>5.</td>
<td><strong>Ash Test:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>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>Appearance of yellow colour flame</td>
<td>Absence of sodium</td>
</tr>
</tbody>
</table>

**Preparation of Extract:**

5gm of sample was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 10 minutes. Then it is cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation is used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it.
<table>
<thead>
<tr>
<th>S.No</th>
<th>EXPERIMENT</th>
<th>OBSERVATION</th>
<th>INFEERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Test For Acid Radicals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Test For Sulphate:</td>
<td>Presence of cloudy appearance</td>
<td>Sulphate present</td>
</tr>
<tr>
<td></td>
<td>2ml of the above prepared extract was taken in a test tube and 2ml of 4% dil. ammonium oxalate solution was added.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Test For Chloride:</td>
<td>Presence of cloudy appearance</td>
<td>Chloride present</td>
</tr>
<tr>
<td></td>
<td>2ml of the above prepared extracts was added with 2ml of dil-HNO₃ until the effervescence ceases off. Then 2 ml of silver nitrate solution was added.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Test For Phosphate:</td>
<td>Absence of yellow precipitate</td>
<td>Phosphate absent</td>
</tr>
<tr>
<td></td>
<td>2ml of the extract was treated with 2ml of con.HNO₃ and 2ml of dil.ammoniummolybdate solution.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Test For Carbonate:</td>
<td>Absence of cloudy appearance</td>
<td>Carbonate absent</td>
</tr>
<tr>
<td></td>
<td>2ml of the extract was treated with 2ml dil. magnesium sulphate solution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Test For Nitrate:</td>
<td>Brown gas was not evolved</td>
<td>Nitrate absent</td>
</tr>
<tr>
<td></td>
<td>1gm of the substance was heated with copper turning and concentrated H₂SO₄ and viewed the test tube vertically down.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Test For Sulphide:</td>
<td>Rotten Egg Smelling gas was not evolved</td>
<td>Sulphide absent</td>
</tr>
<tr>
<td></td>
<td>1gm of the substance was treated with 2ml of con. HCL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Test For Fluoride &amp; Oxalate:</td>
<td>Absence of cloudy appearance</td>
<td>fluoride and oxalate were absent</td>
</tr>
<tr>
<td></td>
<td>2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil.calcium chloride solution and heated.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
8. **Test For Nitrite:**
   3 drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution were placed.
   - Characteristic changes not appeared
   - Nitrite absent

II. **Test For Basic Radicals**

1. **Test For Lead:**
   2ml of the extract was added with 2ml of dil.potassium iodine solution.
   - Yellow precipitate was not obtained.
   - Lead absent

2. **Test For Copper:**
   One pinch (25mg) of substance was made into paste with con. HCl in a watch glass and introduced into the non-luminuous part of the flame.
   - Blue colour precipitate was not formed.
   - Copper absent

3. **Test For Aluminium:**
   In the 2ml of extract dil.sodium hydroxide was added in 5 drops to excess.
   - Yellow colour was not formed
   - Aluminium absent

4. **Test For Iron:**
   a. To the 2ml of extract add 2ml of dil.ammonium solution
   - Presence of brown precipitate
   - Iron present
   b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added
   - Mild red colourformed

5. **Test For Zinc:**
   In 2ml of the extract dil.sodium hydroxide solution was added in 5 drops to excess and dil.ammonium chloride was added.
   - White precipitate was not formed
   - Zinc absent
<table>
<thead>
<tr>
<th></th>
<th>Test For Calcium:</th>
<th>Cloudy appearance was formed</th>
<th>Calcium present</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>2ml of the extract was added with 2ml of 4% dil.ammonium oxalate solution</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Test For Magnesium:</th>
<th>white precipitate not formed</th>
<th>Magnesium absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>In 2ml of extract dil.sodium hydroxide solution was added in drops to excess.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Test For Ammonium:</th>
<th>Brown colour not formed</th>
<th>Ammonium absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>In 2ml of extract 1 ml of Nessler’s reagent and excess of dil.sodium hydroxide solution were added.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Test For Potassium:</th>
<th>Yellowish precipitate was formed</th>
<th>Potassium present</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>A pinch (25mg) of substance was treated with 2ml of dil.sodium nitrite solution and then treated with 2ml of dil.cobalt nitrate in 30% dil.glacial acetic acid.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Test For Sodium:</th>
<th>Yellow colour flame was appeared</th>
<th>Sodium present</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2 pinches (50mg) of the substance was made into paste by using HCl and introduced into the blue flame of Bunsen burner.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Test For Mercury:</th>
<th>Yellow precipitate not formed</th>
<th>Mercury Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Test For Arsenic:</th>
<th>Brownish red precipitate not formed</th>
<th>Arsenic absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### III. Other constituents

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Procedure</th>
<th>Result</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Starch</td>
<td>2ml of extract was treated with weak dil.iodine solution</td>
<td>Blue colour developed</td>
<td>Starch present</td>
</tr>
<tr>
<td>2.</td>
<td>Reducing Sugar</td>
<td>5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes.</td>
<td>The was no specific change in colour</td>
<td>Reducing sugar absent</td>
</tr>
</tbody>
</table>
| 3. | Alkaloids | a) 2ml of the extract is treated with 2ml of dil.potassiumlodide solution.  
    b) 2ml of the extract is treated with 2ml of dil.picric acid. | Reddish brown precipitation was formed  
    Yellow precipitation was formed | Alkaloid Present |
| 4. | Tannic Acid | 2ml of extract was treated with 2ml of dil.ferric chloride solution | Black precipitate not formed | Tannic acid absent |
| 5. | Unsaturated Compound | In the 2ml of extract 2ml of dil.Potassium permanganate solution was added. | Potassium permanganate was decolourised | unsaturated compounds present |
| 6. | Amino Acid | 2 drops of the extract was placed on a filter paper and dried well, then 20ml of Biurette reagent was added in it. | Violet colour not developed | Amino acids absent |
| 7. | Phenols | 2ml of the extract was treated with 2 ml of dil.ferric chloride solution. | No specific colour formation | Phenols absent |
4.2.4 TLC/HPTLC finger print analysis

4g of the sample was soaked in 40ml of alcohol, boiled, filtered, concentrated and made up 10 ml in a 10ml standard flask. 5µl, 10Fµl of the solution was applied on Merck Aluminium plate pre-coated with Silica gel 60 F 254 of 0.2 mm thickness. The plate was developed in Toluene: Ethyl acetate (8:2). 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 finger print was taken before dipping in Vanillin-Sulphuric acid reagent\textsuperscript{43, 44}.

4.2.5 ESTIMATION OF HEAVY METALS (AAS)

Instrument details:

Thermo FisherM Series, 650902 V1.27 model Atomic Absorption Spectrometer (AAS) was used for the analysis. The operating parameters\textsuperscript{45, 46}

**Lead and Cadmium:**

<table>
<thead>
<tr>
<th>Instrument technique</th>
<th>Flame technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (Lead)</td>
<td>217 nm</td>
</tr>
<tr>
<td>Wavelength (Cadmium)</td>
<td>228.8 nm</td>
</tr>
<tr>
<td>Slit width</td>
<td>0.5 mm</td>
</tr>
<tr>
<td>Lamp current (Pb)</td>
<td>4.0 mA</td>
</tr>
<tr>
<td>Lamp current (Cd)</td>
<td>3.0 mA</td>
</tr>
<tr>
<td>Carrier gas and flow rate</td>
<td>Air and Acetylene, 1.1 L/min</td>
</tr>
<tr>
<td>Flow rate</td>
<td>2 ml/min</td>
</tr>
</tbody>
</table>

**Mercury:**

<table>
<thead>
<tr>
<th>Instrument technique</th>
<th>Cold vapour technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>253.7 nm</td>
</tr>
<tr>
<td>Slit width</td>
<td>0.5 mm</td>
</tr>
<tr>
<td>Lamp current</td>
<td>3.0 mA,</td>
</tr>
<tr>
<td>Carrier gas and flow rate</td>
<td>Argon, 1.1 L/min</td>
</tr>
<tr>
<td>Flow rate</td>
<td>5ml/min</td>
</tr>
</tbody>
</table>

The Hallow cathode lamp for Pb, Cd and Hg analysis were used as light source to provide specific wavelength for the elements to be determined.
4.2.6 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\textsuperscript{42i}. (The results expressed in Table – 03)

4.2.6 ELEMENTAL ANALYSIS

\textbf{Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)}

The ICP- OES is a trace-level elemental analysis technique that uses the emission spectra of a sample to identify and quantify the elements present. The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

\textbf{Introduction}

The elemental composition of a sample is often an important part of the information needed to assess its properties. Hence there is a need for sensitive 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 metalloids at trace, minor and major concentrations.

\textbf{Principal}

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 intensities of the individual wavelength can be measured. The number of photons emitted is directly proportional to the concentration of the element. The photon may be detected either sequentially or simultaneously. Quantitative analysis is achieved by measuring the intensity of these specific wavelengths and after performing the calibration using known standards.
Sample preparation – Microwave Digestion

Weigh 0.25g of *Seenthil chooranam* and transfer into a liner provided with the instrument and add 9ml of Nitric acid or Sulphuric acid slowly such that no piece of sample sticks on the slides. Mix gently and allow reacting sometimes, placing the liner in the vessel jacket and closing the screw cap hand tight and sealing it and placed in the rotor fixed in microwave. Set temperature to 180°C for 10 minutes, then allowed to cool down to a vessel interior temperature below 60°C and a vessel surface temperature (IR) below 50°C before removing the rotor.

The drug *Seenthil Chooranam* underwent microwave digestion for sample preparation.

The digested sample was made up to 100ml with Millipore water if visible insoluble particles exist, solution could be filtered through whatmann filter paper and transfer the digested solution into plastic containers and label them properly.

4.2.8 ANALYSIS OF PARTICAL SIZE

Scanning Electron microscopy (SEM)

The partical size of the *Seenthil Chooranam* was determined using Scanning electron microscopy (SEM). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

SEM:

The SEM analysis is carried out by using FEI-Quanta FEG 200-HighResolution Instrument.

- **Resolution**: 1.2 nm gold particle separation on a carbon substrate
- **Magnification**: From a min of 12 X to greater than 1,00,000 X.
- **Application**: To evaluate grain size, particle size distributions, material Homogeneity and inter metallic distribution

**Calculation of the particle size:**

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

5.1. ACUTE ORAL TOXICITY-EXPERIMENT PROCEDURE

Acute toxicity studies were carried out according to the OECD (Organization of Economic Co-operation and Development) guidelines 423. Healthy female rats, weighing 150–200 g, were selected and oral administration of the single doses of *Seenthil Chooranam* were done aseptically by suspending ghee (melted and used as vehicle).\textsuperscript{47,48}

**Administration of doses:**

*Seventhil Chooranam* in Ghee 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 a further 3–4 hours. The principle of laboratory animal care was followed. The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved Number: KKCP/2015/034.

Observations were made and recorded systematically and continuously observed as per the guideline after substance administration. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed – dosing of three additional animals with be the same dose – dosing of three additional animals at the next higher or the next lower dose level. The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

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, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. They were deprived of food, but not water...
12 h prior to the administration of the test substance. Finally, the number of survivors was 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.

**Number of animals and dose levels:**

Since this test drug has been under practice for long time and likely to be non-toxic, a limit test at one dose level of 2000 mg/kg body weight will be carried out with 3 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.

**Limit test**

The limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step. The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

**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 of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal. Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somato motor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded.
5.2. REPEATED DOSE 28-DAYS ORAL TOXICITY STUDY OF 
SEENTHIL CHOORANAM (OECD – 407 GUIDELINES)

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into 3 groups of 10 animals (5 male and 5 female). Group I served as control (fixed volume of Ghee) and Group-II and III were treated with Seenthil Chooranam at the dose of 900 & 1800 mg/kg/day for 28 days. The toxic symptoms such as signs of toxicity, mortality and body weight changes were monitored. Rats were anesthetized with ether at the end of the treatment period. All rats were sacrificed after the blood collection

Test Substance : Seenthil Chooranam (Ghee as vehicle)

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 Sai meera foods Pvt Ltd, Bangalore

Water : Aqua guard portable water in polypropylene bottles ad libitum.

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

Housing temperature : Between 20 & 24°C,

Relative humidity : Between 30% and 70%,

Dark and light cycle : Each of 12 hours.
**Justification for Dose Selection:**

The results of acute toxicity studies in rats indicated that *Seenthil Chooranam* was non toxic and no behavioral changes was observed up to the dose level of 2000mg/kg body weight in acute treatment. In the literature, therapeutic dosage for *Seenthil Chooranam* in human is mentioned as 2gm per day. The oral route was selected for use because oral route is considered to be a proposed therapeutic route. As per OECD guideline dose level of at least 1000mg/kg produces no observable toxic effects the limit test applies based on that two dose levels were selected for the study. They were, mid dose (5X) and high dose (10X). X was calculated by multiplying the therapeutic dose (2000 mg) and the body surface area of the rat (0.018). i.e , 5X dose was 180mg/animal,10X dose was 360 mg/animal.

**Preparation and administration of dose:**

*Seenthil Chooranam* was suspended in melted ghee. The suspension was administered to animals at the dose levels of 900 and 1800 mg/kg. The test substance suspensions were freshly prepared every day for 28 days. The control animals were administered vehicle (10ml/kg) only. Administration was by oral (gavage), once daily for 28 consecutive days.

**METHODOLOGY**

**Randomization, Numbering and Grouping of Animals:**

Ten Rats (Five Male and Five Female) in each group randomly divided into three groups for dosing up to 28 days. Animal’s acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved Number **KKCP/ 2015/034** 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:
Body Weight:

Weight of each rat was recorded on day 0 and at 7 days intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated. (Table-10)

Food and water Consumption:

The quantity of food consumed by groups consisting of six animals of for different doses was recorded at weekly interval. Food consumed per animal was calculated for control and the treated dose groups. (Table-11 and 12)

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.

TERMINAL STUDIES:

Laboratory Investigations:

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 slightly anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations:

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

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

**Necropsy:**

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

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

**Histopathology:**

Histopathological investigation of the vital organs was done. The organ pieces (3-5μm thick) 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 melted 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 brain, heart, kidneys, liver and lungs 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, and hematology and blood chemistry were subjected to One-way Anova. Followed by dunnet’t’ test using a computer software programme. (Graph Pad Prism5).
5.3. REPEATED DOSE 90-DAYS ORAL TOXICITY STUDY OF SEENTHIL CHOORANAM (OECD GUIDELINE - 408)\textsuperscript{50}

<table>
<thead>
<tr>
<th>Test Substance</th>
<th>SEENTHIL CHOORANAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Source</td>
<td>King Institute ,Guindy, Chennai.</td>
</tr>
<tr>
<td>Animals</td>
<td>Wistar Albino Rats (Male -12, and Female-12)</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 consists of 6 animals (Male 3, and Female-3). Group I treated as a control and other three groups 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.
The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved Number: NIS/ IAEC-I/ 2016/ 08. 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 (2000mg) and the body surface area of the rat (0.018). i.e X dose is 36 mg/animal, 5X dose is 180 mg/animal,10X dose is 360 mg/animal.

**Preparation and Administration of Dose:**

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

**OBSERVATIONS:**

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

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

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

- **Mortality:**
  
  All animals were observed twice daily for mortality during entire course of study.
Laboratory Investigations:

Following laboratory investigations were carried out on day 91 in animals' fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for 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:

All the vital organs obtained were immersed in 10% formalin for 24 h–48h for histopathological examination. After standard processing, the cut tissue was embedded in paraffin (Leica TP1020 tissue processor) and cut into 5 μm thick sections in a rotary microtome (Leica RM2255 - Fully Automated Rotary Microtome). The sections were stained with haematoxylin-eosin (Merck). Histological measurement and photographs were taken with Olympus CX31, Trinocular Biological Microscope (magnification 10x & 40 x).

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, haematology and blood chemistry were subjected to One-way ANOVA followed by dunnet’t’ test using a computer software programme (Graph Pad Prism5).
6. PHARMACOLOGICAL STUDIES

6.1. ANTIDIABETIC ACTIVITY OF SEENTHIL CHOORANAM

Aim:

To study the Antidiabetic activity of *Seenthil Chooranam* in Wistar albino rats by Steptozotocin-induced method.

Materials and methods:

Test Substance : *Seenthil Chooranam*

Animal Source : King institute of preventive medicine, Guindy.

Animals : Wistar Albino Rats (Male-15 + Female-15)

Age : 6-8 weeks

Body Weight : 150-200gm.

Acclimatization : 14 days prior to dosing.

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

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

Diet : Pellet feed

Water : Aqua guard portable water in polypropylene bottles.

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

Housing temperature : 24-28°C

Relative humidity : Between 30% and 70%.

Air changes : 10 to 15 per hour

Dark and light cycle : 12:12 hours.
Selection of animals:

Healthy Wistar albino rats (150-200g) of both sexes were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. IAEC approved no: **KKCP/2015/034**

The animals kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water and libitum. They were feed with standard diet and kept in well ventilated animal house they also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments. The rats were closely observed for any infection and if they show signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Experimental procedure:

Healthy Adult wistar rats weighing (150-200g) were used in the experiment. The rats were divided in to five groups which consisting six animals in each group. Group I formed as control group received the vehicle as ghee. Group II were treated as diabetic control received Streptozocin 40mg/kg, group III were treated with Glabencamid 5mg/kg, group IV and group V formed the drug treated groups and received the Seenthul Chooranam 200,400 mg/kg respectively. All the groups were treated with the drugs for 28 days.

Induction of diabetes:

Diabetes was induced in overnight fasted experimental wistar rats by a single intraperitoneal injection of STZ (40 mg/kg) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5)\(^{51}\). STZ injected animals were allowed to drink 20% glucose solution overnight to overcome the initial drug-induced hypoglycemic mortality. After 96 h, plasma glucose was determined and those rats with fasting blood glucose greater than 250 mg/dl were used in the present study.
Drug administration:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal Control</td>
</tr>
<tr>
<td>Group II</td>
<td>Diabetic control- STZ (40 mg/kg b.w)</td>
</tr>
<tr>
<td>Group III</td>
<td>STZ + glibenclamide (5 mg/kg) for 28 days</td>
</tr>
<tr>
<td>Group IV</td>
<td>STZ + <em>Seenthil Chooranam</em> 200mg/kg for 28 days</td>
</tr>
<tr>
<td>Group V</td>
<td>STZ + <em>Seenthil Chooranam</em> 400mg/kg for 28 days</td>
</tr>
</tbody>
</table>

The initial and final body weight of the rats in each group was recorded at the end of the experimental period, the animals were fasted overnight, anesthetized using ketamine hydrochloride (24 mg/kg intramuscular injection), and sacrificed by cervical decapitation. Blood samples for sugar estimation, Bio chemical parameter and Lipid profile were collected from the rats through retro orbital venous plexus and collected in dry test tubes were allowed to coagulate at ambient temperature for 30 min centrifugation at 2000 rpm for 10 min\(^5\). 

All results were reported as mean ± SEM. They were further analyzed using one way analysis of varients (ANOVA) followed by Tukey’s multiple comparison test. Except Body weight, food and water intake were followed by Dunnet’s test.
6.2. HEPATOPROTECTIVE ACTIVITY OF SEENTHIL CHOORANAM

Aim:

To study the Hepato-protective study of Seenthil Chooranam in Wistar albino rats by CCl4 induced hepatotoxicity method.

Materials and methods:

Test Substance : *Seenthil Chooranam*

Animal Source : King institute of preventive medicine, Guindy.

Animals : Wistar Albino Rats (Male -30)

Age : 6-8 weeks

Body Weight : 150-200gm.

Acclimatization : 14 days prior to dosing.

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

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

Diet : Pellet feed

Water : Aqua guard portable water in polypropylene bottles.

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

Housing temperature : 24-28°C

Relative humidity : Between 30% and 70%.

Air changes : 10 to 15 per hour

Dark and light cycle : 12:12 hours.
Selection of animals:

Healthy male Wistar albino rats (150-200g) were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. IAEC approved no: KKCP/2015/034

The animals kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water and libitum. They were feed with standard diet and kept in well ventilated animal house they also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments. The rats were closely observed for any infection and if they show signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Experimental design:

The wistar rats were randomized into 5 groups comprising 6 animals in each group weighing between 150-200g. Rats in Group I received 10 ml/kg body weight of ghee orally once daily for 9 days. Groups III-IV rats were pre-treated with the extract of Seenthil Chooranam 200 and 400 mg/kg for 7 days once daily by gastric intubation. Hepatic damage was induced in Groups II - V rats as described by \(^{53,54}\) (Saraf and Dixit, 1991 and Mohideen et al., 2003) administration of CCl4 intra peritoneally at the dose of 1.25 ml/kg CCl4 in olive oil (at the ratio of 1:1), 30 min post-dose of Seenthil Chooranam on days 8 and 9 as described by (Oyagbemi and Odetola 2010).\(^{55}\) Group V were fed with standard drug Silymarin 25mg/kg; p.o daily for seven days. The animals were fasted overnight and sacrificed on day 10 by cervical dislocation after collection of blood samples.

Blood Sample Collection and Analysis

Blood samples for haematological analysis were collected from all the rats through the retro-orbital venous plexus under ether-induced anaesthesia, into heparinized tubes while the sample for serum biochemistry was collected into plain tubes. From the blood samples collected, packed cell volume (PCV) was determined by micro haematocrit method, haemoglobin concentration (Hb) by cyanmethaemoglobin method while the red blood cells (RBC) and white blood cells (WBC) were counted using haemocytometer. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the values of
PCV, Hb and RBC count as described by Jain, (1986)

Erythrocyte osmotic fragility was determined according to the method described by Oyewale, (1992) by diluting 0.02ml of blood in test tubes containing 0 – 0.9% NaCl in phosphate buffer at pH of 7.4. The tubes were gently mixed and incubated at room temperature (29°C) for 30 minutes, then centrifuged at 3500rev/min for 10 minutes. The supernatant were decanted and the optical density determined at 540nm using SM22PC Spectrophotometer (Surgienfield Instruments, England). Haemolysis in each tube was expressed as a percentage, taking the tube with the highest haemolysis (i.e. Distilled water with 0.0% NaCl) as 100%

**Serum Biochemistry**

Whole blood was separated with high speed macro-centrifuge at 3,500 rpm for 10 minutes and serum was separated by Pasteur pipette for analysis of the following biochemical assays; Alkaline phosphatase (ALP) as described by Tietz and Shuey (1986), aspartate aminotransferase (AST), (Bergmeyer et al., 1985), alanine aminotransferase (ALT) (Klauke et al., 1983) albumin (Varely, 1994) and total protein (Keller, 1984).

All results were reported as mean ± SEM. They were further analyzed using one way analysis of varients (ANOVA) followed by Tukey’s multiple comparison test.
6.3. ANTI-INFLAMMATORY ACTIVITY OF SEENTHIL CHOORANAM

Aim:

To study the Anti-inflammatory of *Seenthil Chooranam* in Wistar albino rats by Carrageenan-induced rat paw edema.

Materials and methods:

Test Substance : *Seenthil Chooranam*

Animal Source : King institute of preventive medicine, Guindy.

Animals : Wistar Albino Rats (Male -12, female -12)

Age : 6-8 weeks

Body Weight : 140-160gm.

Acclimatization : 14 days prior to dosing.

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

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

Diet : Pellet feed

Water : Aqua guard portable water in polypropylene bottles.

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

Housing temperature : 24-28°C

Relative humidity : between 30% and 70%.

Air changes : 10 to 15 per hour

Dark and light cycle : 12:12 hours.
Selection of animals:

Healthy Wistar albino rats (140-160g) of both sexes were used for this study with the approval of the Institutional Animal Ethics Committee and obtained from the animal laboratory. IAEC approved no: KKCP/2015/034

The animals kept in plastic cages and maintained at 24-28°C. All the rats were housed individually with free access to food, water and libitum. They were feed with standard diet and kept in well ventilated animal house they also maintained with alternative dark-light cycle of 12hrs throughout the studies. Rats were allowed an acclimatization period of 14 days before actual experiments. The rats were closely observed for any infection and if they show signs of infection they were excluded from the study. The animal experiment was performed with accordance legislation on welfare.

Experimental protocol

Both sex of Adult wistar Albino rats weighing (140-160g) were used in this study. Rats were divided in to 4 groups, consisting six animals for each group. Group I were treated with ghee as a Vehicle, Group II treated with Seenthil Chooranam 200mg/kg, Group III treated with Seenthil Chooranam 400mg/kg and Group IV treated with standard drug Indomethacin 10mg/kg.

Acute inflammation was induced by carrageenan 30min after the drug treatment. Carrageenan was administrated by sub-planter injection of 0.1 ml freshly prepared 1% suspension in right hind paw in rats\textsuperscript{63}. The paw volume was measured initially and then at 1, 2, 3 and 4 h after the carrageenan injection by using plethysmographic method\textsuperscript{64}.

All the results were reported as mean + SEM. They were further analyzed using Two way analysis of variants (ANOVA) followed by Tukey’s multiple comparison test.
Results
Many studies have been carried out to bring the efficacy and potency of the drug *Seenthil Chooranam*. This study includes literary collections, organoleptic character, physicochemical and phytochemical analysis, Toxicityological study and pharmacological study. The drug *Seenthil Chooranam* has been selected from the text “*Agathiyar Paripuranam 400*”.

- Botanical aspect explains the active principle and medicinal uses of the plants.
- Gunapadam review brings the effectiveness of the drug in the management of Diabetes Mellitus.
- The pharmacological review explains about the Evaluation Of Anti Diabetic, Hepatoprotective and Anti-Inflammatory Activities.

**STANDARDIZATION OF THE TEST DRUG**

Traditional remedies is advantageous, it does suffer some limitations. The main limitation is the lack of standardisation of raw materials, of processing methods and of the final products, dosage formulation, and the non-existence of criteria for quality control. Standardization of the drug is more essential to derive the efficacy, potency of the drug by analysing it through various studies. Following tables and charts are the results of physicochemical and chemical analysis. Physical characterization and estimation of basic and acidic radicals have been done and tabulated. Toxicityological results of the drug and pharmacological activity of the drug were derived. Its result has been tabulated below.

### 7.1 ORGANOLEPTIC CHARACTER

**Table: 1. Organoleptic characters of Seenthil Chooranam**

<table>
<thead>
<tr>
<th>Colour</th>
<th>Yellowish Brown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour</td>
<td>Aromatic with rotten</td>
</tr>
<tr>
<td>Taste</td>
<td>Bitter</td>
</tr>
<tr>
<td>Texture</td>
<td>Fine powder</td>
</tr>
<tr>
<td>Particle size</td>
<td>Completely pass through sieve no 80</td>
</tr>
</tbody>
</table>
7.2 Pharmacognostical study

Powder Microscopy of Seenthil Chooranam

Starch grains and prismatic crystals of calcium oxalate.

Sclereid

Fragment of parenchyma cells.

Prismatic crystals of calcium oxalate under polarizer light.

Stone cells embedded with prismatic crystals of calcium oxalate under polarizer light.

Fragment of parenchyma and annular vessel cells

Figure no - 02
Fragment of fibre.

Fragment of reticulate vessel

Fragment of spiral vessel.

Fragment of cork cells in surface view.

Seta.

Pollen grain.

Figure no - 03
Fragment of bordered pitted vessel and stone cells.

Striking trichome

Fragment of reticulate and spiral vessel

Fragment of anomocytic and anisocytic stomata with subsidiary cells

Fragment of anomocytic and anisocytic stomata with subsidiary cells

Figure no - 04
Powder Microscopy

Under microscope shows numerous fragments of stone cells embedded with prismatic crystals of calcium oxalate; fragments of thick walled polygonal cork cells in surface view; fragments of bordered pitted vessels; numerous simple, irregularly ovoid or elliptical shaped starch grains with concentric striations having hilum in centre and sometimes compound with 2-4 components measuring 12.2 to 51.8µ in diameter, numerous unicellular, uniseriate, warty, tubercles, pointed with basal in different sizes of trichomes; a few fragments of epidermis with anomocytic, anisocytic stomata and cicatrix; a few fragments of parenchyma cells; a few spherical shaped pollen grains with spines or warty surface; a few fragments of lignified spiral and reticulate vessels; a few fragments of non-lignified septate fibers; a few fragment of thick walled sclereid with narrow lumen and pits; a few fragments of parenchyma and annular vessels; a very few fragments of lamina with veins and vein- islets in surface view and a few entire seta.

7.3 Physico-chemical Analysis

Table: 2. Physico-chemical properties of *Seenthil Chooranam*

<table>
<thead>
<tr>
<th>No</th>
<th>Physico-Chemical Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Loss on drying at 105° C</td>
<td>10.30%</td>
</tr>
<tr>
<td>2.</td>
<td>Ash values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Total ash</td>
<td>11.93%</td>
</tr>
<tr>
<td></td>
<td>b. Acid insoluble ash</td>
<td>2.49%</td>
</tr>
<tr>
<td>3.</td>
<td>Extract values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a. Alcohol extract</td>
<td>13.76%</td>
</tr>
<tr>
<td></td>
<td>b. Water extract</td>
<td>21.02%</td>
</tr>
<tr>
<td>4.</td>
<td>PH</td>
<td>7.07</td>
</tr>
</tbody>
</table>
7.4 Determination of Microbial Load in *Seenthil Chooranam*

Table: 3. Microbial load test results of *Seenthil Chooranam*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Results</th>
<th>Permissible Limit for Internal use&lt;sup&gt;R&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Bacterial Count (TBC)</td>
<td>0.5x10^3 cfu/g</td>
<td>10^3 cfu/g</td>
</tr>
<tr>
<td>2</td>
<td>Total Fungal Count (TFC)</td>
<td>Less than 10 cfu /g</td>
<td>10^3 cfu/g</td>
</tr>
<tr>
<td>3</td>
<td>Enterobacteriaceae</td>
<td>Absent</td>
<td>10^3 cfu/g</td>
</tr>
<tr>
<td>4</td>
<td>Escherichia coli</td>
<td>Absent</td>
<td>10 cfu/g</td>
</tr>
<tr>
<td>5</td>
<td>Salmonella Spp</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>6</td>
<td>Staphylococcus aureus</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>7</td>
<td>Pseudomonas aeruginosa</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

7.5 Chemical analysis

The Chemical analysis shows the presence of *Silicate, Sulphate, Chloride, Calcium, Iron, Pottasium, Sodium, and Alkaloids* in *Seenthil Chooranam*.

Table: 4. Chemical Analysis of *Seenthil Chooranam*

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Silicate</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Sulphate</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Chloride</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>Phosphate</td>
<td>Absent</td>
</tr>
<tr>
<td>5</td>
<td>Carbonate</td>
<td>Absent</td>
</tr>
<tr>
<td>6</td>
<td>Nitra</td>
<td>Absent</td>
</tr>
<tr>
<td>7</td>
<td>Sulphide</td>
<td>Absent</td>
</tr>
<tr>
<td>8</td>
<td>Oxalate</td>
<td>Absent</td>
</tr>
<tr>
<td>9</td>
<td>Nitrite</td>
<td>Absent</td>
</tr>
<tr>
<td>10</td>
<td>Borate</td>
<td>Absent</td>
</tr>
<tr>
<td>11</td>
<td>Lead</td>
<td>Absent</td>
</tr>
<tr>
<td>12</td>
<td>Copper</td>
<td>Absent</td>
</tr>
<tr>
<td>13</td>
<td>Aluminium</td>
<td>Absent</td>
</tr>
</tbody>
</table>

Interpretation

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

---

60
### Table: 5. Chemical Analysis of Seenthil Choornam

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.</td>
<td>Iron</td>
<td>Present</td>
</tr>
<tr>
<td>15.</td>
<td>Zinc</td>
<td>Absent</td>
</tr>
<tr>
<td>16.</td>
<td>Calcium</td>
<td>Present</td>
</tr>
<tr>
<td>17.</td>
<td>Magnesium</td>
<td>Absent</td>
</tr>
<tr>
<td>18.</td>
<td>Ammonium</td>
<td>Absent</td>
</tr>
<tr>
<td>19.</td>
<td>Potassium</td>
<td>Present</td>
</tr>
<tr>
<td>20.</td>
<td>Sodium</td>
<td>Present</td>
</tr>
<tr>
<td>21.</td>
<td>Mercury</td>
<td>Absent</td>
</tr>
<tr>
<td>22.</td>
<td>Arsenic</td>
<td>Absent</td>
</tr>
<tr>
<td>23.</td>
<td>Starch</td>
<td>Present</td>
</tr>
<tr>
<td>24.</td>
<td>Reducing sugar</td>
<td>Absent</td>
</tr>
<tr>
<td>25.</td>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>26.</td>
<td>Tannic acid</td>
<td>Absent</td>
</tr>
</tbody>
</table>

**Interpretation**

The basic radical test shows the presence of, Iron, Calcium, Potassium, Sodium and Alkaloids and absence of heavy metals such as lead, arsenic and mercury.
### 7.6 TLC and HPTLC Results

Table: 6. Rf. values for the alcohol extract

<table>
<thead>
<tr>
<th>S.No</th>
<th>254nm</th>
<th>366nm</th>
<th>Dipped in Vanillin-Sulphuric Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colour</td>
<td>Rf</td>
<td>Colour</td>
</tr>
<tr>
<td>1</td>
<td>Green</td>
<td>0.12</td>
<td>Red</td>
</tr>
<tr>
<td>2</td>
<td>Green</td>
<td>0.15</td>
<td>Red</td>
</tr>
<tr>
<td>3</td>
<td>Green</td>
<td>0.34</td>
<td>Red</td>
</tr>
<tr>
<td>4</td>
<td>Green</td>
<td>0.39</td>
<td>Red</td>
</tr>
<tr>
<td>5</td>
<td>Green</td>
<td>0.44</td>
<td>Red</td>
</tr>
<tr>
<td>6</td>
<td>Green</td>
<td>0.51</td>
<td>Violet</td>
</tr>
<tr>
<td>7</td>
<td>Green</td>
<td>0.56</td>
<td>Red</td>
</tr>
<tr>
<td>8</td>
<td>Green</td>
<td>0.59</td>
<td>Blue</td>
</tr>
<tr>
<td>9</td>
<td>Red</td>
<td>0.54</td>
<td>Grey</td>
</tr>
<tr>
<td>10</td>
<td>Red</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Yellow</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Blue</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Blue</td>
<td>0.95</td>
<td></td>
</tr>
</tbody>
</table>
HPTLC finger print profile

The finger print chromatogram was recorded at 254 nm. It showed 16 peaks of which two peaks at Rf. 0.12, 0.51 and 0.54 were major peak and others were moderately smaller peaks.
Rf Table for the HPTLC finger print:

<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>0.02 Rf</td>
<td>0.4 AU</td>
<td>0.03 Rf</td>
<td>22.1 AU</td>
<td>1.49 %</td>
<td>0.04 Rf</td>
<td>0.1 AU</td>
<td>227.3 AU</td>
<td>5.54 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>2</td>
<td>0.04 Rf</td>
<td>9.6 AU</td>
<td>0.08 Rf</td>
<td>111.6 AU</td>
<td>7.56 %</td>
<td>0.10 Rf</td>
<td>0.3 AU</td>
<td>2522.5 AU</td>
<td>6.02 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>3</td>
<td>0.10 Rf</td>
<td>82.0 AU</td>
<td>0.12 Rf</td>
<td>177.3 AU</td>
<td>11.08 %</td>
<td>0.15 Rf</td>
<td>0.1 AU</td>
<td>1969.0 AU</td>
<td>9.46 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>4</td>
<td>0.15 Rf</td>
<td>20.4 AU</td>
<td>0.16 Rf</td>
<td>25.3 AU</td>
<td>2.39 %</td>
<td>0.19 Rf</td>
<td>0.2 AU</td>
<td>749.6 AU</td>
<td>1.79 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>5</td>
<td>0.20 Rf</td>
<td>0.1 AU</td>
<td>0.23 Rf</td>
<td>16.9 AU</td>
<td>1.14 %</td>
<td>0.25 Rf</td>
<td>0.1 AU</td>
<td>359.7 AU</td>
<td>0.66 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>6</td>
<td>0.25 Rf</td>
<td>0.1 AU</td>
<td>0.30 Rf</td>
<td>42.4 AU</td>
<td>2.67 %</td>
<td>0.32 Rf</td>
<td>0.3 AU</td>
<td>1275.2 AU</td>
<td>3.04 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>7</td>
<td>0.33 Rf</td>
<td>24.8 AU</td>
<td>0.35 Rf</td>
<td>52.9 AU</td>
<td>4.04 %</td>
<td>0.37 Rf</td>
<td>0.4 AU</td>
<td>1514.6 AU</td>
<td>3.61 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>8</td>
<td>0.37 Rf</td>
<td>31.5 AU</td>
<td>0.41 Rf</td>
<td>146.6 AU</td>
<td>9.00 %</td>
<td>0.44 Rf</td>
<td>0.4 AU</td>
<td>5325.4 AU</td>
<td>12.70 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>9</td>
<td>0.45 Rf</td>
<td>23.2 AU</td>
<td>0.47 Rf</td>
<td>103.6 AU</td>
<td>7.41 %</td>
<td>0.49 Rf</td>
<td>0.3 AU</td>
<td>2922.8 AU</td>
<td>6.93 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>10</td>
<td>0.50 Rf</td>
<td>60.3 AU</td>
<td>0.51 Rf</td>
<td>168.8 AU</td>
<td>11.35 %</td>
<td>0.53 Rf</td>
<td>0.5 AU</td>
<td>2699.5 AU</td>
<td>6.94 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>11</td>
<td>0.52 Rf</td>
<td>122.1 AU</td>
<td>0.54 Rf</td>
<td>222.9 AU</td>
<td>16.74 %</td>
<td>0.56 Rf</td>
<td>0.7 AU</td>
<td>609.2 AU</td>
<td>14.33 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>12</td>
<td>0.55 Rf</td>
<td>19.0 AU</td>
<td>0.62 Rf</td>
<td>33.0 AU</td>
<td>2.23 %</td>
<td>0.64 Rf</td>
<td>0.3 AU</td>
<td>1127.0 AU</td>
<td>2.69 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>13</td>
<td>0.66 Rf</td>
<td>21.4 AU</td>
<td>0.72 Rf</td>
<td>61.0 AU</td>
<td>4.12 %</td>
<td>0.77 Rf</td>
<td>0.5 AU</td>
<td>3904.2 AU</td>
<td>9.31 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>14</td>
<td>0.78 Rf</td>
<td>25.4 AU</td>
<td>0.83 Rf</td>
<td>58.6 AU</td>
<td>3.96 %</td>
<td>0.87 Rf</td>
<td>0.5 AU</td>
<td>3284.0 AU</td>
<td>7.78 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>15</td>
<td>0.86 Rf</td>
<td>33.4 AU</td>
<td>0.96 Rf</td>
<td>101.7 AU</td>
<td>6.87 %</td>
<td>0.98 Rf</td>
<td>0.6 AU</td>
<td>4373.1 AU</td>
<td>10.43 %</td>
<td>unknown *</td>
</tr>
<tr>
<td>16</td>
<td>0.96 Rf</td>
<td>99.1 AU</td>
<td>1.06 Rf</td>
<td>102.5 AU</td>
<td>6.91 %</td>
<td>1.09 Rf</td>
<td>1.1 AU</td>
<td>1681.4 AU</td>
<td>3.59 %</td>
<td>unknown *</td>
</tr>
</tbody>
</table>
7.7 Heavy metal analysis

Table: 7. Heavy Metals Analysis of Seenthil Choornam

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the Element</th>
<th>Results</th>
<th>Permissible Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lead</td>
<td>5.15 ppm</td>
<td>10 ppm (WHO)</td>
</tr>
<tr>
<td>2</td>
<td>Cadmium</td>
<td>Not Detected</td>
<td>0.3 ppm (WHO)</td>
</tr>
<tr>
<td>3</td>
<td>Mercury</td>
<td>0.23 ppm</td>
<td>1 ppm (API)</td>
</tr>
</tbody>
</table>

7.8 Elemental analysis

Table: 8. Elemental Analysis of Seenthil Choornam

<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>07.390</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>Iron</td>
<td>Fe 238.204</td>
<td>18.346</td>
</tr>
<tr>
<td>6</td>
<td>Lead</td>
<td>Pb 220.353</td>
<td>BDL</td>
</tr>
<tr>
<td>7</td>
<td>Mercury</td>
<td>Hg 253.652</td>
<td>BDL</td>
</tr>
<tr>
<td>8</td>
<td>Nickel</td>
<td>Ni 231.604</td>
<td>BDL</td>
</tr>
<tr>
<td>9</td>
<td>Potassium</td>
<td>K 766.491</td>
<td>03.811</td>
</tr>
<tr>
<td>10</td>
<td>Phosphorus</td>
<td>P 213.617</td>
<td>76.341</td>
</tr>
<tr>
<td>11</td>
<td>Sodium</td>
<td>Na 589.592</td>
<td>14.310</td>
</tr>
</tbody>
</table>
7.9 HR SEM analysis

HR SEM Micrograph of Seenthil Chooranam

Figure no - 07

Inference:

- The particles are in spherical form.
- The particle size of Seenthil Chooranam ranges from 329.58 -366.2nm
7.10. ACUTE TOXICITY STUDIES

Results:

Wistar albino rat was treated with the test drug *Seenthil Chooranam* of single dose of 2000mg/kg in melted ghee. This study was conducted as per the OECD guidelines. The result of acute Toxicity of *Seenthil Chooranam* has been tabulated below.

Table: 9. Dose finding experiment and its behavioural Signs of Toxicity

<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>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>


(+) indicates the presence.
(-) indicates the absence.

Interpretation

The acute Toxicity result shows no mortality rate up to dose level of 2000mg/kg. It showed changes in alertness, grooming, touch response and grip strength. The normal behavioural changes were observed in first four hours and no mortality was reported after 14 days observation. Hence the test drug *Seenthil Chooranam* is a safe up to the dose of level of 2000 mg/Kg in oral administration.
7.11 RESULTS OF REPEATED DOSE 28 DAYSS ORAL TOXICITY STUDY IN WISTAR RATS

Table: 10. Changes of Body weight (g) of albino rats exposed to Seenthil Chooranam for 28 Dayss

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0th Days</th>
<th>7th Days</th>
<th>14th Days</th>
<th>21th Days</th>
<th>28th Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>170.83±6.84</td>
<td>173.50±6.28</td>
<td>179.83±6.31</td>
<td>185.66±6.46</td>
<td>188.66±5.70</td>
</tr>
<tr>
<td>Mid dose</td>
<td>163.16±6.38</td>
<td>163.33±6.60</td>
<td>167.83±6.610</td>
<td>171.16±7.32</td>
<td>174.83±7.37</td>
</tr>
<tr>
<td>High dose</td>
<td>150.83±9.58</td>
<td>151.66±9.09</td>
<td>155.01±9.56</td>
<td>158.53±9.31</td>
<td>163.16±9.67</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 10 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test

Chart- 01
Table: 11. Water intake (ml/Days) of albino rats exposed to *Seenthil Chooranam* for 28 Days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0th Days</th>
<th>7th Days</th>
<th>14th Days</th>
<th>21th Days</th>
<th>28th Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.0±0.48</td>
<td>32.16±0.22</td>
<td>34.13±0.33</td>
<td>35.2±0.16</td>
<td>35.46±0.34</td>
</tr>
<tr>
<td>Mid dose</td>
<td>31.73±0.89</td>
<td>34.52±1.22</td>
<td>35.41±1.11</td>
<td>37.45±1.05</td>
<td>38.64±1.42</td>
</tr>
<tr>
<td>High dose</td>
<td>32.66±1.15</td>
<td>35.01±1.64</td>
<td>38.33±1.77</td>
<td>40.16±1.43</td>
<td>40.5±1.52</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 10 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test

Chart- 02

Table: 12. Food (g/Days) intake of albino rats exposed to *Seenthil Chooranam* for 28 Days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0th Days</th>
<th>7th Days</th>
<th>14th Days</th>
<th>21th Days</th>
<th>28th Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.83±1.8</td>
<td>37.50±1.67</td>
<td>40.83±1.31</td>
<td>41.66±1.46</td>
<td>42.66±1.50</td>
</tr>
<tr>
<td>Mid dose</td>
<td>40.16±0.38</td>
<td>42.33±0.60</td>
<td>42.83±0.61</td>
<td>43.16±1.32</td>
<td>44.13±1.37</td>
</tr>
<tr>
<td>High dose</td>
<td>41.63±0.58</td>
<td>42.66±1.09</td>
<td>43.01±1.56</td>
<td>43.53±1.31</td>
<td>44.16±1.67</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 10 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test
Table: 13. Relative Organ Weight of albino rats exposed to *Seenthil Chooranam* for 28 Days

<table>
<thead>
<tr>
<th>Dose</th>
<th>Liver (g)</th>
<th>Kidney (g)</th>
<th>Brain (g)</th>
<th>Lungs (g)</th>
<th>Heart (g)</th>
<th>Spleen (g)</th>
<th>Ovary (g)</th>
<th>Testis (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.80±0.10</td>
<td>0.66±0.02</td>
<td>0.38±0.01</td>
<td>0.29±0.01</td>
<td>0.15±0.01</td>
<td>1.27±0.01</td>
<td>1.15±0.01</td>
<td></td>
</tr>
<tr>
<td>Mid dose</td>
<td>2.89±0.10</td>
<td>0.65±0.02</td>
<td>0.42±0.01</td>
<td>0.30±0.02</td>
<td>0.17±0.01</td>
<td>1.35±0.01</td>
<td>1.19±0.01</td>
<td></td>
</tr>
<tr>
<td>High dose</td>
<td>3.01±0.03</td>
<td>0.66±0.01</td>
<td>0.42±0.01</td>
<td>0.31±0.01</td>
<td>0.16±0.01</td>
<td>1.38±0.01</td>
<td>1.18±0.01</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 10 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test
Table: 14. Haematological Parameter of *Seenthil Chooranam*

<table>
<thead>
<tr>
<th>Haematological parameter</th>
<th>Control</th>
<th>Seenthil Chooranam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mid dose</td>
<td>High dose</td>
</tr>
<tr>
<td>Total R.B.C. count (×10⁶ mm⁻³).</td>
<td>9.05±0.15</td>
<td>9.10±0.12</td>
</tr>
<tr>
<td></td>
<td>9.12±0.16</td>
<td></td>
</tr>
<tr>
<td>Total W.B.C. Count (×10³ mm⁻³).</td>
<td>12.67±0.22</td>
<td>12.12±0.48</td>
</tr>
<tr>
<td></td>
<td>11.23±0.12</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (Hb) (g/dl)</td>
<td>15.61±0.36</td>
<td>14.87±0.27</td>
</tr>
<tr>
<td></td>
<td>15.48±0.78</td>
<td></td>
</tr>
<tr>
<td>Platelets (×10⁵ mm⁻³).</td>
<td>834.91±24.01</td>
<td>845.21±16.55</td>
</tr>
<tr>
<td></td>
<td>863.58±16.25</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes(%)</td>
<td>77.7±1.32</td>
<td>79.28±2.63</td>
</tr>
<tr>
<td></td>
<td>78.8±5.49</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>20.6±0.65</td>
<td>19.6±1.25</td>
</tr>
<tr>
<td></td>
<td>18.95±0.65</td>
<td></td>
</tr>
<tr>
<td>PCV (%)</td>
<td>43.34±1.08</td>
<td>46.08±1.34</td>
</tr>
<tr>
<td></td>
<td>47.12±0.45</td>
<td></td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>54.34±5.17</td>
<td>55.58±1.83</td>
</tr>
<tr>
<td></td>
<td>55.17±2.79</td>
<td></td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>21.1 ± 0.4</td>
<td>20.2 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>20.5 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>MCHC g/dl</td>
<td>36.81±3.41</td>
<td>34.55±1.18</td>
</tr>
<tr>
<td></td>
<td>36.12±2.17</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 10 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test.

The mean value of T.RBC of control and treated groups of wistar albino rats exposed to *Seenthil Chooranam in Repeated Oral 28 Days Toxicity study.*

Chart - 04
The mean value of T.WBC and Platlets of control and treated groups of wistar albino rats exposed to *Seenthil Chooranam* in Repeated Oral 28 Days Toxicity study.

Chart - 05

<table>
<thead>
<tr>
<th></th>
<th>T.WBC</th>
<th>Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.4</td>
<td>14.6</td>
</tr>
<tr>
<td>Mid Dose</td>
<td>14.8</td>
<td>15</td>
</tr>
<tr>
<td>High Dose</td>
<td>15.2</td>
<td>15.4</td>
</tr>
<tr>
<td></td>
<td>15.6</td>
<td>15.8</td>
</tr>
</tbody>
</table>

The mean value of Hb of control and treated groups of wistar albino rats exposed to *Seenthil Chooranam* in Repeated Oral 28 Days Toxicity study

Chart - 06

<table>
<thead>
<tr>
<th></th>
<th>Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.4</td>
</tr>
<tr>
<td>Mid Dose</td>
<td>15.2</td>
</tr>
<tr>
<td>High Dose</td>
<td>15.6</td>
</tr>
</tbody>
</table>
The mean value of Lymphocytes and Neutrophils of control and treated groups of wistar albino rats exposed to Seenthil Chooranam in Repeated Oral 28 Days Toxicity study.

Chart - 07

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Seenthil chooranam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mid dose</td>
</tr>
<tr>
<td>Total protein (gm/dl)</td>
<td>8.40±0.26</td>
<td>8.12±0.35</td>
</tr>
<tr>
<td>Albumin (gm/dl)</td>
<td>4.40±0.41</td>
<td>4.30±0.35</td>
</tr>
<tr>
<td>Globulin (gm/dl)</td>
<td>3.12±0.21</td>
<td>3.07±0.15</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>121.41±2.68</td>
<td>118.3±1.67</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>69.40±1.57</td>
<td>69.01±2.32</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>112.6±4.67</td>
<td>115.01±1.21</td>
</tr>
<tr>
<td>T. Bilirubin (mg/dl)</td>
<td>0.25±0.32</td>
<td>0.3±1.12</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 10 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test
The mean value of Total protein, Albumin and Globulin of control and treated groups of wistar albino rats exposed to *Seenthil Chooranam* in Repeated Oral 28 Days Toxicity study

**Chart - 08**

**T.Protein, Albumin and Globulin**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mid Dose</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.Protein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Globulin</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean value of AST, ALT and ALP of control and treated groups of wistar albino rats exposed to *Seenthil Chooranam* in Repeated Oral 28 Days Toxicity study

**Chart - 09**

**AST, ALT and ALP**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Mid Dose</th>
<th>High Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The mean value of T. Bilirubin of control and treated groups of wistar albino rats exposed to Seenthil Chooranam in Repeated Oral 28 Days Toxicity study

Chart - 10

Table: 16. Blood sugar and Lipid profile of wistar albino rats exposed to Seenthil Chooranam in Repeated Oral 28-Days Toxicity study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Seenthil Chooranam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mid dose</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>112.6±4.67</td>
<td>98.01±2.22*</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>49.60±1.21</td>
<td>51.46±1.08</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>52.20±1.13</td>
<td>50.23±1.08</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>12.80 ± 2.70</td>
<td>11.5 ± 2.82</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>39.5 ± 2.13</td>
<td>42.5 ± 2.11</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>14.05 ± 3.90</td>
<td>15.5 ± 2.04</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 10 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test
The mean value of Glucose of control and treated groups of wistar albino rats exposed to *Seenthil Chooranam* in Repeated Oral 28 Days Toxicity study

Chart - 11

![Glucose Chart]

The mean value of T.Cholesterol and Triglyceride of control and treated groups of wistar albino rats exposed to *Seenthil Chooranam* in Repeated Oral 28 Days Toxicity study

Chart - 12

![T.Cholesterol and Triglyceride Chart]
The mean value of HDL, LDL, VLDL of control and treated groups of wistar albino rats exposed to Seenthil Chooranam in Repeated Oral 28 Days Toxicity study

Chart - 13

Table: 17. RFT results of wistar albino rats exposed to Seenthil Chooranam in Repeated Oral 28-Days Toxicity study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Seenthil Chooranam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mid dose</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.58 ±0.07</td>
<td>0.65±0.04</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>15.30 ± 0.47</td>
<td>14.33±0.49</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.20±0.21</td>
<td>1.30±0.25</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 10 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test
The mean value of Creatinine, Urea and Uric acid of control and treated groups of wistar albino rats exposed to *Seenthil Chooranam* in Repeated Oral 28 Days Toxicity study

**Chart - 14**
HISTOPATHOLOGY OF VITAL ORGANS

Control               High Power Magnification 40X        High Dose

Brain                               Figure 8.1                               Brain

Heart                               Figure 8.2                               Heart

Kidney                               Figure 8.3                               Kidney
Interpretation:

Liver:
Section of liver from control animals showed No degeneration of hepatocytes, focal steatosis, and congestion of central vein and inflammation of portal tract.
Liver of treated groups showed No degeneration of hepatocytes, focal steatosis, and congestion of central vein and inflammation of portal tract.

Kidney:
Section of kidney from control animals showed normal size of glomeruli with normal tubules. Kidney of treated animals showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney.
**Lungs:**
Section of lungs from control animal showed normal architecture of a section and the drug treated group shows mild paraseptal thickening with inflammation.

**Brain:**
Section of brain from control animals showed brain cortex with normal architecture. Treated animals showed brain cortex with normal architecture.

**Heart:**
Section of heart from control animal showed normal muscle fibers with acidophilic cytoplasm and centrally located nuclei. In treated group showed normal muscle fibers with acidophilic cytoplasm and centrally located nuclei with normal structure.

Heart, Lungs, brain, liver and kidney showed no cellular architecture in treated groups. From the histopathological study no related changes in vital organs are observed in treated groups.

**Results:**
All animals from control and all the treated dose groups survived throughout the dosing period of 28 Days for sub acute Toxicity study. The results for body weight determination of animals from control and different dose groups show comparable body weight gain throughout the dosing period of 28 Days. During dosing period, the quantity of food and water consumed by animals from different dose groups was found to be comparable and normal with that by control animals.

The results of haematological investigations conducted on Days 29th Days revealed no significant changes in the haematological values when compared with those of respective controls. This gave clear justification that bone marrow and spleen were not influenced by *Seenthil Chooranam*.

Results of Biochemical investigations conducted on Dayss 29 and recorded in revealed the no significant changes in the values of different parameters studied when compared with those of respective controls; Urea, SGOT,SGPT, Bilirubin were within the limits, blood glucose significant compared to control group.

The other cardio vascular risk markers were also within normal ensured that *Seenthil Chooranam* did not influence the Cardio vascular system.
Group Mean Relative Organ Weights are recorded. Comparison of organ weights of treated animals with respective control animals on Days 29 was found to be comparable with respective control group. Gross pathological examination of animals in control as well as the treated groups did not reveal any abnormalities.

The vital organs such as liver, heart, kidneys, lungs and brain were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Gross pathological investigation was carried out and histopathology of vital organ revealed normal histological appearance when compared with the control. According to these results, *Seenthil chooranim* could be concluded as no-observed-adverse-effect level (NOAEL). It showed the safety of the drug which proved its utility in long time administration without any harm to the human being.
7.12 RESULTS OF REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY IN WISTAR RATS

Table 18: Changes of Body weight (g) of albino rats exposed to Seenthil Chooranam for 90 Days

<table>
<thead>
<tr>
<th>DAYS</th>
<th>Control</th>
<th>Low dose</th>
<th>Mid dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>160.6±33.68</td>
<td>147.1 ± 21.11</td>
<td>155.6± 13.57</td>
<td>158.5± 28.75</td>
</tr>
<tr>
<td>15</td>
<td>172.8 ± 28.87</td>
<td>161.5 ± 21.71</td>
<td>165.7 ± 29.01</td>
<td>165.8 ± 32.48</td>
</tr>
<tr>
<td>30</td>
<td>180.8 ± 28.31</td>
<td>175.7 ± 14.88</td>
<td>180.8 ± 32.11</td>
<td>180 ± 28.94</td>
</tr>
<tr>
<td>45</td>
<td>204.5± 27.73</td>
<td>194.5± 29.76</td>
<td>203.7± 19.75</td>
<td>207.3± 22.75</td>
</tr>
<tr>
<td>60</td>
<td>217.6±33.68</td>
<td>213.6±23.68</td>
<td>225.6±33.68</td>
<td>214.6±23.78</td>
</tr>
<tr>
<td>75</td>
<td>235.8± 26.85</td>
<td>225.8± 28.87</td>
<td>248.0± 28.87</td>
<td>223.8±26.87</td>
</tr>
<tr>
<td>90</td>
<td>248± 27.320</td>
<td>238.5± 27.320</td>
<td>250.4± 27.320</td>
<td>236.3±36.32</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 6 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test

Chart – 15
Table 19: Haematological parameters of Wistar albino rats group exposed to *Seenthil Chooranam*.

<table>
<thead>
<tr>
<th>Category</th>
<th>Control</th>
<th>Low dose</th>
<th>Mid dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.RBC</td>
<td>7.20±0.30</td>
<td>7.33±0.33</td>
<td>7.20±0.74</td>
<td>7.30±0.30</td>
</tr>
<tr>
<td>T.WBC (cells/cu.mm)</td>
<td>11.05±0.76</td>
<td>11.50±0.33</td>
<td>11.25±0.46</td>
<td>11.93±0.22</td>
</tr>
<tr>
<td>Platelets</td>
<td>3.61±0.30</td>
<td>3.70±0.27</td>
<td>3.65±0.16</td>
<td>3.41±0.43</td>
</tr>
<tr>
<td>PCV</td>
<td>37.74±.88</td>
<td>38.84±2.43</td>
<td>37.92±1.71</td>
<td>39.22±2.25</td>
</tr>
<tr>
<td>Hb (%)</td>
<td>12.58±0.29</td>
<td>12.95±0.82</td>
<td>12.65±0.58</td>
<td>13.08±0.75</td>
</tr>
<tr>
<td>MCV (%)</td>
<td>88.50±3.10</td>
<td>91.50±2.73</td>
<td>91.50±1.87</td>
<td>92.33±3.33</td>
</tr>
<tr>
<td>MCH (%)</td>
<td>31.83±1.72</td>
<td>30.83±2.79</td>
<td>31.67±3.72</td>
<td>31.50±1.05</td>
</tr>
<tr>
<td>MCHC</td>
<td>34.33±1.86</td>
<td>36.33±4.88</td>
<td>34.50±2.59</td>
<td>33.17±2.13</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 6 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test

**The mean value of T.WBC of control and treated groups of wistar albino rats exposed to Seenthil Chooranam**

Chart – 16
The mean value of Hb and T.RBC of control and treated groups of wistar albino rats exposed to Seenthil Chooranam

Chart – 17

<table>
<thead>
<tr>
<th></th>
<th>Hb gm</th>
<th>T.RBC million</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>356000</td>
<td>358000</td>
</tr>
<tr>
<td>low dose</td>
<td>360000</td>
<td>362000</td>
</tr>
<tr>
<td>Mid dose</td>
<td>364000</td>
<td>366000</td>
</tr>
<tr>
<td>High dose</td>
<td>368000</td>
<td>370000</td>
</tr>
</tbody>
</table>

The mean value of Platelet of control and treated groups of wistar albino rats exposed to Seenthil Chooranam

Chart – 18

<table>
<thead>
<tr>
<th></th>
<th>Platelet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>356000</td>
</tr>
<tr>
<td>low dose</td>
<td>370000</td>
</tr>
<tr>
<td>Mid dose</td>
<td>364000</td>
</tr>
<tr>
<td>High dose</td>
<td>362000</td>
</tr>
</tbody>
</table>
The mean value of PCV, MCHC and MCV of control and treated groups of wistar albino rats exposed to *Seenthil Chooranam*

Chart – 19

Table 20: Biochemical Parameters of of Wistar albino rats group exposed *Seenthil Chooranam*

<table>
<thead>
<tr>
<th>BIOCHEMICAL PARAMETERS</th>
<th>CONTROL</th>
<th>LOW DOSE</th>
<th>MID DOSE</th>
<th>HIGH DOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUCOSE (R) (mg/dl)</td>
<td>130.83±3.54</td>
<td>91.0±7.37*</td>
<td>81.83±8.18*</td>
<td>74.33±11.11*</td>
</tr>
<tr>
<td>T.CHOLOSTEROL (mg/dl)</td>
<td>108.67±11.43</td>
<td>116.00±11.01</td>
<td>107.33±11.31</td>
<td>117.17±5.11</td>
</tr>
<tr>
<td>TGL (mg/dl)</td>
<td>134.17±6.18</td>
<td>137.50±6.09</td>
<td>133.83±11.30</td>
<td>131.83±6.82</td>
</tr>
<tr>
<td>HDL</td>
<td>39.00±6.13</td>
<td>43.67±2.80</td>
<td>43.50±3.78</td>
<td>44.67±8.09</td>
</tr>
<tr>
<td>LDL</td>
<td>42.83±13.86</td>
<td>44.83±11.39</td>
<td>32.33±11.83</td>
<td>50.83±5.53</td>
</tr>
<tr>
<td>VLDL</td>
<td>26.83±1.23</td>
<td>27.50±1.22</td>
<td>26.76±2.26</td>
<td>26.87±1.64</td>
</tr>
<tr>
<td>ALP</td>
<td>83.50±11.45</td>
<td>80.67±16.56</td>
<td>74.67±17.67</td>
<td>81.00±16.48</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 6 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test
The mean value of Blood sugar, Total cholesterol and Triglycerides of control and treated groups of wistar albino rats exposed to *Seenthil Chooranam*

Chart – 20

![Graph showing Blood sugar, T. cholesterol and Triglycerides](chart.png)

**Table 21: Renal function test of of Wistar albino rats group exposed to *Seenthil Chooranam***

<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>30.50±10.01</td>
<td>24.67±2.50</td>
<td>30.17±9.15</td>
<td>26.17±6.34</td>
</tr>
<tr>
<td>CREATININE(mg/dl)</td>
<td>0.57±0.15</td>
<td>0.50±0.12</td>
<td>0.53±0.12</td>
<td>0.46±0.13</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 6 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test
The mean value of Urea and Creatinine of control and treated groups of wistar albino rats exposed to Seenthil Chooranam

Chart – 21

Table 22: Liver Function Test of of Wistar albino rats group exposed to  Seenthil Chooranam

<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>T.BILIRUBIN(mg/dl)</td>
<td>0.58±0.15</td>
<td>0.48±0.07</td>
<td>0.66±0.16</td>
<td>0.76±0.12</td>
</tr>
<tr>
<td>SGOT(U/dl)</td>
<td>24.00±2.28</td>
<td>25.83±2.92</td>
<td>27.83±4.66</td>
<td>24.67±3.67</td>
</tr>
<tr>
<td>SGPT(U/dl)</td>
<td>23.33±6.37</td>
<td>24.50±2.73</td>
<td>25.33±2.66</td>
<td>23.33±5.20</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM (n = 6 for each group), *P < 0.05, **P<0.01 were considered significant using One way ANOVA followed by Dunnett’s test.
The mean value of Total bilirubin, SGPT and SGOT of control and treated groups of Wistar albino rats exposed to Seenthil Chooranam

Chart – 23
Histopathology of Control group animals

Heart

Low Power Magnification 10X  High Power Magnification 40X

Figure 9.1

Kidney

Low Power Magnification 10X  High Power Magnification 40X

Figure 9.2
Liver

Low Power Magnification 10X

High Power Magnification 40X

Figure 9.3

Lung

Low Power Magnification 10X

High Power Magnification 40X

Figure 9.4
Brain

Low Power Magnification 10X

High Power Magnification 40X

Figure 9.5

Histopathology of High dosed group animals

Heart

Low Power Magnification 10X

High Power Magnification 40X

Figure 9.6
Kidney

Low Power Magnification 10X

High Power Magnification 40X

Figure 9.7

Liver

Low Power Magnification 10X

High Power Magnification 40X

Figure 9.8
Lung

Low Power Magnification 10X

High Power Magnification 40X

Figure 9.9

Brain

Low Power Magnification 10X

High Power Magnification 40X

Figure 9.10
Interpretation

Kidney
- Appearance of glomeruli, tubules, interstitium and lumen was normal in both the samples with no signs of degeneration.
- Interstitial connective tissue of both the sample appear normal.

Heart
- Perfectly arranged myocardial fibers, clear transverse striation and normal structure were observed.
- Appearance of cardiomyocyte was normal with dark nuclear region. The nuclei of muscle fibers appear oval arrangement.

Liver
- Hepatocyte appears with dark pigment chromatin in centri lobular and periportal region.
- Hepatic sinusoid and hepatic cord was normal.

Lung
- Lung parenchyma appears normal with regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis.
- Perivascular region appears normal, Alveolar septa and wall appeared widen and normal.
- No signs of lymphocyte cuffing.
- No signs of airway secretion and bronchial secretion.
- Bronchial blood vessels and connective tissue appears normal with no signs of pulmonary edema.

Brain
- Arrangement of the neurons appears intact with no signs of degeneration or apoptotic changes in both the samples.
- Cortex region showed normal neurons with polygonal to round cell bodies containing dense cytoplasm. No signs of ischemia or lesion were observed.
Results & Interpretation of sub-chronic Toxicityity study:

Sub-chronic oral Toxicityity repeated dose of *Seenthil chooranam* on rats were conducted. All animals from the treated dose survived throughout the dosing period of 90 Days. Various parameters were studied and the interpretation of the study result is discussed below.

**Body weight**

The result of the body weight of rats exposed to control and the *Seenthil chooranam* of different dose groups exhibited overall weight gain throughout the dosing period of 90 Days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

**Haematological investigation interpretation:**

The haematological investigation results of the rats conducted on 91st Days 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 90th Days and the result is produced. The results revealed there are no significant changes in the values of different parameters with that of the control. But sugar levels were reduced significantly. Other values were within the normal biological and laboratory limits.

**Gross & Histopathology**

Gross pathological examination of animals in control as well as the treated groups did not reveal any abnormalities. The vital organs such as liver, heart, kidneys, lungs and brain were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Gross pathological investigation was carried out and histopathology of vital organ reveled normal histological appearance when compared with the control.
7.13 PHARMACOLOGICAL RESULTS OF ANTIDIABETIC ACTIVITY

Tabl:23. Effect of Seenthil Chooranam on body weight, food and water intake in Streptozotocin induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Feed Intake (g/rat Per Days)</th>
<th>Water Intake (ml/rat per Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>Group I</td>
<td>155.3±4.05</td>
<td>168.3±2.02</td>
<td>19.0±1.52</td>
</tr>
<tr>
<td>Group II</td>
<td>152.6±4.66</td>
<td>121.6±3.75***</td>
<td>22.12±1.76</td>
</tr>
<tr>
<td>Group III</td>
<td>146.8±4.37</td>
<td>175.0±2.88***</td>
<td>28.00±1.15</td>
</tr>
<tr>
<td>Group IV</td>
<td>149.0±3.51</td>
<td>155.0±7.50</td>
<td>25.33±1.76</td>
</tr>
<tr>
<td>Group V</td>
<td>140.0±1.15</td>
<td>167.6±1.45**</td>
<td>32.00±2.30</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of 6 animals. Statistical significance test for comparison between intial Vs final treatment between the groups was done by one way ANOVA followed by Dunnet’s test. Whereas * p<0.05, ** p<0.01, ***p<0.001.

Chart - 24
Chart - 25

Feed Intake (g/rat per day)

- Control
- Diabetic Control
- Glibenclamide 5 mg/kg
- S. Chooranam 200 mg/kg
- S. Chooranam 400 mg/kg

Initial vs Final feed intake across different treatments.

Chart - 26

Water Intake (ml/rat per day)

- Control
- Diabetic Control
- Glibenclamide 5 mg/kg
- S. Chooranam 200 mg/kg
- S. Chooranam 400 mg/kg

Initial vs Final water intake across different treatments.
Oral glucose tolerance test: (OGTT)

Normal rats were divided into four groups containing six animals in each group. All animals fasted before treatment. Group I was kept as vehicle control which received ghee, group II received Glibenclamide 5mg, group III received Seenthil chooranam 200 mg/kg, and group IV received Seenthil chooranam 400 mg/kg respectively. All the rats were loaded with glucose (3 g/kg, p.o.) 30 minutes after drug administration. Blood were collected and estimated. The blood glucose levels were determined by using glucometer by using strips. Blood samples were collected from puncturing the retro orbital sinus just prior to drug administration, and 30, 60, 120 minutes after loading glucose.5

Table: 24 Results of Oral glucose tolerance test (OGTT)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Oral Glucose Tolerance Test</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O min</td>
<td>30 min</td>
<td>60 min</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>91.33± 1.76</td>
<td>131.16± 6.14</td>
<td>122.33 ± 5.20</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td></td>
<td>89.09± 4.65</td>
<td>114.56± 1.78 **</td>
<td>98.66 ± 6.35 ***</td>
</tr>
<tr>
<td>S. Chooranam 200 mg/kg</td>
<td></td>
<td>88.16± 6.41</td>
<td>135.18± 3.24 $</td>
<td>120.66 ± 1.76 ***, $$$</td>
</tr>
<tr>
<td>S. Chooranam 400 mg/kg</td>
<td></td>
<td>91.16± 1.28</td>
<td>120.26± 6.24 **</td>
<td>105.33 ± 2.40 ***, $$$</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of 6 animals. Whereas * p<0.05, ** p<0.01, ***p<0.001 compared to control rats. $ p<0.05, $ $ p<0.01, $ $$ p<0.001 compared to glibenclamide groups was done by one way ANOVA followed by Tukey’s multiple comparison test.
Collection of blood and determination of glucose content

For blood glucose determination, the blood was obtained by snipping the tail by means of a sharp razor. Blood glucose level (BGL) was determined by means of a one touch horizon glucometer. Levels of glucose were expressed in mg/dl

Table: 25. Estimation blood glucose level on different Days interval

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days -0</td>
</tr>
<tr>
<td>Control</td>
<td>101.9±3.58</td>
</tr>
<tr>
<td>STZ rats</td>
<td>421.0±14.62***</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>375.60±1.82###</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>----------------------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Blood glucose (mg/dl)</strong></td>
<td>383.0± 4.26 ###</td>
</tr>
<tr>
<td>S. Chooranam 200 mg/kg</td>
<td>368.0 ± 2.28 ###</td>
</tr>
<tr>
<td>S. Chooranam 400 mg/kg</td>
<td>352.1 ± 4.09##</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of 6 animals. a- Group I Vs Groups II. Whereas * p<0.05, ** p<0.01, ***p<0.001. Whereas # p<0.05, ## p<0.01, ###p<0.001. b- Group II Vs Group III- V. Statistical significance test for comparison was done by one way ANOVA followed by Tukey’s multiple comparison test.

Chart – 28
Estimation of biochemical parameters on *Seenthil Chooranam* in rats

The SGOT, SGPT, creatinine, urea, uric acid, insulin were estimated in serum by kits specific for the test using autoanalyser. Serum insulin was determined by radioimmunoassay method.

### Table: 26 Biochemical parameters on *Seenthil Chooranam*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine</th>
<th>SGOT</th>
<th>SGPT</th>
<th>Urea</th>
<th>Uric acid</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.46±1.22</td>
<td>45.00±1.73</td>
<td>31.33±1.76</td>
<td>22.67±1.76</td>
<td>1.33±0.06</td>
<td>8.56±0.202</td>
</tr>
<tr>
<td>STZ rats</td>
<td>0.93±0.12</td>
<td>125.7±3.48***</td>
<td>54.33±2.02***</td>
<td>98.67±1.76***</td>
<td>4.99±0.15***</td>
<td>2.86±0.17***</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>0.40±1.31</td>
<td>52.00±1.15###</td>
<td>28.00±1.15###</td>
<td>28.00±0.57###</td>
<td>1.43±0.09###</td>
<td>7.76±0.08###</td>
</tr>
<tr>
<td>S. Chooranam 200 mg/kg</td>
<td>0.59±1.018</td>
<td>72.67±2.90###</td>
<td>34.67±2.667###</td>
<td>72.33±1.45###</td>
<td>3.40±0.28###</td>
<td>5.70±0.15###</td>
</tr>
<tr>
<td>S. Chooranam 400 mg/kg</td>
<td>0.50±2.01</td>
<td>58.67±1.76###</td>
<td>32.00±2.30###</td>
<td>42.00±5.29###</td>
<td>2.53±0.14###</td>
<td>6.86±0.17###</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of 6 animals. a- Group I Vs Groups II. Whereas * p<0.05, ** p<0.01, ***p<0.001. Whereas # p<0.05, ## p<0.01, ###p<0.001. b- Group II Vs Group III- V. Statistical significance test for comparison was done by one way ANOVA followed by Tukey’s multiple comparison test.
Chart – 31

SGPT (IU/L)

Control  STZ rats  STZ + Glibenclamide  STZ + S. Chooranam 200 mg/kg  STZ + S. Chooranam 400 mg/kg

Chart – 32

Urea

Control  STZ rats  STZ + Glibenclamide  STZ + S. Chooranam 200 mg/kg  STZ + S. Chooranam 400 mg/kg
Chart – 33

[Graph showing uric acid levels across different groups:]
- Control
- STZ rats
- STZ + Glibenclamide
- STZ + S. Chooranam 200 mg/kg
- STZ + S. Chooranam 400 mg/kg

Chart – 34

[Graph showing serum insulin levels across different groups:]
- Control
- STZ rats
- STZ + Glibenclamide
- STZ + S. Chooranam 200 mg/kg
- STZ + S. Chooranam 400 mg/kg
Table 27: Estimation of serum lipid profile on Scenthil Chooranam in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>113.3± 5.69</td>
<td>130.7±2.40</td>
<td>63.33±2.40</td>
<td>31.33±1.76</td>
<td>26.00±2.30</td>
</tr>
<tr>
<td>STZ rats</td>
<td>152.3±4.33 ***</td>
<td>161.3±4.80 ***</td>
<td>42.67±3.52</td>
<td>61.00±1.528 ***</td>
<td>32.67±4.05 ***</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>108.7±4.05 ###</td>
<td>134.3±2.33 ###</td>
<td>56.67±2.90 #</td>
<td>30.67±1.45 ###</td>
<td>28.00±3.46</td>
</tr>
<tr>
<td>S. Chooranam 200 mg/kg</td>
<td>116.7±2.40 ###</td>
<td>148.7±1.76</td>
<td>45.67±2.02</td>
<td>28.67±1.76 ###</td>
<td>25.33±2.02</td>
</tr>
<tr>
<td>S. Chooranam 400 mg/kg</td>
<td>106.3±3.38 ###</td>
<td>139.7±2.02 #</td>
<td>52.00±2.30</td>
<td>21.00±1.15 ###</td>
<td>27.33±3.18</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of 6 animals. a- Group I Vs Groups II. Whereas * p<0.05, ** p<0.01, ***p<0.001. Whereas # p<0.05, ## p<0.01, ###p<0.001.b- Group II Vs Group III- V. Statistical significance test for comparison was done by one way ANOVA followed by Tukey’s multiple comparison test.

Chart – 35
Histopathology of pancreas

Pancreatic tissues were fixed in 10% formalin, dehydrated with 50-100% ethanol solution and embedded in paraffin. The section of 5µm thick were cut and stained with haemotoxyllin eosin then examined under light microscope.
Group IV- STZ + S. Chooranam 400mg/kg

- Group I- Normal Ghee treated rats showed using with Haematoxylin & Eosin stained section shows pancreas with normal islets and acini
- Group II- STZ treated rats showed damaged and atrophic islet with acini.
- Group III- STZ + Glibenclamide rats showed preserved and regenerated cells of islet with acini
- Group IV- STZ + S. Chooranam 200mg/kg showed partial damaged of pancreatic islet cells.
- Group V- STZ + S. Chooranam 400mg/kg showed small damaged and regenerating pancreatic islet cells
Interpretation of Anti-Diabetic activity

The Anti-Diabetic Activity of the Seenthil Chooranam has been estimated in the streptozotocin induced diabetes in Wistar albino rat. Increase in blood glucose level is the important feature in diabetes.

S. Chooranam at the dose of 200 mg/kg, 400mg/kg for four weeks were able to produce reduce the glucose level compared with streptozotocin treated Group. Experimental groups (III- V) were compared with diabetic control rats - Values are statistically significant at P<0.001.

The diabetic hyperglycemia induces elevation of serum urea the results showed significant elevation in the levels of serum urea in the diabetic groups. The elevation of urea level observed in the diabetic rats was declined to normal by the administration of S. Chooranam at the dose of 200 mg/kg and 400mg/kg significantly.

Conclusion:

From these results, it could be concluded that the S. Chooranam is effective in the impaired diabetic renal function in addition to its hypoglycemic effect.
### 7.14 PHARMACOLOGICAL RESULTS OF HEPATOPROTECTIVE ACTIVITY

**Table: 28 Effect of Seenthil Chooranam and Silymarin on haematological parameters of CCl₄ induced liver damage in rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCl₄</th>
<th>CCl₄ + S. Chooranam 200 mg/kg</th>
<th>CCl₄ + S. Chooranam 400 mg/kg</th>
<th>CCl₄ + Silymarin 25 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>45.33±1.76</td>
<td>22.67±1.76 (###)</td>
<td>29.67±1.20</td>
<td>32.33±2.02 *</td>
<td>34.67±1.76 **</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>16.00±0.57</td>
<td>10.60±0.70 (###)</td>
<td>12.67±0.33</td>
<td>13.53±0.17 **</td>
<td>14.43±0.23 ***</td>
</tr>
<tr>
<td>RBC (x 10⁶/µ L)</td>
<td>7.33±0.17</td>
<td>5.00±0.11 (###)</td>
<td>5.96±0.12 *</td>
<td>6.03±0.18 *</td>
<td>6.43±0.26 **</td>
</tr>
<tr>
<td>Platelets (x 10³/µ L)</td>
<td>164.0±2.30</td>
<td>97.67±3.18 (###)</td>
<td>116.7±2.40 **</td>
<td>121.3±1.76 **</td>
<td>126.7±2.90 ***</td>
</tr>
<tr>
<td>MCV</td>
<td>63.67±0.88</td>
<td>41.33±3.52 (###)</td>
<td>47.00±2.08</td>
<td>51.33±1.20 *</td>
<td>54.00±2.00 *</td>
</tr>
<tr>
<td>MCH</td>
<td>24.33±1.85</td>
<td>14.33±1.20 (#)</td>
<td>13.67±0.88</td>
<td>15.33±1.76</td>
<td>16.67±2.40</td>
</tr>
<tr>
<td>MCHC</td>
<td>35.00±1.73</td>
<td>45.00±1.73 (#)</td>
<td>41.67±0.81</td>
<td>40.67±2.18</td>
<td>36.67±2.40</td>
</tr>
<tr>
<td>WBC X 10³</td>
<td>6.93±0.59</td>
<td>9.86±0.17 (###)</td>
<td>9.10±0.17</td>
<td>8.16±0.14 #</td>
<td>7.23±0.20 ###</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM; n = 6 animals in each group: ≠P<0.05, ≠≠ P< 0.01, ≠≠≠ P<0.001 is considered significant when compared with group I; *P<0.05, **P< 0.01, ***P<0.001 is considered significant when compared with group II by Tukey multiple comparison test.
Chart – 39

PCV (%)

Chart – 40

Hb (g/dl)
Table: 29 Effect of Seenthil Chooranam and Silymarin on serum enzymes (SGPT, SGOT and SALP), Total bilirubin and Total protein on CCl4 induced liver damage in rats

<table>
<thead>
<tr>
<th>Group and Treatment</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>SALP (IU/L)</th>
<th>Total Bilirubin (mg/dl)</th>
<th>TOTAL PROTEIN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghee 10ml/kg</td>
<td>62.00±8.08</td>
<td>34.67±4.66</td>
<td>22.67±2.40</td>
<td>1.63±0.58</td>
<td>15.33±1.76</td>
</tr>
<tr>
<td>CCl4 Rats 1.25ml/kg (ip)</td>
<td>167.3±34.65#</td>
<td>119.3±14.89###</td>
<td>41.33±1.76###</td>
<td>10.03±1.20###</td>
<td>3.66±0.88##</td>
</tr>
<tr>
<td>Seenthil chooranam 200mg/kg+ CCl4 1.25ml/kg (ip)</td>
<td>93.33±10.73</td>
<td>78.00±8.71*</td>
<td>28.67±1.76**</td>
<td>7.33±0.35</td>
<td>7.66±1.20</td>
</tr>
<tr>
<td>Seenthil chooranam 400mg/kg+ CCl4 1.25ml/kg (ip)</td>
<td>76.67±6.36*</td>
<td>62.00±3.46**</td>
<td>23.33±2.40***</td>
<td>6.06±0.75*</td>
<td>9.33±0.88</td>
</tr>
<tr>
<td>Silymarin 25 mg/kg 25 mg/kg+ CCl4 1.25ml/kg (ip)</td>
<td>54.67±4.05**</td>
<td>56.67±4.66**</td>
<td>21.33±1.76***</td>
<td>3.46±0.40***</td>
<td>11.00±2.08*</td>
</tr>
</tbody>
</table>

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

Chart – 47
Table: 30 Effect of Seenthil Chooranam and Silymarin on serum Creatinine and Urea, on CCl4 induced liver damage in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CCl4</th>
<th>S. Chooranam 200 mg/kg</th>
<th>S. chooranam 400 mg/kg</th>
<th>Silymarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.84±0.05</td>
<td>1.60±0.11</td>
<td>1.23±0.20</td>
<td>1.36±0.26</td>
<td>1.10±0.10</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>16.67±2.18</td>
<td>84.67±4.80</td>
<td>56.33±4.91 ***</td>
<td>43.00±4.35 ###</td>
<td>35.33±4.80 ###</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. ***P<0.001, **P<0.01, *P<0.05 compared to control rats. ### P<0.001, ##P<0.01, #P<0.05 compared with group II by Tukey multiple comparison test.
Histopathology of Liver

Group 1: Control

Group 2: CCl₄ treated

Group 3: S. Chooranam

200mg/kg + CCl₄

Group 4: S. Chooranam

400mg/kg + CCl₄

Group 5: Silymarin 25mg + CCl₄

- Group 1: Photomicrograph of liver tissue of control rats showing normal hepatic cells with central vein (CV) and sinusoidal dilation (S)
- Group 2: Photomicrograph of liver tissue of rats treated with CCl₄ showing severe Centrilobular necrosis (N) with disappearance of nuclei
- Group 3: Photomicrograph of liver tissue of rats treated with *S. Chooranam* at 200mg/kg showing mild degree of necrosis (N) with mild inflammatory cells

- Group 4: Photomicrograph of liver tissue of rats treated with *S. Chooranam* at 400mg/kg showing normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area (M)

- Group 5: Photomicrograph of liver tissue treated with Silymarin at 25mg/kg showing normal hepatocytes, portal vein (V) and portal artery

**Interpretation of Hepatoprotective activity**

The serum marker enzymes, SGOT, SGPT and SALP more specific index of liver cell damage and oxidative stress, which stimulate the release of amino transferases from hepatocytes into the blood. This study reveals that increase in the activity of the serum enzymes SGOT, SGPT and ALP were detected in mouse treated with CCl₄ (Group II). However, the activities of these serum enzymes were significantly (P < 0.001) lower in treated with *S. Chooranam* (Group 3 and 4) than in Group II. This present study confirmed in both the doses of *S. Chooranam* treatment (200 and 400 mg/kg body wt.) significantly improved the effect of CCl₄ induced liver damage.

The Histopathological studies showed that CCl₄ administered rat caused pathological changes in liver including severe centrilobular necrosis with disappearance of nuclei (Group 2). The liver with mild change in showing mild degree of necrosis with mild inflammatory cells of rats treated with *S. Chooranam* 200mg/kg and CCl₄ (Group3), the liver was almost has normal appearance of rats treated with *S. Chooranam* at 400mg/kg and CCl₄ (Group4), showing normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area. Indicating that the administration of *S. Chooranam* decreased the hepatocyte damage and silymarin also has the same effect (Group5). Control rats showed the normal appearance of liver without any histological alterations (Group1).

**Conclusion:**

In the present study the above parameters analyzed, it may concluded that *S. Chooranam* has Significantly produced hepatoprotective activity against CCl₄ induced rat.
7.15 PHARMACOLOGICAL RESULTS OF ANTI-INFLAMMATORY ACTIVITY

Table: 31 Inhibitory effect of Seenthil Chooranam against carrageenin induced paw oedema in albino Wistar rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage of inflammation after carrageenan injection at hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>45.10±1.08</td>
</tr>
<tr>
<td>S. Chooranam</td>
<td>33.16±1.27 b</td>
</tr>
<tr>
<td>200mg/kg</td>
<td></td>
</tr>
<tr>
<td>S. Chooranam</td>
<td>24.82±2.16 *</td>
</tr>
<tr>
<td>400mg/kg</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>16.74±7.26 **</td>
</tr>
<tr>
<td>10mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

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

Chart – 53
Results

Seenthil Chooranam 200 and 400 mg/kg doses have demonstrated considerable anti-inflammatory activity with the statistical significance of p<0.05 when compared to control rats. The Seenthil Chooranam at 400mg/kg dose have shown better anti-inflammatory response (p<0.01) control rats among the study doses and are shown in Table31.

Conclusion:

Based on the results it can be concluded that the Seenthil Chooranam used in this study in different dose level were effective in the anti-inflammatory activity.
Discussion
8. DISCUSSION

Qualitative, Quantitative, Toxicological and Pharmacological studies of *Seenthil chooranam* were performed in this study. Qualitative analysis includes pharmacognostical, chemical analysis and Quantitative analysis using sophisticated analytical equipments such as AAS, ICP-OES, TLC/HPTLC and HR-SEM, besides that evaluation of acute, 28 days repeated oral dose toxicity, 90 days repeated oral dose toxicity studies were carried out in Wistar albino rats as per OECD guidelines (423, 407 and 408 respectively). And also the evaluation of Antidiabetic, Hepatoprotective and Anti-inflammatory activities were carried out in wistar albino models.

In **physiochemical analysis** the pH of the drug *Seenthil Chooranam* was observed as 7.07 (Table-1). It denotes it is slightly alkaline. Hence, in the oral administration of the drug it may indicates that the drug will get ionized in stomach and will be absorbed in intestine and directly sent to portal system.

Loss on drying of *Seenthil choorana m* at 105°C is 10.30 % (Table-1). It indicates the loss of volatile substances along with the water this reveals that drug will not lose much of its volume and volatile substance. It shows that the drug has more stability.

Ash value 11.93 % (Table-1) it is the residue remaining after incineration that determines the inorganic substances present in the drug. Similarly it can also detect the nature of the material, whether it is adulterate or not. Hence, determination of the ash value provides an idea for judging the identity and purity of the drug.

The **microbial load** of the drug *seenthil chooranam*, the total bacterial count and fungal count are presence within the permissible limits. So, it is safe for the internal use.

Qualitative Analysis of *Seenthil Chooranam* showed presence of Silicate, Sulphate, Chloride, Calcium, Iron, Pottasium, Sodium, and Alkaloids.(Table-4,5).

By **AAS** the results of the trail drug *seenthil chooranam* possess that the heavy metals such as lead, cadmium, mercury are all detected within the permissible limits as per WHO guidelines.

In **ICPOES** analysis can conclude that the presence of the element such as arsenic, cadmium, copper, lead, mercury, nickel were determined as BDL as per WHO permissible limits in this *Seenthil chooranam* sample. Hence, it may be safe for human consumption. (Table-8). Also the study results exhibits that the presence of the elements in the sample such as phosphorus, iron, sodium, calcium, potassium were observed at the level of 76.341mg/L, 18.346 mg/L, 14.310 mg/L, 07.390mg/L, 03.811mg/L respectively. The insufficient intake of
phosphorus leads to liver diseases normally. The drug *seenthil chooranam* rich in phosphorus thus can explain this drug may help in the treatment of liver diseases.

**TLC/HPTLC** is one of the important parameter used for detecting the adulteration for judging the quality of drugs. If the drug is adulterated there might be appearance of the other compounds present in adulterant, in turn may increase the number of spots and number of waves. (Table 06), Figure (5, 6)

**HR SEM** analysis of *Seenthil chooranam* reveals the particle size as -329.5 to 366.2 nm. The particles are not arranged uniformly because can observe the aggregated particles in these SEM images of *Seenthil chooranam* sample. The aggregation of particles possess that the sample is in compound form. The particles are present in nano sizes thus can enhance the bioavailability of the drug (Figure 07).

In **Acute toxicity study** there is no mortality was observed in animals. Based on OECD 423 the trail drug *Seenthil Chooranam* is considered as non toxic up to the dose of 2000mg/kg.

**Repeated oral toxicity study** was conducted for about 28 days as per the OECD guideline-407 animals were observed throughout the period. The results of haematological investigations conducted on day 29th day revealed no significant changes in the haematological values when compared with those of respective controls. This possesses clear justification that bone marrow and spleen were not influenced by *Seenthil Chooranam*.

Results of Biochemical investigations conducted on days 29 and recorded in revealed the no significant changes in the values of different parameters studied when compared with those of respective controls; Urea, SGOT, SGPT, Bilirubin were within the limits, blood glucose significant compared to control group.

Group Mean Relative Organ Weights were recorded Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable with respective control group.

The vital organs such as liver, heart, kidneys, lungs and brain were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Gross pathological investigation was carried out and histopathology of vital organ reveals normal histological appearance when compared with the control. According to these results, *Seenthil chooranam could be concluded as No-Observed-
adverse-effect level (NOAEL). It confirms the safety of the drug which proved its utility in long time administration without any harm to the human being.

Sub-chronic oral toxicity 90 days repeated oral dose of Seenthil chooranam on rats were conducted. All animals from the treated dose survived throughout the dosing period of 90 days.

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

The haematological results were within the normal biological and laboratory limits. The biochemical results revealed there were no significant changes in the values of different parameters with that of the control. But sugar levels were reduced significantly compares to the control group, other values were within the normal biological and laboratory limits.

Gross pathological examination of animals in control as well as the treated groups did not reveal any abnormalities. The vital organs such as liver, heart, kidneys, lungs and brain were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Gross pathological investigation was carried out and histopathology of vital organ revealed normal histological appearance when compared with the control.

The Anti-Diabetic Activity of the Seenthil Chooranam has been estimated in the streptozotocin induced diabetes in Wistar albino rat. Increase in blood glucose level is the important feature in diabetes.

S. Chooranam at the dose level of 200 mg/kg, 400mg/kg for four weeks were able to produce reduce the glucose level compared with streptozotocin treated Group. Experimental groups (III- V) were compared with diabetic control rats - Values are statistically significant at P<0.001.

The diabetic hyperglycemia induces elevation of serum urea the results showed significant elevation in the levels of serum urea in the diabetic groups. The elevation of urea level observed in the diabetic rats was declined to normal by the administration of S. Chooranam at the dose of 200 mg/kg and 400mg/kg significantly. From these results, it could be concluded that the S. Chooranam is effective in the impaired diabetic renal function in addition to its hypoglycemic effect.
The serum marker enzymes, SGOT, SGPT and SALP more specific index of liver cell damage and oxidative stress, which stimulate the release of amino transferases from hepatocytes into the blood.

This study reveals that increase in the activity of the serum enzymes SGOT, SGPT and ALP were detected in mouse treated with CCl₄ (Group II). However, the activities of these serum enzymes were significantly (P < 0.001) lower in treated with S. Chooranam (Group 3 and 4) than in Group II. This present study confirmed in both the doses of S. Chooranam treatment (200 and 400 mg/kg body wt.) significantly improved the effect of CCl₄ induced liver damage.

The Histopathological studies showed that CCl₄ administered rat caused pathological changes in liver including severe centrilobular necrosis with disappearance of nuclei (Group 2). The liver with mild change in showing mild degree of necrosis with mild inflammatory cells of rats treated with S. Chooranam 200mg/kg and CCl₄ (Group.3), the liver was almost has normal appearance of rats treated with S. Chooranam at 400mg/kg and CCl₄ (Group.4), showing normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area indicating that the administration of S. Chooranam decreased the hepatocyte damage and silymarin also has the same effect (Group. 5). Control rats showed the normal appearance of liver without any histological abnormalities (Group. 1).

In the present study the above parameters analyzed, it may concluded that S. Chooranam has significantly produced Hepatoprotective activity against CCl₄ induced rat.

In anti-inflammatory activity the trail drug Seenthil Chooranam at 200 and 400 mg/kg doses of have demonstrated considerable anti-inflammatory activity with the statistical significance of p<0.05 when compared to control rats. The Seenthil Chooranam at 400mg/kg dose have shown better anti-inflammatory response (p<0.01) control rats among the study doses and are shown in Table1 and Figure1.

Based on these results can conclude that the drug seenthil Chooranam showed potent anti-inflammatory activity in various doses.

Based on the above results, It can be assumed that the Seenthil Chooranam has scientifically validated.
Summary
9. SUMMARY

- The test drug *Seenthil Chooranam* was selected from the siddha literature “*Agasthiyar paripuranam* – 400” for its anti-Diabetic, Hepatoprotective and anti-inflammatory activities. The dissertation started with an introduction explaining about the siddha concept, prevalence of diabetes and role of the test drug in treating diabetes.

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

- Review of literature in various categories was carried out. Siddha aspect, botanical aspect and pharmaceutical review disclosed about the drug and the disease. Pharmacological review was done to establish the methodologies.

- The drug was subjected to analysis such as pharmacognostical, physicochemical, phytochemical, chemical and also the chemical fingerprint was engaged by using modern analytical techniques like Atomic Absorption Spectrometer (AAS), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and that the particle size and qualitative analysis of chemical elements of *Seenthil chooranam* were also assessed by Scanning Electron Microscope (SEM) 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 the acute, repeated dose 28-day oral toxicity and repeated dose 90-day oral toxicity study. *Seenthil chooranam* was confirmed as no-observed-adverse-effect level (NOAEL). It showed the safety of the drug which proved its utility in long time administration without any harm to the human being.

- In conclusion, *Seenthil chooranam* was evaluated to be non-toxic in the tested doses and experimental states.

- The final Discussion and conclusion chapters analyzed the dissertation. The conclusion chapter also provides a discussion of the verification and validity of the research results carried out. The most vital part of some experience of the findings in the dissertation is also discussed and thereafter invites the reader to further studies and future research possibilities.
Pharmacological study was done. It revealed the Anti-diabetic, Hepatoprotective and Anti-inflammatory activities in animal model Wistar albino rats. This present study suggests *Seenthil Chooranam* has remarkable medicinal value in the treatment of Diabetis and Liver diseases.

Results and discussion gives the necessary justifications to prove the potency of the drug.

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

Thus the herbal formulation *Seenthil Chooranam* is validated for its safety and efficacy for treating diabetes mellitus and Liver diseases it would be a great drug of choice.
Conclusion
10. CONCLUSION

- *Seenthil Chooranam* was prepared as per the siddha literature.
- When it was subjected into analysis it fulfills all the standardization parameters of chooranam as mentioned in AYUSH guidelines.
- From the results of SEM images it confirms the spherical shape and aggregated particle morphology obtained for this *Seenthil Chooranam* sample.
- Based on OECD 423 the trail drug *Seenthil Chooranam* is considered as non toxic up to the dose of 2000mg/kg. This observation reveals that the LD$_{50}$ of *Seenthil chooranam* is greater than 2000mg/kg.
- *Seenthil Chooranam* could be confirmed as no-observed-adverse-effect level (NOAEL) drug as it acts harmlessly under the current normal usage and this phenomenon is considered to be non toxic.
- The **Anti-Diabetic, Hepatoprotective and Anti-Inflammatory activity** of *SEENTHIL CHOORANAM* was scientifically validated in animal models.
- Hence, it can be concluded as a therapeutically effective drug in Diabetes and Liver diseases by further evaluation.
Annexure
This certificate is awarded to

Dr. S. Usha Kanthan

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

"Research Methodology & Biostatistics"

organised by the Department of Siddha

M.G.R. Medical University from 5th to 9th May 2014.

Dr. N. Kabilan M.D. (Siddha)

Reader, Dept. of Siddha

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

#69, Anna Salai, Guindy, Chennai-600 032.
NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drug used in Siddha formulation “Seenthil chooranaam” taken up for Post Graduation Dissertation studies by Dr. S. Ushakanthan, M.D.(S), II year, Department of Gunapadam, 2015, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

_Tinospora cordifolia_ (Willd.) Meirs (Menispermaceae), Stem
_Eclipta alba_ Linn. (Asteraceae), Whole plant

Certificate No: NISMB2152016

Date: 14-8-2015

Authorized Signatory

Dr. D. ARAVIND, M.D.(S), M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha
Chennai - 600 047, INDIA
CERTIFICATE OF ZOOLOGICAL AUTHENTICATION

This is to certify that the following animal was used in the Siddha formulation “Seenthil Choornam” (Internal) taken up for Post Graduation Dissertation by Dr. S. USHAKANTHAN, M.D(S), II year, Department of Gunapadam, during 2015-16, is identified and authenticated through visual inspection / Experience / Morphology / Microscopical / Taxonomical methods.

1. *Eudrilus eugeniae* Kinberg, 1867

References:


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Specialization: Biodiversity
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Govt. Arts College, C. Mutlur
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Phone: 04144 231770

*V. Thangamani, M.Sc., M.Phil., PGDCA.,
Associate Professor & Head,
Department of Zoology,
Govt. Arts College, C. Mutlur,
Chidambaram-608 102.*
CERTIFICATE

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

[DR.R.MURUGESAN]

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

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e-mail : saif@iitm.ac.in http://www.saif.iitm.ac.in
Ref: 4530/KKCP/2015

Date: 10.08.2015

APPROVAL CERTIFICATE

This is to certify that the project title “Safety and pharmacological profile of SEENTHIL CHOORANAM” 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/2015/034</td>
<td>Wistar Albino rat</td>
<td>49 Male + 55 female</td>
<td>150–200gms</td>
<td>108</td>
<td>104</td>
</tr>
</tbody>
</table>

Wistar Albino rats - One hundred and four only

Chairman IAEC
(Prof. A. Meena)

CPCSEA Nominee
(Dr. C. Kathirvelan)

Veterinary Officer
(V. VAIDHYALINGAM)

Members

Dr. K. Sadasivan Pillai
CERTIFICATE

This is certify that the project title: "SAFETY PROFILE OF .................

"SENTHIL CHORAHAM" (12 Male, 12 Female, (History, Alcohol, etc.)

has been approved by the IAEC. (No: NIS/IAEC-1/2016/08)

Name of Chairman/Member Secretary IAEC: Name of CPCSEA

Dr. B.R. SENTHILKUMAR

Signature with date

Chairman/Member Secretary of IAEC: CPCSEA nominee:

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

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Acknowledgement
ACKNOWLEDGEMENT

- This dissertation is one of the milestones in the journey of Siddha – drug research. Thus I came across this task which kept on completed with the support and encouragement of numerous people. So I take great pleasure in thanking all the people who made this dissertation study a valuable and successful one, which I owe to treasure it.

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