ANTI-INFLAMMATORY ACTIVITY OF SIDDHA POLYHERBAL FORMULATION SEVVYADHI CHOORANAM ON CARRAGEENAN INDUCED PAW EDEMA IN WISTAR ALBINO RATS

SHAMSHALNIHA S.*, ANBU N.
Department of Post Graduate Maruthuvam, Government Siddha Medical College, Arumbakkam-600106, Chennai, India
*Corresponding author: Shamshalniha S.; Email: nihaasiddha@gmail.com
Received: 12 Aug 2023, Revised and Accepted: 13 Sep 2023

ABSTRACT
Objective: The aim of the study was to explore the anti-inflammatory activity of Siddha polyherbal formulation Sevviyadhi chooranam in Carrageenan induced paw edema in wistar albino rats, and compared with the standard drug Indomethacin.

Methods: The Siddha polyherbal formulation Sevviyadhi chooranam indicated for sinusitis was prepared based on GMP (Good Clinical Practice) guidelines. Study procedure was approved by Institutional Animal Ethics Committee (IAEC). The experimental animals were measured for paw edema volume at 1, 2, 3, 4, 5 h using Plethysmometer (Model 7150 UGO Basile, Italy). Edema was expressed as mean increase in paw volume relative to control animals. And then, findings were compared with Indomethacin (Standard drug).

Results: The findings revealed that test drug Sevviyadhi chooranam at higher dosage 200 mg/kg (Group V) had equal effect on anti-inflammatory activity with percentage protection 93.2% when compared with the standard drug Indomethacin at about 40 mg/kg (Group III) with percentage protection 93.2%. However, the test drug Sevviyadhi chooranam at a higher dosage 200 mg/kg (Group V) with a percentage protection 93.2% was highly effective when compared with lower dosage about 100 mg/kg (Group IV) with a percentage protection 72.12%. Hence, the study resulted that the Siddha polyherbal formulation Sevviyadhi chooranam has an optimistic anti-inflammatory activity with more therapeutic value.

Conclusion: The study concluded that the Siddha polyherbal formulation Sevviyadhi chooranam has a promising anti-inflammatory activity, probably due to the presence of biologically active phytocompounds. However, it is important to admit that there are some scientific evidences of the potential actions of these phytocompounds in anti-inflammatory activity.

Keywords: Siddha system, Sevviyadhi Chooranam, Sinusitis, Anti-inflammatory activity, Carrageenan, Wistar albino rats

INTRODUCTION

The Siddha system of medicine is the oldest traditional treatment system generated from Dravidian culture and it is flourished in the period of Indus Valley Civilization [1]. It is an ancient system that is practiced in Tamil Nadu in South India and other Tamil-speaking regions of the world. Siddha system of medicine focuses on addressing the root cause of the disease rather than treating the disease symptoms [2]. Herbal plants play an important role in preventing and treating of human diseases [3]. Herbal medicine derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian Traditional System of medicine” [4].

“Sinusitis is the inflammation of sinuses, which are air-filled cavities in the skull. It can be acute or chronic. Types of sinuses are maxillary, frontal, ethmoid and sphenoid. The maxillary sinuses are most commonly affected. Aetiology of sinusitis are both infectious and non-infectious. Infectious aetiology includes viral, bacterial and fungal. Non-infectious aetiology includes allergic rhinitis (with either mucosal or polyp obstruction), barotraumas (deep sea diving or air travel), exposure to chemical irritants [5].

Carrageenan was used to induce paw edema volume in Wistar albino rats for the study. During past decade, carrageenan has become much used experimentally mainly for its ability to induce an acute inflammation [6]. Paw edema induced by carrageenan was described by Winter et al [7]. Cardinal signs of inflammation such as edema, hyperalgesia and erythema develop immediately following sub-planter injection of carrageenan into hind paw, as a result of the action of pro-inflammatory agents such as bradykinin, histamine, prostaglandins, thromboxane, reactive oxygen etc. that can be generated at the site of the insult by infiltrating cells [8-11].

Over 50 y ago, Indomethacin emerged as an extremely potent non-steroidal anti-inflammatory drug (NSAID) during massive effort to find effective anti-inflammatory and analgesic medications [12]. It is used as a potent antipyretic, analgesic and anti-inflammatory activity that has been effectively used in the management of mild to moderate pain since the mid-1960s [13]. Hence Indomethacin was used as a standard drug of the current study.

New drug development process must continue through several stages in order to make a medicine that is safe, effective and has approved all regulatory requirements [14]. The process of developing a novel drug is time-consuming and costly. To increase the chances of successfully completing a clinical trial leading to the approval of a new drug, the choice of appropriate preclinical models is of utmost importance. Identifying a safe, potent, and efficacious drug requires thorough preclinical testing, which evaluates aspects of pharmacodynamics, pharmacokinetics and toxicology in vitro and in vivo settings. Nevertheless, merely a small fraction of investigational new drugs tested in clinical trials after passing pre-clinical evaluation eventually led to a marketed product. Hence, there is a need for optimizing current standard preclinical approaches to better mimic the complexity of human disease mechanisms [15].

The Siddha system of medicine contains many peculiar herbal, mineral and herbo-mineral combinations for the treatment and management of sinusitis. Among that one such distinct polyherbal formulation was Sevviyadhi Chooranam indicated for the treatment and management of sinusitis in Siddha literature “Anupava Vaidhya Dheva Ragasayam-Moondram paagam” [16]. This polyherbal combination consists of 12 herbal drugs.

The present study was focused on the pharmacological evaluation of Siddha polyherbal formulation Sevviyadhi chooranam for its anti-inflammatory activity on Carrageenan-induced paw edema in Wistar albino rats. Hence, the objective of the study was to explore and...
validate the formulation on its capability to reduce the inflammation induced by Carrageenan in Wistar albino rats.

**MATERIALS AND METHODS**

**Study drug**

**Collection and authentication of drugs**

The ingredients present in the formulation were acquired from an indigenous raw drug store. These raw drugs were verified and authenticated by the Botanist, Department of Medicinal Botany, Government Siddha Medical College, Chennai (Voucher number GSMC/MB 579-590).

**Composition of Sevviyadhi chooranam**

The Siddha polyherbal formulation Sevviyadhi chooranam consists of 12 herbal drugs as per Siddha literature “Anupava Vaidhya Dheva Ragasiyam-Moordram Pagam (Pg. no-466)” [16] as follows

1. *Piper nigrum* (black pepper root)-250g
2. *Zingiber officinale* (Dried ginger)-250g
3. *Piper longum* (Long pepper)-250g
4. *Abies spectabilis* (East Himalayan fir)-250g
5. *Cuminum cyminum* (Cumin)-250g
6. *Phyllanthus emblica* (Indian gooseberry)-250g
7. *Plumbago indica* (Indian leadwort)-250g
8. *Cinnamomum verum* (Cinnamon)-250g
9. *Cinnamomum tamala* (Indian bark)-250g
10. *Elettaria cardamomum* (Cardamom)-250g
11. *Piper nigrum* (black pepper)-250g
12. *Bambusa arundinaceae* (bamboo salt)-250g

**Purification and preparation of sample**

**Purification of herbal drugs**

Prior to preparation, all the herbal raw drugs of *Sevviyadhi chooranam* were purified as per Siddha literature “Sikitcha Rathna Deepam Ennum Vaidhya Nool” [17] as follows

- **Black pepper root** (*Seviyam*)
  - Purified by peeling out the outer skin and sun-dried.
- **Dried ginger** (*Chukku*)
  - A part of the dried ginger was treated and bleached with 2 parts of limestone solution (kal sumambu) for 3 h, washed, dried and the external scale leaf was peeled off.
- **Long pepper** (*Thippili*)
  - Soaked and treated in leaf juice of *P. indica* for 24 min and sun-dried.
- **East Himalayan Fir** (*Thalisapathiri*)
  - Purified by washing and sun-dried.
- **Cumin** (*Seeragam*)
  - Soil and dust particles were removed and dried in sunlight.
- **Indian gooseberry** (*Nellivatral*)
  - Boiled in cow milk and then seeds were removed and sun dried.
- **Indian leadwort** (*Chithiramoolam*)
  - Outer bark was removed, powdered and boiled with steaming method in cow milk and dried.
- **Cinnamon** (*Lavangapattai*)
  - Unwanted dust particles were removed and dried under sunlight.

- **Indian bark** (*Lavangapattai*)
  - Cleaned and dried under sunlight
- **Cardamom** (*Elakkai*)
  - Unwanted soil and dust were removed and sun-dried
- **Black pepper** (*Milaçu*)
  - Soaked and treated with buttermilk (sour) for 3 h and dried under sunlight.
- **Bamboo salt** (*Moongiluppu*)
  - Dissolved in clear water and dried under sunlight to obtain the salt principate.

**Sample preparation**

The polyherbal Siddha formulation *Sevviyadhi Chooranam* was prepared as per Siddha literature “Anupava Vaidhya Dheva Ragasiyam-Moordram Pagam (Pg. no 466)” [16]

- After purification, all the ingredients were grounded individually in an iron mortar by using a pestle and sieved with the sieving cloth.
- And then all the grounded ingredients were mixed together and stored in an airtight container.

**Ethical approval**

Before the initiation of preclinical evaluation, ethical approval for the study procedure was obtained from Institutional Animal Ethics Committee (IAEC) at Department of Pharmacy, C. L. Baid Metha college of Pharmacy, Thorapakkam, Chennai-6000092. All the study procedures were performed as per the guidelines and ethical principles of ethics committee for experimentation using animals under proper care and control (IAEC NO. 9/31/PO/Re/S/01/CPCSEA/dated 06/04/2022)

**Experimental animals**

**Selection of experimental animals**

For study the healthy, young adult Wistar albino female rats were taken. They were nulliparous, non-pregnant about 6-8 w old with weight of about 150-200 gm, the weight of the animals fell in the mean interval of +20% of mean weight. Female rats were chosen because of their sensitivity to the treatment [18]. Experimental animals were obtained from Maes biotech, Chennai.

**Housing and feeding conditions of experimental animals**

Animals involved in the experiment were housed in polypropylene cages, with husk bedding. The temperature maintained was about 22 °C+3 °C and relative humidity about 50-60%. In 24 h, 12 h of light cycle and 12 h of dark cycle were maintained. Conventional laboratory feeds were fed with an unlimited supply of drinking water. Prior to the administration of drugs, acclimatization was done followed by a veterinary examination of all the experimental animals. And then, all the experimental animals were kept as group caged with proper study procedure.

**Preparation of experimental animals**

For an experiment, the animals were randomly selected and kept in individual cages marked with picric acid for identification 7 d before to inducing inflammation. The animals were divided into 5 groups (each group contains 6 animals). They were grouped as follows

- Group I (Control)-received 3% of gum acacia 10 ml/kg per oral administration.
- Group II (Carrageenan)-received 0.1 ml of 1% w/v suspension of Carrageenan (Subcutaneous injection)
- Group III (Standard)-received Indomethacin 40 mg/kg per oral administration.
- Group IV (Low dose)-received Sevviyadhi Chooranam 100 mg/kg per oral administration.
- Group V (High dose)-received Sevviyadhi Chooranam 200 mg/kg per oral administration.
Induction of paw edema volume

The study was performed at C. L. Baid Metha College of Pharmacy, Chennai. After the preparation of animals, all the drugs were administered orally and the volume of the medicaments were kept constant at 10 ml/kg body weight of the experimental animals. Thus, Group I received 3% of gum acacia orally, Group II received 0.1 ml of 1% w/v suspension of Carrageenan injected subcutaneously, Group III received the standard drug Indomethacin 40 mg/kg orally, Group IV received low dose (100 mg/kg) of test drug Sevviyadhi chooranam orally and Group V received high dose (200 mg/kg) of Sevviyadhi chooranam orally. After 1 h of dosing, 0.1 ml of 1% w/v suspension of carrageenan was injected into the sub-plantar region of the left hind paw to all the groups involved in the study. Then the paw edema volume was measured in 1, 2, 3, 4 and 5 h using a Plethysmometer (Model 7150 UGO Basile, Italy). Edema was expressed as the mean increase in paw volume relative to control [19].

Analysis of reduction in paw edema volume

After injection of carrageenan to the subcutaneous region of left hind paw, the paw edema volume was analyzed for the experimental animals. The paw volume was measured up to the tibiotarsal articulation measured at 0, 1, 2, 3, 4, 5 h and denoted as mean±SEM. The percentage protection of test drug Sevviyadhi chooranam was calculated by formulae (T2-T1/T2) X 100, Where T1 denotes normal control and T2 denotes drug used for test.

Statistical analysis

The observations were statistically analyzed using One-way ANOVA followed by Dunnett’s test. The study drug was statistically significant (p<0.05) when compared with the standard drug.

RESULTS

The paw edema volume of experimental animals at different time intervals were indicated in table 1 and the percentage protection of test drug Sevviyadhi chooranam were described in table 2 and fig. 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Initial paw volume</th>
<th>Change in paw edema (mm) at different time intervals</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
<td>1h</td>
<td>2h</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>1.20+1.14</td>
<td>1.20+1.14</td>
<td>1.20+1.14</td>
</tr>
<tr>
<td>II</td>
<td>Carrageenan</td>
<td>1.23+2.18</td>
<td>1.96+2.12</td>
<td>2.31+1.11</td>
</tr>
<tr>
<td>III</td>
<td>Indomethacin</td>
<td>1.33+1.14</td>
<td>2.25+1.26</td>
<td>1.89+1.14</td>
</tr>
<tr>
<td>IV</td>
<td>Low dose</td>
<td>1.46+1.23</td>
<td>1.68+1.12</td>
<td>1.79+2.22</td>
</tr>
<tr>
<td>V</td>
<td>High dose</td>
<td>1.33+1.32</td>
<td>1.68+1.33</td>
<td>1.73+2.21</td>
</tr>
</tbody>
</table>

Paw edema volume measurements denoted as Mean±SEM; n=6 rats, ’p value is less than 0.05 (p<0.05), ”p value<0.01; statistically analyzed through One way ANOVA test followed by Dunnett’s test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Initial paw volume</th>
<th>Paw volume in 5 h</th>
<th>Difference in paw volume in ml</th>
<th>Percentage protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>1.20+1.14</td>
<td>1.20+1.14</td>
<td>0.00</td>
<td>100</td>
</tr>
<tr>
<td>II</td>
<td>Carrageenan</td>
<td>1.23+2.18</td>
<td>2.68+1.23</td>
<td>1.45</td>
<td>-43.21</td>
</tr>
<tr>
<td>III</td>
<td>Indomethacin</td>
<td>1.33+1.14</td>
<td>1.27+2.12</td>
<td>0.06</td>
<td>93.21</td>
</tr>
<tr>
<td>IV</td>
<td>Low dose</td>
<td>1.46+1.23</td>
<td>1.59+2.24</td>
<td>0.13</td>
<td>27.12</td>
</tr>
<tr>
<td>V</td>
<td>High dose</td>
<td>1.33+1.32</td>
<td>1.27+3.31</td>
<td>0.06</td>
<td>93.2</td>
</tr>
</tbody>
</table>

Paw edema volume measurements denoted as mean+SEM; n=6 rats.

DISCUSSION

Carrageenan-induced rat paw edema is used widely as a working model of inflammation in the search for new anti-inflammatory drug [20]. From the results obtained, the test drug at the lower dose of 100 mg/kg have mild inhibition on inflammation induced by carrageenan when compared to standard drug with percentage protection of 27.12% at 5th h. However, at higher dose, the test drug Sevviyadhi chooranam had equal effect on reducing inflammation with percentage protection of 93.2%, when compared with standard drug Indomethacin contains the percentage protection of 93.21% at 5th h. This ensured an anti-inflammatory activity of Siddha polyherbal formulation Sevviyadhi chooranam in Carrageenan induced paw edema volume in Wistar albino rats.
The development of edema in the paw of the rat after the injection of carrageenan is due to release of histamine, serotonin and prostaglandin [21]. Thus, the anti-inflammatory effect of *sevviyadi chooranam* may be due to the presence of many phyto-compounds in its ingredients. Many prior studies were published regarding the anti-inflammatory activity of individual drugs in *sevviyadi chooranam*. In *P. nigrum*, piperine possesses inhibition of prostaglandin release mediated anti-inflammatory properties [22]. The small and long varieties of *Pippali* (*P. longum*) produced considerable suppression of edema formation against carrageenan-induced paw edema in rats [23]. The unique ability of *Z. officinalis* to inhibit the synthesis of PG2 and TXB2, and to lower serum cholesterol levels is clinically important, because its daily intake for a prolonged period will neither lead to side-effects nor to complications as normally occurs with non-steroidal anti-inflammatory drugs [24].

The copper oxide nano Particles in Abies spectabilis (AS-CuONPs) is effective against different stimuli induced nociception and it act as a potent anti-inflammatory agent without rendering any side effects [25]. The aqueous extracts of *C. cyminum* seeds show predominantly anti-inflammatory activity [26]. The anti-inflammatory and analgesic activities of the standardized water extract from the fruit of *P. emblica* seem to be similar to NSAIDs [27]. *P. zeylanica* extract showed significant action against carrageenan-induced rat paw edema in a dose-dependent manner [28]. The cinnamon extract from *C. verum*, its active compounds trans-cinnamaldehyde and p-cymene or combinations increase potency of central active compounds opening up novel treatment strategy for diverse inflammatory diseases [29]. *C. tamala* possesses anti-inflammatory activity and has therapeutic potential for the treatment of inflammatory diseases [30]. *E. cardamom* extracts have a therapeutic potential against periodontal infections through their anti-bacterial and anti-inflammatory properties [31]. Sarilang is a bamboo salt soy sauce which has been demonstrated to exert anti-inflammatory and anti-tumour activity [32]. The phytocompounds present in herbal drugs of *sevviyadi chooranam* individually contains anti-inflammatory property by its inhibitory effects in histamine, prostaglandin and serotonin. These scientific evidences acquired from research articles further ensured the anti-inflammatory activity of the Siddha polyherbal formulation *Sevviyadi chooranam*.

**CONCLUSION**

From the results and discussion, the Siddha polyherbal formulation *Sevviyadi chooranam* has potent anti-inflammatory activity without any adverse effects. This activity mainly due to the presence of phytocompounds of herbal drugs present in Sevviyadi chooranam which has an inhibitory action over pro-inflammatory agents such as prostaglandins, serotonin and thromboxanes. By this the current study concluded and ensured the anti-inflammatory activity of the Siddha polyherbal formulation *Sevviyadi chooranam*. Hence, it will be a promising drug of choice for the management and treatment of sinusitis and various other inflammatory diseases.

**ACKNOWLEDGEMENT**

This publication is a part of the MD program in Government Siddha Medical College, Arumbakkam, Chennai-106 of The Tamil Nadu Dr. MGR Medical University, Guindy, Chennai, Tamil Nadu.

**FUNDING**

Nil

**AUTHORS CONTRIBUTIONS**

Dr. S. Shamshehniha has designed, conducted the study and wrote the manuscript, Dr. N. Anbu reviewed and approved the manuscript.

**CONFLICT OF INTERESTS**

Authors have declared no competing interests exist.

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