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Research Article

SCIENTIFIC EVALUATION OF ANTI ANALGESIC AND ANTI-INFLAMMATORY EFFICACY OF A SIDDHA DRUG, *SIVATHAI CHOORANAM*

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ABSTRACT

Siddha medicine offers an unceasing development in medical practice for a number of metabolic diseases and lifestyle disorders. The present study was to evaluate the anti analgesic and anti-inflammatory potential of *Sivathai chooranam*. The animals were divided into 5 groups of 6 animals each {control, standard - Indomethacin (5mg/kg, i.p), *Sivathai chooranam* 0.108mg/kg(po), *Sivathai chooranam* 0.44 mg/kg(po) and *Sivathai chooranam* 2.7mg/kg(po). The analgesic activity of the extracts was evaluated using acetic acid induced writhing method and *in vitro* anti-inflammatory activity was evaluated using HRBC membrane stabilization assay and egg albumin denaturation assay. In acetic acid induced writhing model of analgesia, the number of writhes (in 30 minutes) was highest in control group (49.5±1.707) and lowest in Indomethacin group (26.25±0.629). Analgesic activity in *Sivathai chooranam* at 2.7mg/kg concentration has registered lowest number of writhes (26.25±0.629) compared other drug concentrations tested and it was statistically significant as compared to control group. The concentration of 250µg/ml of *Sivathai chooranam* has exhibited a maximum percentage of haemolysis (88.24±0.73%) and albumin (protein) denaturation (84.51±0.66%) while the same concentration of standard drug of hydrocortisone represented the haemolysis was 88.94±1.27% and Diclofenac sodium showed the 89.18±0.76% of protein denaturation. The results of the work indicate that the Siddha medicine, *Sivathai chooranam* has possessed remarkable anti analgesic and anti inflammatory potential and can be applied as alternative in the treatment of painful conditions and inflammatory complications.

INTRODUCTION

Pain is a debilitating side effect of numerous medical conditions and it is a distressing sensory and emotional experience caused by actual or potential tissue damage[1,2]. Often, it causes discomfort and causes many adverse effects despite giving a warning and being primarily protective in nature[3].

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It is caused by many biochemical mediators like prostaglandins, bradykinins, and substance P acting on the nociceptors.

These mediators are considered the immediate cause of pain and are either chronic or acute. Acute pain is defined as having a sudden onset and short duration that lasts for hours, but chronic pain is characterized by persistent pain over an extended length of time[4,5,6]. Therefore, pain management is one of the most critical therapeutic priorities.

The process of inflammation is characterized by a complex biological response of the vascular tissues to harmful stimuli. Additionally, inflammation is associated with pain and involves increased protein denaturation, increased vascular permeability, and altered membranes, among other things[7]. As well as

inactivating or eliminating the invading stimuli or organisms, inflammation is also characterized as removing irritants and preparing the body to repair itself, and the process is accelerated by the release of chemical mediators from injured cells or tissues and migrating cell[8]. A crucial role in the inflammatory response is played by leukocyte migration from the venous system to the site of injury and by the release of cytokines. These chemicals cause blood capillaries to widen (vasodilate) and become permeabilized, leading to an increase in blood flow to the injured area[9].

For treating pain and inflammation, NSAIDs (Non-steroidal Anti-Inflammatory Drugs) and steroidal drugs are both used. However, long-term use of NSAIDs can have adverse effects and damage the liver, gastrointestinal tract, and so on. Moreover, they lead to cardiovascular problems and kidney failure[10-13]. As a result of steroids, the immune system may be suppressed and erectile dysfunction, manic depression, hypertension, cramps, and dizziness can occur, as well as the appearance of dormant diabetes, skin atrophy, and reduced bone density, stomach ulcers with possible perforations, irregular menstruation, eye problems and allergies[14]. Moreover, opioid analgesics are often prescribed for pain relief, but they may cause side effects such as addiction, constipation, and breathing difficulties[15]. To overcome these side-effects, there is a need for further research into alternative painkillers and anti-inflammatory drugs. Plant based medicines may provide safer, more effective, and better quality medications for treating pain and inflammatory conditions than conventional medicine. Therefore, the purpose of this study was to assess the analgesic and antiinflammatory properties of a Siddha plant based medicine called *Sivathai chooranam*.

MATERIALS AND METHODS

Drug Selection

Sivathai Chooranam (SC) was taken as a compound drug from the literature, Siddha Vaidhya Thiraddu, Indian Medicine and Homeopathy, 2009.

Ingredients

Operculina turpethum, Cinnamomum zeylanicum, Terminalia chebula, Terminalia chebula, Termunalia bellirica, Zingiber officinale, Piper nigrum, Piper longum, Elettaria repens, Mesua ferrea and *Cyperus rotundas*.

Source of Collection

All the raw drugs were bought from Country drug shop at Parry's corner, Chennai, Tamil Nadu, India.

Identification and Authentication of the drug

All the raw drugs were identified and authenticated by the Gunapadam experts in Government Siddha Medical College, Arumbakkam, Chennai.

Preparation of the trial drug: *Sivathai Chooranam*

Procedure

All the above ingredients were powdered in an iron mortar separately and it was sieved by a cotton cloth. *Operculina turpethum* is 4 part and other ingredients are used in equal proportions while preparing *Chooranam* mentioned in Siddha Vaidhya Thirattu.

Storage of the drug

The prepared test drug was stored in a clean, air tight glass container. The contents were inspected frequently to avoid moisture and insects.

Evaluation of Analgesic activity-Acetic acid induced Writhing test

Test Animals and Test Conditions

Swiss albino rats (20-25g) were obtained from Animal house of the KMCH College of Pharmacy, Coimbatore. All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet.

Preparation of Animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

Procedure - Acetic acid induced Writhing test

Swiss albino rats were divided into five groups (n = 6). Group I received acetic acid (1% v/v, 0.1ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 minutes. Group II received Indomethacin (5 mg/kg b.w.p.o.) Group III, IV and V received *Sivathai chooranam* at the doses of 0.108mg/kg, 0.44mg/kg and 2.7mg/kg b.w., p.o. respectively. 30 min after Indomethacin and *Sivathai chooranam* administration, group II, III, IV and V received acetic acid (1% v/v, 0.1ml/kg b.w., i.p.) and writhing reflex was noted for the period of 15 min. Control animals received the same volume of vehicle. The writhing episodes were recorded for 30 minutes; stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted.

Anti-inflammatory activity – *In vitro* study

HRBC Membrane Stabilization Method

The human red blood cell (HRBC) membrane stabilization method was used for this study. The blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with iso-saline and a

10% suspension was made. Drug was prepared by 4 g of *Sivathai chooranam* was macerated with 10ml of hyposaline (0.36% NaCl) and extracts is centrifuged at 3000 rpm. From this, various concentrations of *Sivathai chooranam* were prepared (10, 25, 50, 100 and 250µg/ml) using distilled water and to each concentration 1ml of phosphate buffer, 2ml hyposaline and 0.5ml of HRBC suspension were added. Test solution was incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm. 10–250µg/ml of Hydrocortisone (50mg) were used as reference standard and a control was prepared omitting the extracts.

a) Inhibition of albumin denaturation

The reaction mixture was consisting of *Sivathai chooranam* (10–250µg/ml) and 1% aqueous solution of bovine albumin fraction, pH (7.2) of the reaction mixture was adjusted using small amount of 1N HCl. The samples were incubated at 37°C for 20 min and then heated at 51°C for 20 min. After cooling the samples, the turbidity was measured spectro-photometrically at 660nm. Diclofenac sodium was taken as a standard drug of varied concentration 10–250µg/ml. The experiment was performed in triplicate. % of protein denaturation was calculated as follows:

$$\text{Inhibition} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}}$$

RESULTS

In vivo analgesic activity

Acetic Acid Induced Writhing Reflex

The effect of *Sivathai chooranam* on the acetic acid- induced abdominal constrictions in swiss albino rat is presented in Table 1. The result shows that the drug (0.54mg/kg, 2.7mg/kg and 13.5mg/kg Bwt), and the reference drug indomethacin (5mg/kg) significantly ($P < 0.05$) reduced abdominal writhing in animal when compared to the negative control group reducing the mean number of writhing from 49.5±1.70 in the negative group to 26.0±0.81 at the dose of 5mg/kg. The reduction was in a dose dependent manner. Also the test drug caused a dose dependent increase in inhibition of abdominal writhing, increasing it from 0% in negative control group to 46.96% at the dose 13.5mg/kg. Furthermore, posthoc analysis did not detect any significant difference between the *Sivathai chooranam* at the doses of 0.54mg/kg, 2.7mg/kg versus reference drug (indomethacin) and control group.

In vitro anti-inflammatory activity

The *in vitro* anti-inflammatory profile of *Sivathai chooranam* appeared to be somewhat consistent, according to the results in Fig. 1. The *Sivathai chooranam* displayed IC50 values that were comparable to or even lower than the reference drugs for the suppression of heat-induced and hypotonic solution-induced human RBC haemolysis. At 250 µg/ml concentration, the test drug recorded the highest percentage of hemolysis (78.10±0.92%), which was close to the standard (82.24±1.15%), while 10 µg/ml concentration of test drug has registered the lowest haemolysis (09.12±0.09%). The IC50 value of RBC haemolysis activity of standard was 276.30µg/ml and the *Sivathai chooranam* was 305.08µg/ml.

Table 1: Analgesic potential of *Sivathai Chooranam*

Group	No of Writhing (30 min)	Inhibition (%)
Control	49.5±1.70[d]	-----
Indomethacin (5mg/kg, i.p)	26±0.81[a]	47.47 %
Sivathai chooranam (0.54mg /kg)	39±1.29[c]	21.21 %
Sivathai chooranam (2.7mg/kg)	32±0.816[b]	35.35 %
Sivathai chooranam (13.5mg /kg)	No of Writhing (30min)	46.96 %

In vitro anti-inflammatory activity

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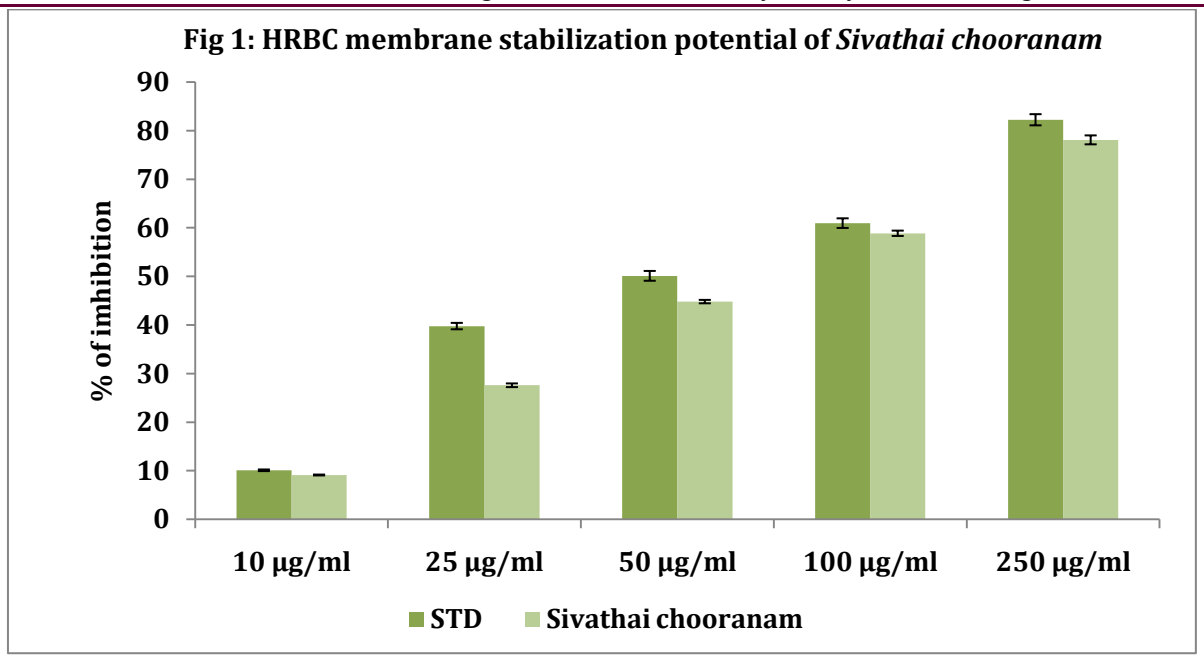
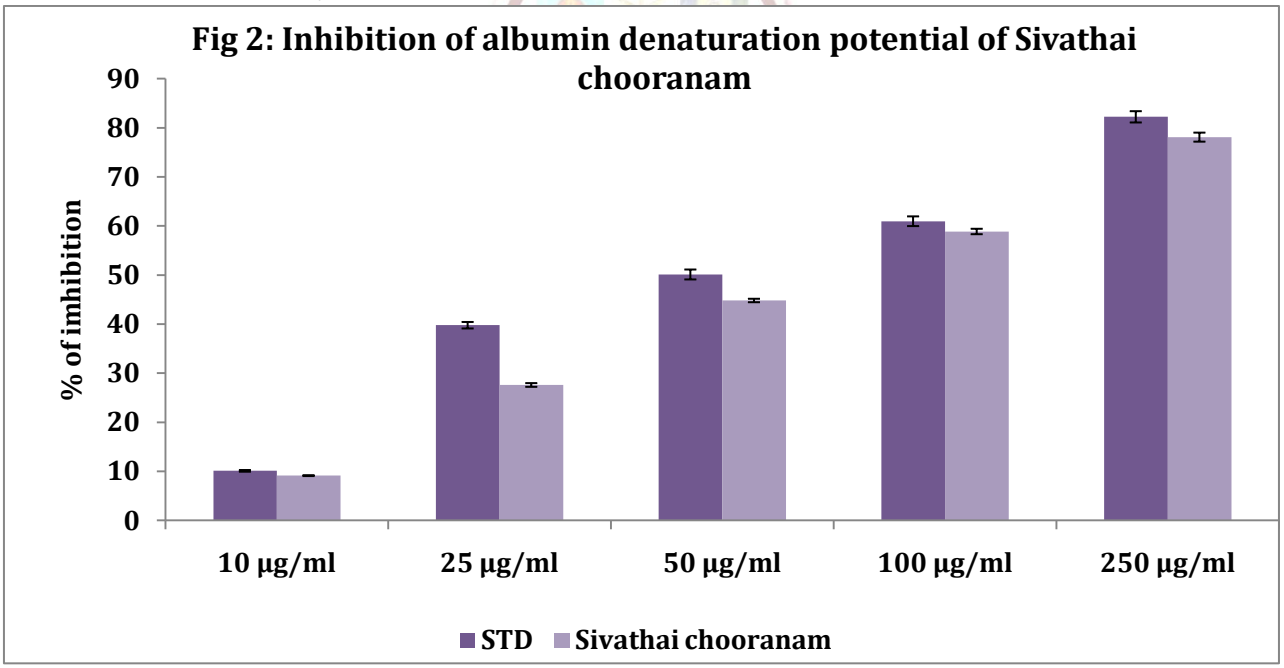


Fresh egg albumin stabilisation against heat-induced protein denaturation also demonstrated significant *in vitro* anti-inflammatory activity (Fig 2). The extracts were found to be effectual in diverse concentrations in inhibiting the heat-induced hemolysis. It indicates the percentage of inhibition against concentration in the range of 10–250µg/ml for both standard and *Sivathai chooranam*. The *Sivathai chooranam* showed maximum percentage of albumin denaturation at 250µg/ml concentration (77.83±0.90 %) and it was lower than that of the standard (80.46±1.51%). The IC50 value of albumin denaturation activity of Standard was 255.56µg/ml and the *Sivathai chooranam* exhibited as 307.68µg/ml.



DISCUSSION

The chemical agent acetic acid is known to stimulate the production of noxious substances within the peritoneum resulting in writhing response[16]. It is a simple, rapid and reliable model and especially suitable to evaluate peripheral type of analgesic action of a drug[17]. The results of the analgesic activity suggest that *Sivathai chooranam* exhibited significant inhibition of pain response in acetic acid-induced pain model. The effect of *Sivathai chooranam* was comparable to aspirin in amelioration of acetic acid-induced pain in rats which suggest the role of *Sivathai chooranam* in inhibition of cyclooxygenase or lipoxygenase pathway which is the general pathway of common peripherally acting analgesic drugs[18]. Similarly, Sugunthan *et al*.[19] has investigated that the administration of villaiver kudineer drug showed significant analgesic activity on peripheral nervous system revealed through suppression of acetic acid

induced writhing. Also, Thanikaiselvi and Antony Duraichi[20], as evaluated the analgesic activity of *Kaalakodi rasam* was done by acetic acid Induced Writhing Test method in Swiss albino mice. Doses of different proportions 100mg, 200mg of *Kaalakodi rasam* powder suspension were given to the animals for a stipulated period of time. *Kaalakodi rasam* is having significant analgesic activity against the acetic acid-induced writhing in mice.

Stabilization of liposomal membrane is important in limiting the inflammatory response by inhibiting the release of liposomal constituents of activated neutrophil such as bactericidal enzymes and protease, which cause further tissue inflammation and damage upon extracellular release[21]. In the present study, the *Sivathai chooranam* showed maximum percentage of haemolysis at 250µg/ml concentration (78%) which is lower than that of the standard (82%). Denaturation of proteins is responsible for the cause of inflammation. Neutrophils are known to be a rich source of proteinases which carry in their lysosomal granules, and it was previously reported that leukocytes proteinase plays an important role in the development of tissue damage during the inflammatory response [22]. So, prevention of protein denaturation may help in preventing inflammatory conditions[23,24]. In the present investigation, the *Sivathai chooranam* showed maximum percentage of albumin denaturation at 250µg/ml concentration (77%) which lower than that of the standard (80%). In this result is supported by previous *in vitrov* antiflammatory works of Solanaceae plants done viz., *S. xanthocarpum* [25,26] and *S. torvum* [27].

CONCLUSION

From the above study it was concluded that the Siddha drug, *Sivathai chooranam* has significant anti-analgesic and anti inflammatory activities (membrane stabilization property, albumin denaturation). However further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and helpful in projecting Siddha drug of *Sivathai chooranam* as a therapeutic target in anti-analgesic and anti-inflammatory research.

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