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Research Article

Anti-Inflammatory of Ayapoonaga Chenduram and Seenthil Choornam

# Evaluation of Anti-Inflammatory and Immunomodulatory Activities of Ayapoonaga Chenduram, Seenthil Choornam and their Combination in Wistar Rats

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**Abstract:** *Ayapoonaga chendooram* (APNC) and *Seenthilchoornam* (SC) are two polyherbal Siddha formulations that traditionally have been used as a folklore medicine to treat rheumatism, orchitis, bronchitis, asthma, tuberculosis (TB), and cough. In the present study, an attempt has been made to demonstrate the anti-inflammatory and immunomodulatory activities of individual APNC and SC and also in their combination (APNC+SC). The acute anti-inflammatory effect of APNC, SC and their combination was evaluated in carrageenan-induced hind paw edema in wistar rats. Diclofenac sodium was used as reference drug for comparison. The immunomodulatory activity was evaluated in rats by studying the hemagglutination antibody (HA) titer, delayed type hypersensitivity (DTH) reactions and the Ig E levels. The cyclophosphamide is used as an immunosuppressant.Diclofenac Sodium (50mg/kg) significantly inhibited the carrageenan-induced hind paw edema, indicating its strong anti-inflammatory activity. APNC (65 mg/kg b.w.*p.o*) at 2nd hour and APNC+SC (13+200 mg/kg b.w.) at 3rd hour had shown moderate inhibition of paw edema, indicating their mild-moderate anti-inflammatory activity but statistically, no significant difference was observed ($p>0.05$). Oral administration of APNC, SC, and APNC + SC for 21 days increases the left paw volume at 1$^{st}$ hour and 2$^{nd}$ hour resulting in hypersensitivity reaction in rats. The agglutination (dilution factor 1:200) was visually observed in APNC 130mg/kg b.w. (moderate agglutination) and in APNC + SC (high agglutination) results in humoral immunity. Mild increased levels of Ig E were observed in APNC (30 mg/kg b.w.), SC (200 mg/kg b.w.) and in APNC + SC (65+1000 mg/kg b.w.). Under the conditions of the present study, single oral administration of APNC, SC and their combination has shown mild anti-inflammatory activity against carrageenan induced paw edema in experimental rats. The immunity has been stimulated by APNC (13mg/kg b.w.) and SC (2000mg/kg b.w.).

**Keywords:** *AyapoonagaChendooram, SeenthilChoornam,* Anti-Inflammatory Activity, Immunomodulatory Activity and Hypersensitivity Reaction.

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## 1. INTRODUCTION

Conventional Systems of medicine are playing a major role in meeting global healthcare desires[1]. India is practicing seven traditional systems of medicine; Ayurveda, Siddha, Unani, Yoga, Naturopathy, Homoeopathy and Sowa-Rigpa[2]. Among all the systems, Siddha system of medicine is inimitable and widely practiced in south Asia, especially in Tamilnadu. Siddha system of medicine is providing several effective preparations with very minimal side effects compared with the allopathic system of medicine to treat a number of diseases[3].

### *1.1 SeenthilChoornam (SC)*

The main ingredients of SC are as follows[4]

**Table 1. Composition of APNC**

| S.NO | Siddha Name | Scientific Name | Quantity |
|---|---|---|---|
| 1 | Seenthil | *Tinosporacordifolia* | 10 palam (350gm) |
| 2 | Karisalankanni | *Eclipta Alba* | 10 palam (350gm) |
| 3 | Bhoonagam | *Eudriluseugeniae* (Earthworm) | 3 palam (105mg) |

*Tinosporacordifolia*, also termed Seenthil can stimulate bile secretion, causes constipation, and alleviates thirst, burning sensation, and vomiting[5]. Earlier studies validated the hypoglycemic, immunomodulatory, anti-inflammatory, antioxidant, and other pharmacological activities of Seenthil[4]. *Eclipta alba*, is commonly known as Bhringraj in Sanskrit and Kaikeshi, Karisha-langanni in Siddha. The aerial parts of the plant contain terthienyl derivatives, like formyl terthienyl, and several esterified 5-hydroxy terthienyl derivatives, flavonoids apigenin, and luteolin[4]. Surprisingly Earthworms are holding a key post in traditional systems of medicine, especially in Unani and Siddha[4]. They are referred to as Kharateen in Unani and are utilized both internally and externally as aphrodisiacs[6]. They can treat sore throats, blisters, and wounds. Earthworms are used to reduce fever in China and Japan. They are employed in Myanmar and Laos to treat smallpox[4]. Earthworms are also used to treat rheumatism, bronchitis, fever, and tuberculosis[7]. They are also useful in the treatment of edema-reducing, anti-inflammatory, and anticoagulatory or fibrinolytic[8].Earthworm-dried powder and whole plant extracts of *Eclipta alba*, *Tinosporacordifolia*, and other plants are combined to make *SeenthilChoornam*. It is utilized in the Siddha system of medicine to treat a variety of therapeutic ailments. Significant analgesic and anti-inflammatory effects are present[9]. It is used in hepatotoxicity and safeguards the liver[9]. It treats uterine hemorrhage, piles, insect bites, stings, swellings, and other skin conditions[9]. It also treats abortion and miscarriage[4]. In the treatment of problems including infertility, bone abnormalities, and chronic skin conditions, *SeenthilChoornam* is proven to be quite effective[4].

### *1.2 AyapoonagaChendooram(APNC)*

The key ingredients of APNC are as follows[10-11]

**Table 2. Composition of SC**

| S.NO | Siddha Name | Scientific Name | Quantity |
|---|---|---|---|
| 1 | Bhoonagam | *Eudriluseugeniae* (Earthworm) | - |
| 2 | Irumpu | Iron fillings | - |
| 3 | Madulai | *Punicagranatum* (Promegranate) | - |

The Pomegranate fruit has long been used as a traditional treatment for respiratory diseases, acidosis, dysentery, microbiological infections, diarrhea, and helminth infections[12]. Additionally, it has been demonstrated that Pomegranate seeds contain estrogenic substances like estrone and estradiol[13]. Additionally, the dried pericarp and fruit juice are thought to help treat dental disorders, colic, colitis, menorrhagia, oxyuriasis, headaches, diuretics, acne, piles, and allergic dermatitis[12]. Pharmacologically Pomegranate has been validated for its antioxidant, anti-inflammatory (through blocking pro-inflammatory cytokines), anti-cancer, and anti-angiogenesis activities[13]. *Ayapoonagachendooram*(APNC) has had poor scientific validation till today. Based on the composition we hypothesized that it may give an additive effect with *Seenthilchoornam* (SC). By taking the consideration of the folklore uses of these two formulations, we designed our study to evaluate the anti-inflammatory and immunomodulatory activities. In the present study, we attempted to evaluate their potential of anti-inflammatory and immunomodulatory in Wistar rats.

## 2. MATERIAL AND METHODS

### *2.1 Procurement and Preparation of Test Drugs*

The test drug *Ayapoonagachendooram*was collected from the department of Pharmacy of Siddha Central Research Institute, Arumbakkam, Chennai-106 and *Seenthilchoornam*was collected from The Indian Medical Practitioner's Co-operative Pharmacy & Stores LTD., Thiruvanmiyur, Chennai. The test doses of APNC and SC were prepared by triturating a weighed quantity of test drugs in the required volume of 0.5 % Carboxymethylcellulose (CMC) prepared in 5% v/v honey to obtain a concentration of 13, 65, and 130 mg/kg of APNC and 200, 1000 and 2000 mg/kg of SC and the combination of APNC & SC was prepared by triturating of individual test compounds in the required volume of vehicle.

### *2.2 Experimental Animals and Husbandry*

Wistar rats of both sexes (6 to 8 weeks) with body weight of 150-200 g were procured from Tamil Nadu Veterinary and

Animal Sciences University (TANUVAS), Chennai, Tamil Nadu. This study was performed as per the recommendations of the Committee for the Purpose of Control and Supervision Experiments on Animals (CPCSEA) guidelines for laboratory animal facilities after approval of the Institutional Animal Ethics Committee (IAEC) of Siddha Central Research Institute, Central Council for Research in Siddha (Ministry of AYUSH), Arumbakkam, Chennai-600106 with an approval number 199/PHARMA/SCRI/2018. The rats were placed in Sterilized Polypropylene cages containing corncob bedding material and provided Standard pellet diet (19% crude protein) and Reverse osmosis treated water ad libitum. The animal room was maintained as 12-hour light/12-hour dark with the temperature 18 to 25$^0$c and relative humidity 30% to 65%.

### *2.3 Instruments*

The instruments used are an Electronic weighing balance (Shimadzu-model No. AUX-220), Refrigerator (BPL- model No. F272MVX), and Digital plethysmometer (PANLAB-model No. LE75000, Auto analyzer (Biosystem - model No. BA400) and Centrifuge (REMI- model No. R8M).

### *2.4 Chemicals*

The chemicals used are Carrageenan (Biocorporals), Diclofenac sodium, Cyclophosphamide (Biochem pharmaceutical, Mumbai), and ELISA commercial kit (Biotran Diagnostics, U.S.A.) Carboxymethyl cellulose, Sodium chloride, Formaldehyde, Anesthetic ether, and Sodium ethylenediaminetetraacetic acid (Loba Chemie Pvt. Ltd) of analytical grade were purchased from There's scientific works, Chennai.

### *2.5 Experimental Design*

#### *2.5.1 Anti-Inflammatory Activity*

Evaluation of the Anti-inflammatory activity was performed as per the procedure suggested by Amri O *et al*.,2018[14]. A total of 72 wistar rats (36/sex) were divided into twelve groups, each group consisted of 06 rats (03/sex) based upon descending body weights and physical examinations. Group-I is Normal control, receiving 0.5% CMC. Group II is Disease control, 0.1 mL of 1 % w/v carrageenan was injected into the subplantar region of rats. Group-III is Standard control, rats administered with Diclofenac sodium (50 mg/kg b.w.) followed by 0.1 mL of 1 % w/v Carrageenan. Group-IV to XII animals were administered with a single dose of a low, medium, and high doses of APNC (13, 65, and 130 mg/kg), SC (200, 1000, and 2000 mg/kg), and APNC+SC (13+200, 65+1000 and 130+2000 mg/kg) followed by 0.1 mL of 1 % w/v Carrageenan injection.

#### *2.5.2 Immunomodulatory Activity*

A total of 66 wistar rats (33/sex) were divided into eleven groups, each group consisting of 06 rats (03/sex). The experiment was done as per VachanaMurunikkara*et al.,* 2014[15]. Group-I animals received 0.5% CMC. Group II animals were administered with the standard drug Cyclophosphamide on days 9 and 16 by orally at a dose of 100 mg/kg/day. Group-III to IV were administered with low, medium, and high doses of APNC (13, 65 and 130 mg/kg), SC (200, 1000, and 2000 mg/kg), and APNC+SC (13+200, 65+1000 and 130+2000 mg/kg) for 21 days by orally.

### *2.6 Experimental Procedures for Anti-Inflammatory Activity in Rats*

#### *2.6.1 Induction of Acute Paw Inflammation in Rats*

Carrageenan (1% w/v) was prepared 24 hrs. before administration. 0.1 mL of 1 % w/v Carrageenan was injected into the subplantar tissue of the left hind paw of each rat. Swelling or paw edema was measured at 0, 1, 2, 3, 4, 24 hour using a digital plethysmometer[16]. Rats were treated with test compounds 1hour before the Carrageenan injection. Percentage inhibition of test drugs was calculated in comparison with Disease control. % Inhibition of paw edema was calculated as per the below given formula

$$\% \text{ Inhibition of paw oedema} = \frac{Vd - Vt}{Vd} \times 100$$

Vd = Paw edema of Disease control rats
Vt = Paw edema of drug-treated rats.

#### *2.6.2 Hematological Parameters*

Blood was collected from the retro-orbital plexus of all experimental rats at 24th hours after carrageenan injection and the count of Eosinophils, monocytes, lymphocytes, and neutrophils was analyzed by using a hemato analyzer. Tumor necrosis factor-alpha (TNF-alpha) was also estimated with a commercially available ELISA kit.

### *2.7 Experimental Procedures for Immunomodulatory Activity in Rats*

#### *2.7.1 Preparation of Alsever's Solution*

It is a saline liquid used to prevent coagulation of blood and is composed of 2.05% dextrose, 0.8% sodium citrate, 0.055% citric acid, and 0.42% sodium chloride. For usage, an equal volume of blood is gently, but thoroughly, mixed with this solution. This solution is used to preserve blood cells from other sources[17]

#### *2.7.2 Preparation of Sheep RBC (SRBC)*

Sheep blood was collected in sterile Alsever's solution in 2:1 proportion of Alsever's solution (Freshly prepared). Blood was kept in the refrigerator and processed, for the preparation of the SRBCs batch, by centrifugation at 2000rpm for ten minutes and washing with physiological saline 4-5 times and then suspending into buffered saline for further use[18]

#### *2.7.3 Hem agglutination Assay*

The rats of all groups are pre-treated with test drugs for 21 days. And each rat was immunized with 0.1X10 SRBC/rat by i.p. route, including control rats. The immunization was given on day 7 and day 14. On day 22 the titer was determined by titrating serum dilutions with SRBCs. The microtiter plates were incubated at room temperature for one hour and examined visually for agglutination[18]

#### 2.7.4 Identification of IgE

On day 21, serum was obtained from all the groups of rats to estimate serum Ig E levels with a commercially available ELISA kit. Briefly, 25µ l of serum was added to all the wells, add 100µ l of IgE biotin reagent was then allowed to incubate for one hour. Discarded the contents and added 300 µ l of wash buffer and discarded that it was carried out four times. Then added 100 µ l of IgE enzyme reagent allowed it for incubation of half an hour. Discarded the contents and added 300 µ l of wash buffer and discarded it was carried out 4 times after that 1 ml of working substrate solution was added allowing for 15 minutes of incubation then added 0.05ml of stop solution and the absorbance at 450 nm was recorded[19].

#### 2.7.5 Delayed-Type Hypersensitivity (DTH) Response

On day 21 rats were administered 0.1ml of 1% SRBCs in the left hind footpad by sub-plantar injection, in the right hind footpad administered 0.1 ml of 0.9% normal saline and the increased level of paw volume was measured by Digital plethysmometer in four different time (0 hour, 1 hour, 2 hours, 3 hour) intervals. The thickness between left and right hind paw volume was measured[20]

#### 2.7.6 Measurement of TNF-A Levels

On day 21, blood was collected and serum was separated from all the groups of animals to estimate serum Tumor necrosis factor-alpha (TNF-alpha) levels with a commercially available ELISA kit. An automated microplate reader (SpectraMax® M5) was used for the measurement of the optical density (OD) at 450 nm. Based on optical density and the concentration of the standard, the concentrations of each sample were found.

### 2.8 Termination

After the completion of both studies, all experimental animals were euthanized by using the carbon dioxide asphyxiation method.

## 3. STATISTICAL ANALYSIS

Hematological and biochemical data were analyzed statistically. All the data were expressed as Mean ± SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by Tukey's post hoc using Graph pad prism version-5. The significance level was set at $P<0.05$ for all tests

## 4. RESULTS AND DISCUSSION

### 4.1 Anti-Inflammatory Activity of APNC, SC and their Combination

#### 4.1.1 Paw Edema or Paw Volume

Acute inflammation caused by carrageenan is one of the best test methods to evaluate anti-inflammatory drugs. In a carrageenan-induced paw edema paradigm in rats, the time course of edema development is often illustrated. The measurement of paw edema is a key parameter to evaluate the anti-inflammatory activity of a test substance in animals[21]. Paw edema was measured at 0, 1, 2, 3, 4, 24 hour using a digital plethysmometer. Rats injected with Carrageenan (1% w/v) alone have shown an increased volume of paw edema when compared with normal control groups. Rats treated with Diclofenac Sodium have shown a significant decrease in paw volume ($p<0.05$) at the 1st, the 2nd, 3rd, and 4th hour when compared with the disease control group. APNC at 65 mg/kg b.w. at 2nd hour and a combination of APNC+SC at a low dose in 3rd hour has also had mild anti-inflammatory activity but statistically, no significant difference was observed. The values are presented in table 3. APNC and SC did not significantly reduce paw edema in the early stages of the trial at all three tested doses as indicated in Table 3. Therefore, it may be said that serotonin and histamine are not being inhibited. The effect of non-steroidal anti-inflammatory drugs, which largely block the cyclo-oxygenase involved in prostaglandin synthesis, has been studied using the carrageenan-induced paw edema model in rats, which is known to be sensitive to cyclo-oxygenase inhibitors[22]. It has a significant impact on the onset of the second stage of the inflammatory response, which is measured at the third hour. As shown in Table 1, there is mild percentage inhibition of paw edema in animals treated with the combination of APNC (13, 65 mg/kg) and SC (200, 1000 mg/kg). But the effect was not dose-dependent.

**Table 3. Effect of APNC, SC, and their combination on Mean paw volume (% inhibition) in Anti-inflammatory study**

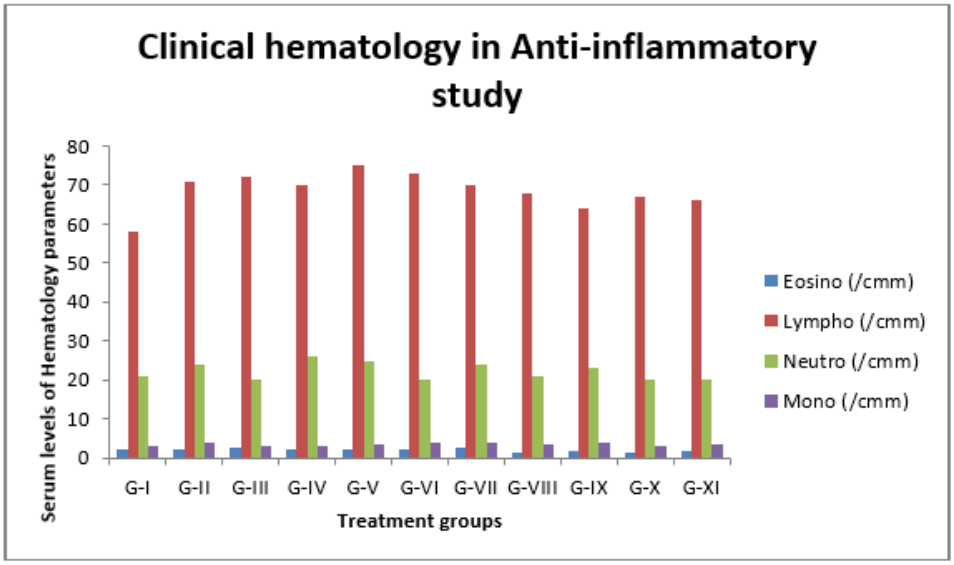
| Group | Dose | Mean paw volume (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0th hour | 1st hour | 2nd hour | 3rd hour | 4th hour | 24th hour |
| I | Normal control | 0.855±0.09 | 0.903±0.07 | 0.900±0.08 | 0.933±0.06 | 0.940±0.06 | 1.015±0.05 |
| II | Disease control | 0.9433±.03 (9.36%) | 1.665±0.10 (45.75%) | 1.915±0.07 (53.00%) | 2.017±0.10 (53.73%) | 2.075±0.07 (54.70%) | 1.582±0.06 (35.84%) |
| III | Standard control (Diclofenac 50mg/kg b.w) | 0.918±0.05 (2.68%) | 1.2080.08 (27.45%) | 1.210±0.08 (36.81%) | 1.280±0.07 (36.54%) | 1.225±0.09 (40.96%) | 1.201±0.17 (24.08%) |
| IV | APNC low dose (13mg/kg b.w) | 1.075±0.08 (-13.96%) | 1.442±0.08 (13.39%) | 2.070±0.23 (-8.09%) | 2.243±0.17 (-11.20%) | 2.212±0.09 (-6.60%) | 1.370±0.08 (13.40%) |
| V | APNC mid dose (65 mg/kg b.w) | 1.103±0.02 (-16.93%) | 1.328±0.08 (20.24%) | 1.792±0.17 (6.42%) | 2.100±0.20 (-4.12%) | 1.865±0.19 (10.12%) | 1.270±0.14 (19.72%) |
| VI | APNC high dose (130 mg/kg b.w) | 1.128±0.05 (-19.58%) | 1.307±0.10 (21.50%) | 1.610±0.08 (15.93%) | 2.122±0.21 (-5.21%) | 1.970±0.16 (5.06%) | 1.408±0.10 (11.00%) |
| VII | SC low dose mid dose (200 mg/kg b.w) | 1.025±0.07 (-8.66%) | 1.280±0.12 (23.12%) | 1.693±0.13 (11.59%) | 2.132±0.08 (-5.70%) | 2.020±0.08 (2.65%) | 1.425±0.33 (9.92%) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **VIII** | SC mid dose (1000 mg/kg b.w) | 1.117±0.17 (-18.41%) | 1.310±0.14 (21.32%) | 1.863±0.27 (2.72%) | 1.917±0.39 (4.96%) | 1.940±0.41 (6.51%) | 1.318±0.30 (16.69%) |
| **IX** | SC high dose (2000 mg/kg b.w) | 1.152±0.09 (-22.12%) | 1.407±0.07 (15.50%) | 1.783±0.17 (6.89%) | 1.930±0.24 (4.31%) | 1.950±0.27( (6.02%) | 1.407±0.15 (11.06%) |
| **X** | APNC (13mg/kg + SC 200 mg/kg b.w) | 0.840±0.04 (10.95%) | 1.357±0.50 (18.50%) | 1.473±0.05 (23.08%) | 1.630±0.03 (19.19%) | 1.633±0.04 (21.30%) | 1.217±0.09 (23.07%) |
| **XI** | APNC (65 mg/kg+ SC 1000 mg/kg b.w) | 0.940±0.03 (0.35%) | 1.480±0.03 (11.11%) | 1.710±0.04 (10.70%) | 1.810±0.11 (10.26%) | 1.958±0.04 (5.64%) | 1.500±0.10 (5.18%) |
| **XII** | APNC (130 mg/kg+ SC 2000 mg/ kg b.w) | 1.037±0.06 (-9.93%) | 1.563±0.11 (6.13%) | 1.772±0.07 (7.47%) | 1.943±0.03 (3.67%) | 2.018±0.04 (2.75%) | 1.468±0.11 (7.21%) |

***All values are expressed as Mean ± SEM, n=6. Values in parenthesis are % inhibition of paw edema***

### *4.1.2 Clinical Hematology*

No compound-related changes and no significant differences were observed in Eosinophils, monocytes, lymphocytes and neutrophils. The values are graphically represented in figure 1.

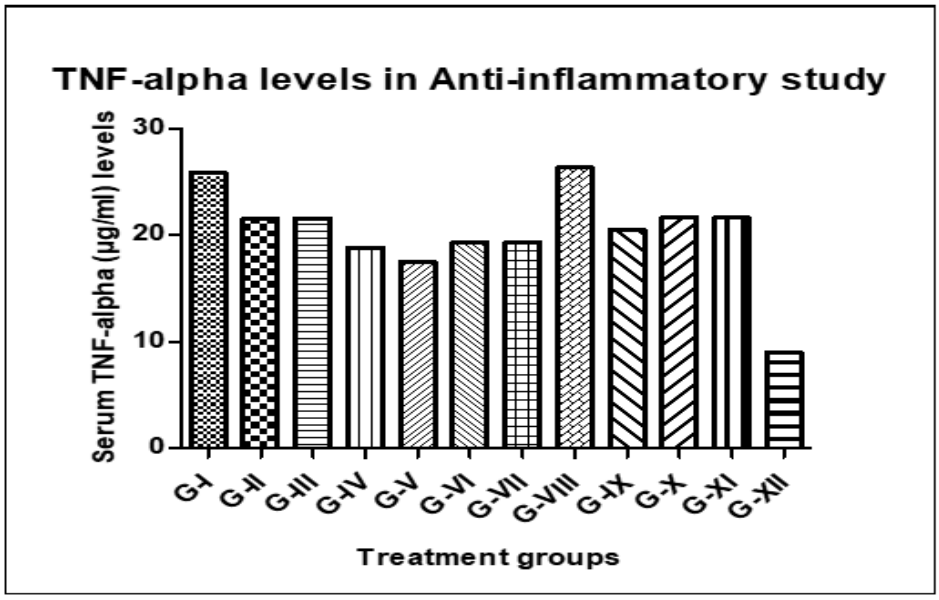



***Eosino=Eosinophils, Lymphoma=Lymphocytes, Neutro= Neutrophils and Mono= Monocytes. All values are expressed as Mean ± SEM, n=6.***

**Fig.1. Effect of APNC, SC, and their combination on clinical hematology in Anti-inflammatory study.**

### *4.1.3 TNF-Alpha Levels*

Proinflammatory cytokine tumor necrosis factor (TNF-a), which is largely produced by macrophages and monocytes, is a powerful mediator of inflammation. TNF –alpha is a powerful paracrine inducer of interleukin-1, interleukin-6, and interleukin 8 as well as an autocrine activator of other inflammatory cytokines. Additionally, TNF causes fibroblasts to express the leukocyte adhesion molecule, which causes an increase in the transport of leukocytes into inflammatory areas, such as the joints of people with rheumatoid arthritis[23-24].24 hours after Carrageenan injection, TNF-alpha levels were measured in all experimental animals. No significant difference was observed among all groups except in the animals treated with the combination of the highest dose of APNC and SC (130+2000 mg/kg), showing a mild decrease in TNF-alpha levels in experimental rats. The values are graphically represented in figure 2.



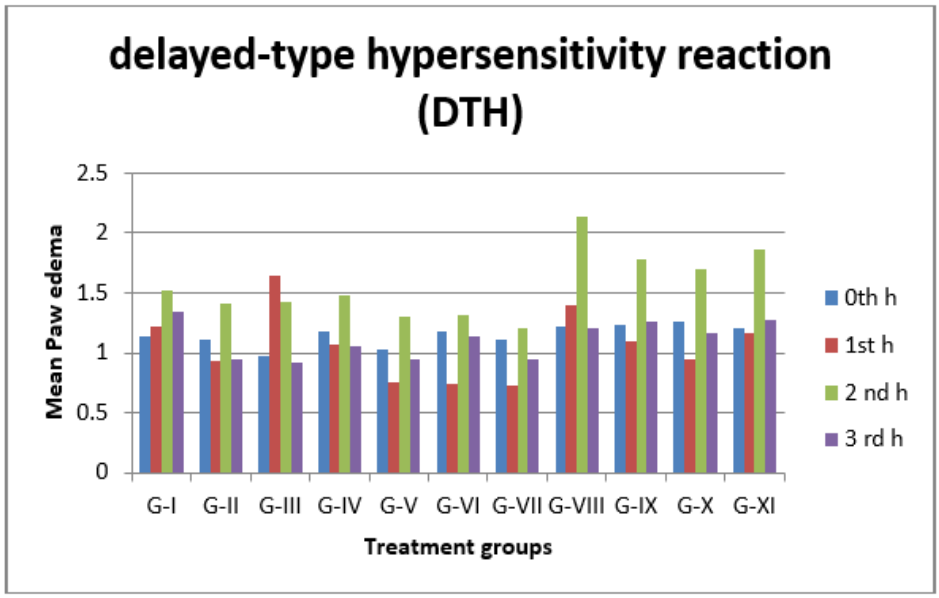

*All values are expressed as Mean ± SEM, n=6.*

**Fig.2. Effect of APNC, SC, and their combination on TNF-alpha levels in Anti-inflammatory study.**

### *4.2 Immunomodulatory Activity of APNC, SC, and Their Combination*

#### *4.2.1 Delayed-Type Hypersensitivity Reactions*

To evaluate the immunosuppressive impact of APNC and SC using a delayed-type hypersensitivity reaction assay, the immunological response of cells against the sheep red blood cell antigen was identified. The DTH edema index is a measurement of the variation in mean foot pad thickness between the right and left hind limbs at the conclusion of the experimental period[25-26]. We measured the Delayed type hypersensitivity of the test compounds. Oral administration of APNC at a dose of 13 mg/kg b.w for 21 days increases the paw volume measured at 1st hour. 2000mg/kg b.w of SC also significantly increases the hind paw volume at 2nd hour ($p<0.05$) when compared with Cyclophosphamide treated rats. While other groups showed no significant differences when compared with the Cyclophosphamide group. The values are graphically represented in figure 3.




*Percentage of foot paw edema in all experimental rats treated with different concentrations of test compounds and measured at 0th h to 2nd h. All values are expressed as Mean ± SEM, n=6.*

**Fig.3. Effect of APNC, SC, and their combination on delayed-type hypersensitivity reaction (DTH) in rats immunized with SRBCs.**

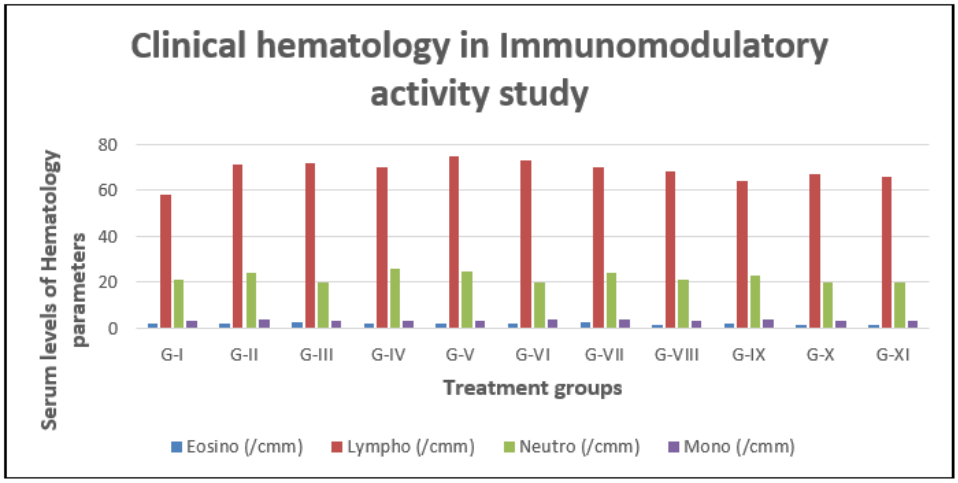
#### *4.2.2 HemagglutinationReaction*

The relative strength of an antibody titer is defined as the reciprocal of the highest dilution which is still capable of causing visible agglutination[27]. The antibody titer is useful to measure the changes in the amount of the antibody in the course of an immune response. The antibody response on day 22 in test drugs treated groups was observed visually when compared with the disease control group. Very minimal/no antibody titer was observed in the

cyclophosphamide-treated group when compared with the control group. The agglutination (dilution factor 1:200) was found in APNC 130mg/kg b.w. (moderate agglutination) and combination of APNC 130mg/kg b.w. +SC 2000mg/kg b.w. (high agglutination) results in humoral immunity.

### 4.2.3 *Clinical Hematology*

No compound-related changes and no significant differences were observed in Eosinophils, monocytes, lymphocytes, and neutrophils.The values are graphically represented in figure 4.

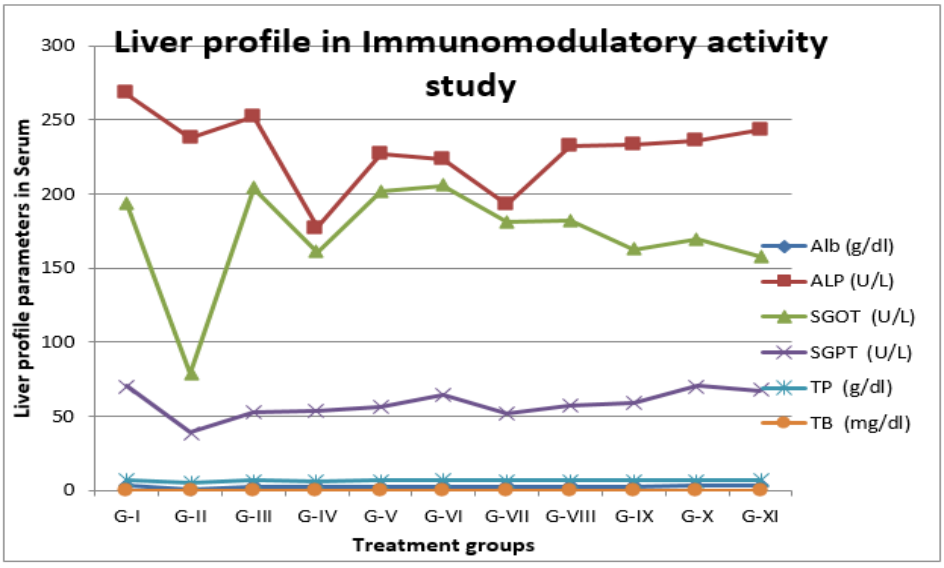



***Eosino=Eosinophils, Lymphoma=Lymphocytes, Neutro= Neutrophils and Mono= Monocytes.***
***All values are expressed as Mean ± SEM, n=6.***

**Fig.4. Effect of APNC, SC and their combination on clinical hematology in Immunomodulatory study.**

### 4.2.4 *Liver Profile*

Mild increases in Total proteins and albumin were observed in all groups (Group-III-XI) when compared with group-II. The levels of SGPT were significantly changed in animals treated with medium and high doses of a combination of APNC (65-130 mg/kg b.w) and SC (1000-2000 mg/kg b.w.). No significant changes were noted among other groups. Animals treated with APNC (130 mg/kg b.w) and APNC+SC (130+2000 mg/kg b.w) showed significant changes in the levels of SGOT when compared with animals treated with Cyclophosphamide. No significant changes in total bilirubin and ALP were recorded in all groups compared with the Cyclophosphamide group. The values are graphically represented in figure 5.

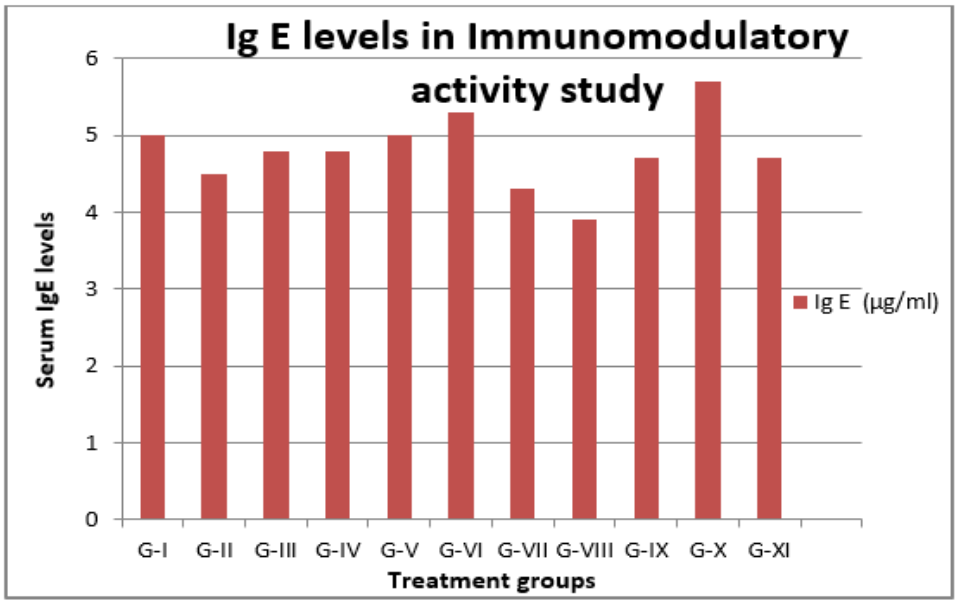



***All values are expressed as Mean ± SEM, n=6.***

**Fig.5. Effect of APNC, SC, and their combination on Liver profile in Immunomodulatory study.**

### 4.2.5 *Immunoglobulin E (Ig E) Levels*

IgE is essential for antigen presentation, mast cell/basophil activation, and the pathophysiology of allergic disorders. So we measured the serum levels of IgE in all experimental animals. No significant changes were observed in Ig E levels of normal control rats and test drugs treated rats when compared with rats treated with Cyclophosphamide. The values are graphically represented in figure 6.




***All values are expressed as Mean ± SEM, n=6.***

**Fig.6. Effect of APNC, SC and their combination on Ig E levels in Immunomodulatory study.**

## 5. CONCLUSION

Under the conditions of the present study, single oral administration of *Ayapoonagachenduram, Seenthilchoornam*and their combination has shown mild anti-inflammatory activity against carrageenan-induced paw edema in experimental rats. The relationship between the dose, dosage, and efficacy needs to be further investigated to understand the impact of each of these variables on the inflammatory process. The immunity has been stimulated by *Ayapoonagachenduram*(13mg/kg b.w.) and Seenthilchoornam (2000mg/kg b.w.). Hence the drug *Ayapoonagachenduram*and *Seenthilchoornam*was found to have moderate immunomodulatory activity. The effects are not dose-dependent. A detailed study is warranted to elucidate the mechanism of drugs at the cellular level.

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## 8. AUTHORS CONTRIBUTION STATEMENT

Dr. P. SathiyaRajeswaran and Dr. M. Kannan conceptualized and designed the study. Dr. G. Dayanand Reddy has prepared the protocol and prepared the original draft for the study. B. Rama Devi and K. Dhanaraj have gathered the literature regarding this study and performed the experimental studies. D. Pichaiah helped in the maintenance of experimental animals and dosing. Dr. R. Ganesan supervised the analysis of biochemical parameters. All authors discussed the methodology and results and contributed the final manuscript.

## 9. CONFLICT OF INTEREST

Conflict of interest declared none.

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