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# ANTI-INFLAMMATORY ACTIVITY OF SIDDHA MEDICINE *AYAVEERA CHENDURAM* ON CARRAGEENAN INDUCED PAW OEDEMA IN WISTAR ALBINO RATS

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## ABSTRACT

Siddha medicines mainly depend on the natural resources for medicine preparation, in which higher order medicines like *Parpam, Chenduram, Kattu* etc., are more effective but which are not validated in scientific aspect. In this study, the anti-inflammatory activity of *Ayaveera Chenduram* (AVC) was evaluated in a carrageenan-induced rat paw edema method and compared with that of indomethacin. Rats treated with indomethacin (10 mg/kg, p.o.) and AVC (200 and 400 mg/kg, p.o.) showed a significant reduction in carrageenan-induced paw edema. Indomethacin inhibited the edema by 54.15 % and 66.54% at 2 and 3 h after carrageenan injection, respectively. The inhibitory effect of AVC began at 2 h or later after carrageenan injection depending upon the administered dose. Low doses of AVC (23 mg/kg) gave significant inhibitory effects of 30.76-53.95%, and higher doses of AVC (46 mg/kg) caused significant inhibition at levels of 35.29-58.63%. The reduction of edema by indomethacin and AVC at 2 h or more after carrageenan injection suggests that both compounds produce anti-inflammatory effects in the second phase of edema, indicating inhibition of prostaglandin synthesis. Therefore, it can be inferred that the inhibitory effect of

*Ayaveera Chenduram* downregulating the proinflammatory cytokines pathway. They were found to be effective and (**p<0.01) significantly decreased the inflammatory reactions.The present results suggest that *Ayaveera Chenduram* suppresses carrageenan-induced paw edema, thus, confirming the anti-inflammatory property of *Ayaveera Chenduram.*

**Keywords: Anti-inflammation, Paw edema, Carrageenan, *Ayaveera Chenduram***

**INTRODUCTION:**

Siddha system emphasizes aetiology and management of various diseases that affecting the mankind. Siddha medicine claimed to revitalize and rejuvenate dysfunctional organs that cause the disease and to maintain three humors ideally. A fundamental concept found in Siddha system of medicine is that of balance between the mind and body; The main effort in Siddha medicine is using of natural resources like herbals, metals, minerals, animal and marine products. The metallic compounds are mainly used in higher order medicine preparation like *Parpam, Chenduram, Chunam.* In past, many metallic formulations of Siddha medicine and the practice were well flourished. The ancient Siddhars had their own methods of standardizing the drugs, by the spiritual knowledge. They detoxify the various metallic compounds in different purification methods and then processed into highly efficacious medicine.

*Ayaveera chenduram* is a Herbo-mineral preparation has high privilege in curing eight types of *Gunmam* (peptic ulcer), *Mega vayu* (venereal disease), *Pakkavatham* (Hemiplegia) and *Pakkasoolai* (throbbing pain) **[1]**.

Anti-inflammatory drugs can interfere in the pathophysiology of inflammation, seeking to minimize tissue damage and provide greater patient comfort. The major classes of anti-inflammatory agents are the glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs) **[2]**. NSAIDs encompass a range of agents and, in general, all their effects are related to the inhibition of COX action in the production of prostaglandins and thromboxanes. **[3–6]**.

The main mechanism of action of NSAIDs is the inhibition of COX, both central and peripheral, interfering in the conversion of arachidonic acid to prostaglandins E2, prostacyclins and thromboxanes. Enzymes related to the action of NSAIDs can be divided into COX-1 and COX-2, acting in different regions. COX-1 appears in most cells, even foetal and amniotic fluid, participates in physiological effects, such as regulatory and protective effects. On the other hand, COX-2 is activated by inflammation and proinflammatory cytokines **[7, 8]**.

There are several ways to classify NSAIDs; according to COX-2 inhibitory potency over COX-1, concentration to achieve clinical effects, among others. NSAIDs can be classified into non-selective NSAIDs (ketoprofen, aspirin, naproxen, flunixin, meglumine, and others), preferential COX-2 inhibitors (meloxican, etodolac, nimesulide), and highly selective COX-2 inhibitors (coxib). Most of the side effects are related to the inhibition of COX-1 due to its performance in several systems related to cell cleansing. Besides, NSAIDs can also be classified according to their chemical structure **[9]**.

The severe side effects of steroidal and nonsteroidal anti-inflammatory drugs evoked us to search for new anti-inflammatory agents from natural resources which may have less side effects.

**MATERIALS AND METHODS:**

**Procurement of Ingredients:**

The ingredients of *Ayaveera chenduram* were purchased from reputed siddha raw drug store, Chennai and authenticated by HOD, Department of Gunapadam, National Institute of Siddha, Chennai– 47.

**Purification:**

***Ayam:***

The purification of iron ore (*Ayam*) was carried out by soaking in fruit juice of *Syzygium cumini* and kept in the sunlight to get dried. This process was repeated for six times **[10]**.

***Veeram:***

Take *Veeram* (Mercury Chloride) and *Piper nigrum* seeds in the ratio of 1:3Grind the piper seeds in rice porridge until paste form. Gently cover the *Veeram* with that paste and tie with a white cloth. Sufficient quantity of tender coconut water was added into a mud pot. It was burned with tender coconut water by *Thulaenthiram* method for 1 ½ hours (1/2 *Saamam*) **[10]**.

***Sunnambu thelineer*:**

Mix the Oyster shell with water in the ratio of 1:4 and leaves it for 4 hours until its sedimentation. Finally, gently take the upper zone of water without vigorous mixing **[10]**.

**Method of Preparation**

The purified *Ayam* and purified *Veeram* were taken and placed in the *Kalvam* for grinding, with adding *Sunnambu thelineer* until 4 *Saamam*, made it into a single pellet and dried under the sunlight. Then the pellet was placed in a mud plate and closed with similar mud plate. The closed mud plate was covered with 5 layers of cloth, dried and kept in the pit under the earth. This entire setup subjected to *Pudam* (calcination) process with cow dung cakes (the weight of the cow dung measures 25 times weight of the prepared mud pot). On Next morning being

cooled, mud plate was removed and processed *Ayaveera chenduram* was collected and stored in an airtight container. It must be identified by observing color which it resembles like the flower of the *Butea frondosa* which is indicated for eight types of *Gunmam* (Gastritis), *Mega Vaayu* (Venereal diseases), *Pakkavatham* (Hemiplegia), *Oduvayu* (Myalgia) and *Pakkasoolai* (Pleural effusion) **[2]** .

**Figure 1:** ***Ayam***

**Figure 2:** ***Veeram***

**Figure 3:** ***Kar Chunnambu***

**Figure 4:** ***Aya Veera Chenduram***

**Toxicity study**: **[11]**

The test drug *Ayaveera Chenduram* was already ensure the Acute oral toxicity study (OECD guideline-423) and 28days repeated dose oral toxicity study (OECD Guideline 407)

In this Study there is no toxic vital signs and mortality were reported.

**Anti-inflammatory activity of *Ayaveera Chenduram*: [12-13]**

**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 from National Institute of Siddha. (IAEC approved no:

NIS/IAEC-V/09082017/11). 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 and 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 any signs of infection they were excluded from the study. The animal experiment was performed with accordance to legislation on welfare.

**Animal grouping:**

Experimental mice were divided in to four groups of 6 animals each. Paw oedema was induced on each rat by injecting 0.1 mL of carrageenan on physiological saline to the left hind paw. They study drug *Ayaveera Chenduram* (AVC) at different concentrations were administered orally 30 minutes prior to carrageenan administration. In which group - I served as control was administered with aqueous distilled water (2 mL/100 g body weight) + carrageenan (0.1ml of 1% w/v). Group - II received Indomethacin (5 mg/kg), p.o + carrageenan (0.1ml of 1% w/v). Group - III received test drug AVC at the dose of 23 mg/kg, p.o + carrageenan (0.1ml of 1% w/v). Animal belongs to group - IV received test drug AVC at the dose of 46 mg/kg, p.o+ carrageenan (0.1ml of 1% w/v).

**Evaluation of Anti- inflammatory potential:**

Paw edema was induced by injecting 0.1 ml of 1% carrageenan in physiological saline into sub plantar tissues of hind paw of each rat. *Ayaveera chenduram* at the dose of 200 and 400 mg/kg were administered orally 30 min prior to carrageenan administration. The paw volume was measured at intervals of 60, 120, 180, and 240 min by the mercury displacement method using a plethysmograph. Percent inhibition (%IE) of edema was calculated using the equation,

$$\%IE = (V_c - V_t)/V_c \times 100,$$

Where, $V_c$ is the inflammatory increase in paw volume in control group of animals and $V_t$ is the inflammatory increase in paw volume in drug treated animals. Inhibition of paw volume in drug-treated group was compared with the carrageenan control group (Group- I), whereas indomethacin (10 mg/kg p.o.) was used as reference drug.

**Data Analysis:**

The Data were expressed in mean± SEM. The results were analyzed using one way ANOVA followed by Dunnet's test. Differences were considered as statistically significant at $P < 0.05$. when compared with control.

**RESULTS:**

Anti-inflammatory activity of *Ayaveera chenduram* was carried out by Carrageenan induced paw oedema in wistar albino rat. The Data were expressed in mean ± SEM and inhibition in percentage are tabulated in **Table 1 & 2 and in Figure 5 & 6**.

**Table 1: Effect of *Ayaveera Chenduram* on Carrageenan induced Paw oedema in Rat.**

| Group | Paw oedema Volume(ml) | | | |
|---|---|---|---|---|
| | 0th hr | 1st hr | 2nd hr | 3rd hr |
| Group I (Carrageenan + Normal saline) | 1.76±0.13 | 2.21±0.14 | 2.53±0.16 | 2.78±0.14 |
| Group II (Carrageenan + AVC 23mgm/kg/bw) | 1.61±0.07 | **1.53±0.08 | **1.38±0.07 | **1.28±0.07 |
| Group III (Carrageenan + AVC46mgm/kg/bw) | *1.56±0.08 | **1.43±0.1 | **1.3±0.08 | **1.15±0.12 |
| Group IV (Carrageenan + Indomethacin 10mgm/kg/bw) | **1.43±0.17 | **1.31±0.16 | **1.16±0.16 | **0.93±0.12 |

**Data are presented as mean± SD (N=6). Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared Standard with the control group. A p values ** < 0.01 were considered as Significant**

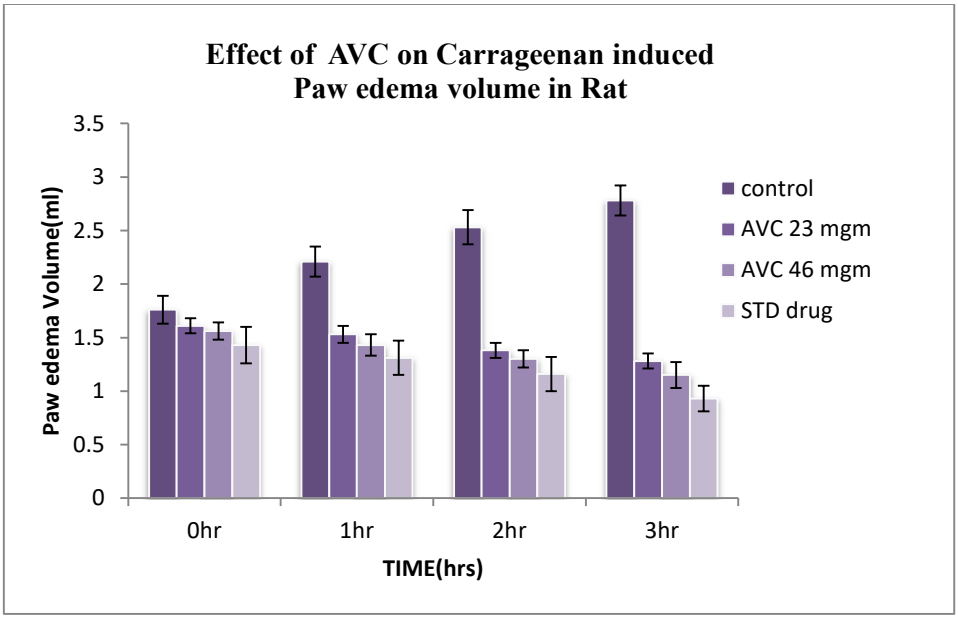


**Figure 5: Effect of *Ayaveera Chenduram* on Carrageenan induced Paw oedema in Rat**

**Table 2: Anti-inflammatory activity effect of *Ayaveera Chenduram* in % of Inhibition of Paw oedema**

| Group | % Inhibition of Paw oedema | | | |
|---|---|---|---|---|
| | 0th hr | 1st hr | 2nd hr | 3rd hr |
| Group II AVC 23mgm/kg/bw | 8.52 | 30.76 | 45.45 | 53.95 |
| Group III AVC 46mgm/kg/bw | 11.36 | 35.29 | 48.61 | 58.63 |
| Group IV Indomethacin 10 mgm/kg/bw | 18.75 | 40.72 | 54.15 | 66.54 |

**Data expressed in percentage (n=6).**

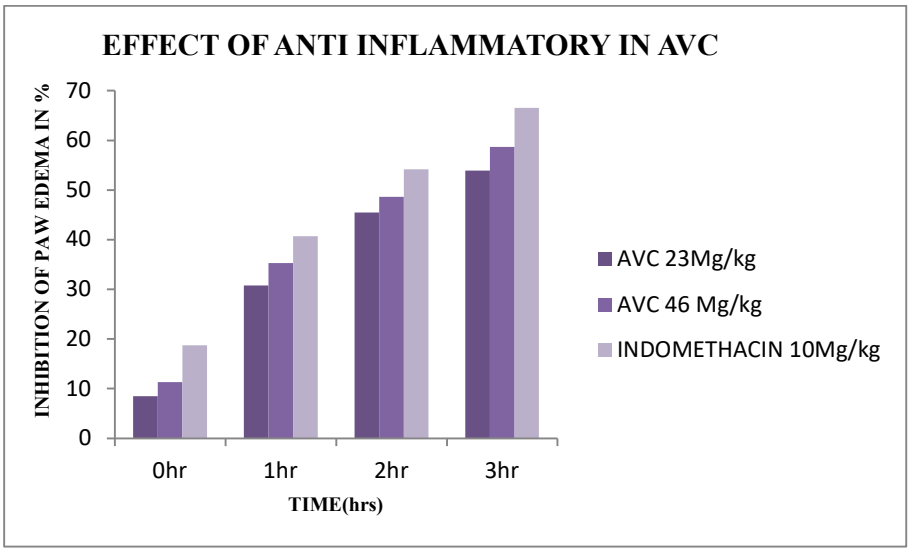


**Figure 7: Anti-inflammatory activity effect of *Ayaveera Chenduram* in Percentage (%) Inhibition of Paw oedema**

## DISCUSSION

Inflammation is a common phenomenon, and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response**.** Carrageenan induced inflammation is a useful model for the estimation of anti-inflammatory effect. The development of oedema in the paw of the rat after the injection of Carrageenan is due to the release of histamine, serotonin, Prostaglandin. The lysosomal enzymes released during inflammation produce a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. This Drug *Ayaveera chenduram* acts either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane **[14]**.

The time course of edema development in carrageenan-induced rat paw edema model in rats is generally represented in mean±SEM 2.78±0.14 in control group, Treatment with trial drug AVC at both the dose level of 23 and 46 mg/kg has 1.28±0.07 and 1.15±0.12, 0.93±0.12 in standard group at 3hrs. As shown in **Table 1**, there was no significant inhibition of paw edema in the early hours of study by the *Ayaveera chenduram* at the dose level of 200 and 400 mg/kg. Carrageenan-induced rat paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of *Ayaveera Chenduram* which primarily inhibits the cyclooxygenase involved in prostaglandin synthesis. It plays a major role in the development of second phase of

inflammatory reaction, which is measured at the second hour. As shown in **Table 2**, the *Ayaveera Chenduram* at the dose of 400 mg/kg showed high significant activity (***P*<0.01) at 3 h, where it caused 58.63% inhibition, as compared to that of 10 mg/kg of indomethacin which exhibited up to 66.54% inhibition. Therefore, it can be inferred that the inhibitory effect of *Ayaveera Chenduram* on carrageenan-induced inflammation may be due to inhibition of the cyclooxygenase leading to inhibition of prostaglandin synthesis. They were found to be significant (**p<0.01) effective in reduction of paw volumer after 3 hrs. So, from above the reasons this study proved the test drug *Ayaveera Chenduram* has Anti-inflammatory activity.

**CONCLUSION:**

The present study concluding that, the preclinical study of *Ayaveera Chenduram* shows its efficacy of Anti-inflammatory activity in animal model. Hence, the drug *Ayaveera Chenduram* may have good efficacy against chronic degenerative disease, venereal diseases and acid peptic disease in human as mentioned in reference literature.

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