



## ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS LEAF EXTRACT OF *ANNONA MURICATA.L* AND *SPERMACOCE ARTICULARIS.L* F AGAINST CARRAGEENAN INDUCED PAW OEDEMA IN RATS

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### ABSTRACT

The present study was designed to evaluate the anti-inflammatory activity of aqueous Leaf extract of *Annona muricata.L* and *Spermocoe articularis.L.f* , at a dose of 200 and 400 mg/kg against carrageenan induced rat paw edema and compared to standard drug Indomethacin (10 mg/kg). The results obtained from present study reveals that the *Spermocoe articularis.L.f* aqueous leaf extract (400 mg/kg) exhibited high significant activity ( $p < 0.001$ ) within 1hr of administration of the extract, while the other groups showed less significant effect in reducing paw volume.

### KEY WORDS

*Annona muricata.L*; anti-inflammatory activity; carrageenan induced rat paw edema, Indomethacin, *Spermocoe articularis. L.f*;

### 1.Introduction

Inflammation is a pervasive phenomenon, which is elicited by the body in response to obnoxious stimuli as a protective measure. However, sustained inflammation leads to several diseases including cancer. Therefore, it is necessary to neutralize inflammation. The inflammation is initiated by a cascade of events including enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown, and repair processes [1]. The inflammation releases white blood cells as a protective measure against injury. These white blood cells synthesize several biomolecules and release them after injury leading to swelling and redness. The inflammation is characterized by induction of pain, redness, and rashes [2]. Cyclooxygenase (COX) is the key enzymes in the synthesis of prostaglandins, prostacyclin's and thromboxane's which are involved in inflammation, pain and platelet aggregation [3].

Inflammatory diseases are major worldwide problem. Chronic inflammation lies at the basis of many diseases of advanced age such as heart attacks, Alzheimer's diseases, and cancer [4]. The drugs used to reduce inflammation are NSAIDs. These drugs block COX-1 and COX-2 enzyme activity. COX enzymes assist with prostaglandin production. NSAIDs, steroidal anti-inflammatory drugs are being used till now, as a result long term uses of these drugs cause adverse side effects and damage human biological system such as liver, gastrointestinal tract, and cause gastric lesions, cardiovascular, renal failure [5]and gastrointestinal damage [6,7]

Now, there is a need for the new safe, potent, nontoxic or less toxic anti-inflammatory drug. Fossil records show evidence for the use of natural products, especially the plants as medicine since Middle Paleolithic (approximately 60,000 years) age [8]. The modern allopathic drugs are single active chemical

molecules and target one specific pathway, whereas herbal medicines contain pleiotropic molecules that work on orchestral approach which are able to target many elements of the complex cellular pathway. Many of the diseases in the modern world are thought to be due to inflammation; therefore, anti-inflammatory agents, anti-inflammatory food and food products are of great interest to contain or reduce inflammation-induced health disorders [9], [10]. The pain and inflammatory conditions are usually managed by either steroidal (corticosteroids) or non-steroidal (aspirin) drugs, which induce toxic side effects at different levels including allergic reactions, occasional hearing loss, and renal failure. These drugs also increase the risk of hemorrhage by negatively altering platelet function [11]. The medicinal plants have been a major source of a wide variety of biologically active compounds for many centuries and have been used extensively in crude form or as pure isolated compounds to treat various disease conditions including inflammation [12]. The study plant *Annona muricata* Linn. belongs to the family Annonaceae, commonly known as "Seemai Mundhiri", "sour soup". It is a slender evergreen tree, 5-10m in height and 15 cm in diameter, trunk straight, and bark smooth, rough and fissured with age. It is widely distributed in tropical region of the world. Young branches are hairy. Leaves are alternate, simple, 7.6-15.2 cm long, leathery, obovate to elliptic, glossy on top, glabrous on underside. The leaves stalk is 4mm-13mm long and without hairs [13]. The leaves of *Annona muricata* L. have astringent, anti-plasmodic and gastric properties. [14]. It is a traditional medicinal plant in Indonesia to treat breast cancer. They are rich in annonaceous acetogenins.

In India, the fruit and flower are employed as remedies against catarrh, while the root-bark and leaves are believed to have antiphlogistic and anti-helminthic activities [15,16].

*Spermocoe articularis*. L.f (Rubiaceae) was popularly known as "Nattaiccuri" in Tamil and "Shaggy button weed" in English. It is widely distributed in the Western Ghats of Kerala [17] and Maruthamalai forest, in Tamil Nadu. *Spermocoe articularis*. L.f. removes old age signs, improves vitality and it was used by the tribals in Western Ghats of Kerala since ancient times [18]. *Spermocoe articularis*. L.f is one of the crude materials used for the treatment of various ailments in the form of various preparations. The plant seed was

used as a remedy to treat nerves and kidney injuries [19]. Its pharmacological properties include antioxidant [20], anti-inflammatory [21]. Bioactive molecules isolated from plants served as the starting materials for isolation and laboratory synthesis of drugs as well as a model for the production of biologically active compounds [22]. The present study has been undertaken to investigate the anti-inflammatory activity of the aqueous extracts of *Annona muricata*.L and *spermocoe articularis*.L.f leaf, against carrageenan induced rat paw edema.

## 2. Materials and Methods

### 2.1 Collection of plant material

The leaves of *Annona muricata* L. and *Spermocoe articularis*.L.f were collected from Anaikatti, Coimbatore District, Tamilnadu, India in the month of November 2015. The plant was identified and authenticated by Botanical Survey of India, Coimbatore. The leaves of *Annona muricata* L. and *Spermocoe articularis*.L.f were washed and cleaned to remove foreign organic matter, cut into small pieces and then kept for drying in shade. The dried plant parts were made into coarse powder. These powders were stored in air tight container and used for further extraction.

### 2.2 Preparation of extract

The extract from leaf powder was taken using Soxhlet extractor with water as a solvent. The collected extract was evaporated to dryness and stored in 4°C for experimental study. The extracts were stored in air tight container and used for further experimental purposes.

### 2.3 Experimental animals

Albino Wistar rats of either sex weighing between 180-200g were used in this study. The animals were obtained from animal house, Nandha College of pharmacy Erode, Tamil Nadu, India. They were maintained in polypropylene cages with paddy husk as bedding at a temperature of 24± 2°C and relative humidity of 30-70% with 12:12 light and dark cycle. Animals were allowed free access to water and fed with standard commercial pelleted rat chow (M/s. Hindustan Lever Ltd, Mumbai). Animals were acclimatized to the laboratory conditions one week before the experiment and fasted overnight before the experiment. Experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) (No.688/PO/Re/S/02/CPCSEA).

## 2.4 Acute toxicity study

Acute toxicity studies were performed according to OECD-423 (Organization of Economic and Cooperation Development) guidelines. The aqueous extract of *Annona muricata.L* and *Spermacoce articularis.L.f* was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for first 24 hrs. and after 72 hrs. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 1000 & 2000 mg/kg) doses of the plant extracts were employed for further toxicity studies. [23].

## 2.5 Carrageenan -induced rat paw edema

The Anti-inflammatory activity of the extract was determined using carrageenan induced rat paw Edema assay [24].

The rats were divided into six groups of 5 each

Group I - Normal control (distilled water, 1ml/Kg)

Group II – Reference control (Indomethacin -10mg/Kg)

Group III – Aqueous leaf extract of *Annona muricata .L*(200mg/Kg)

Group IV –Aqueous leaf extract of *Annona muricata.L* (400mg/Kg)

Group V-Aqueous leaf extract of *Spermacoce articularis.L.f*(200mg/Kg)

Group VI-Aqueous leaf extract of *Spermacoce articularis.L.f*(400mg/Kg)

The test drugs were administered orally using gastric gavages by dissolving in distilled water. After 30 minutes, acute inflammation was produced in the right hind paw of each rat by sub plantar injection of 0.05ml freshly prepared carrageenan suspension (1%) in normal saline. The volumes of the oedematous paws were measured using Plethysmometer following oral administration of the test drugs, 0 min (before carrageenan injection) and at every 1 hr intervals for 5 hr. Oedema was expressed as the increment in paw thickness due to carrageenan administration. The percentage of anti-inflammatory activity was calculated using the formula given below:

$$100 \times \left(1 - \frac{V_t}{V_c}\right)$$

Vc – Control Mean and Vt – Test Mean

## 2.6 Statistical Analysis

All the results were expressed as mean  $\pm$  standard error mean (SEM). The data were analyzed by using one-way analysis of variance (ANOVA) followed by Dunnett's t-test using Graph Pad software of version 3. P values < 0.05 were considered as significant.

## 3.Results

### 3.1 Acute toxicity study

The results of acute toxicity study of aqueous extracts of *Annona muricata .L* and *Spermacoce articularis .L.f* were shown on( Table 1). Mortality was not produced by both aqueous extracts up to 2000mg/kg on oral administration after 24 & 72 hrs. On general behavior, the aqueous extract of *Annona muricata.L* showed moderate analgesic and mild muscle relaxant activity, and all other general behavior observed were normal. Whereas, the aqueous extract of *Spermacoce articularis.L.f* showed mild analgesic activity and other general behavior remains normal. No lethality or toxic reactions were found with both the extracts, during and after the study period

### 3.2 Carrageenan induced rat paw edema

The effects of Aqueous Leaf extracts of *Annona muricata.L* and *Spermacoce articularis .L.f* were studied in albino rats by observing its anti-inflammatory activity induced by Carrageenan. The experiment showed (Table 2) that the leaf extract of *Spermacoce articularis .L.f* exhibited was statistically more significant at a dose of 400 mg/kg within 1 hr of administration of the extract. The Group I normal control is Carrageenan induced which showed an elevated level of paw volume in each hour. At the end of the 5th hr. the paw volume is higher than the Initial Paw Volume. In Group II the Standard Drug Indomethacin showed low paw volume in each hr. (1st to 5th hr.) finally at the end of 5th hr. paw volume showed least value. The other groups showed less significant effect in reducing paw volume compared to standard drug Indomethacin.

**Table 1. Oral acute toxicity study of aqueous extract of *Annona muricata* .L and *Spermacoe articularis* .L.f (2000mg/kg) in mice.**

S.No	General Behaviour	<i>Annona muricata</i>	<i>Spermacoe articularis</i>
1	Sedation	-	-
2	Hypnosis	-	-
3	Convulsion	-	-
4	Ptosis	-	-
5	Analgesia	++	+
6	Stupar Reaction	-	-
7	Motor activity	-	-
8	Muscle Relaxant	+	-
9	CNS Stimulant	-	-
10	CNS Depressant	-	-
11	Pilo Erection	-	-
12	Skin Colour	-	-
13	Lacrimation	-	-
14	Stool Consistency	-	-

‘+’ PRESENT & ‘-’ ABSENT

**Table 2. Anti-inflammatory activity of aqueous extract of *Annona muricata*.L and *Spermacoe articularis* .L.f against Carageenan induced Paw Oedema in rats.**

Drug Treatment	Thickness of Rat Paw (mm)					
	0 hr.	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
Control	9.22±0.17	10.56±0.98	12.37±0.74	14.66±0.28	15.94±1.22	15.90±0.92
Distilled Water (1ml/kg)						
Reference Control	8.56±0.32	9.44±0.87	10.62±0.11*	12.03±0.85**	11.32±0.92**	8.74±0.62***
Indomethacin(10mg/kg)		(10.61%)	(14.15%)	(17.99%)	(28.98%)	(45.03%)
<i>Annona muricata</i> .L (200 mg/kg)	9.03±0.62	10.28±0.54	12.13±0.74	14.32±0.90	15.30±0.80	14.76±1.04
		(2.65%)	(1.94%)	(2.32%)	(4.02%)	(7.17%)
<i>Annona muricata</i> .L (400 mg/kg)	9.15±0.33	10.13±0.82	11.52±0.31	14.02±1.05	14.32±0.67	13.06±0.90*
		(4.07%)	(6.87%)	(4.37%)	(10.16%)	(17.86%)
<i>Spermacoe articularis</i> .L.f (200 mg/kg)	9.42±0.29	10.33±0.91	12.06±0.73	13.98±0.98	14.02±0.32	10.76±0.82**
		(2.18%)	(2.51%)	(4.64%)	(12.05%)	(32.33%)
<i>Spermacoe articularis</i> .L.f (400 mg/kg)	8.94±0.56	9.75±0.32	11.04±0.22*	12.66±0.65*	12.05±0.54**	9.04±0.61***
		(7.67%)	(10.75%)	(13.64%)	(24.40%)	(43.14%)

Percentage Protection was given in Parentheses Values are in mean ± SEM (n=5) \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 Vs. Control

#### 4. Discussion

The carrageenan-induced inflammatory processes are biphasic [25]. The initial phase seen at the first hour is attributed to the release of histamine and serotonin [26] and the second accelerating phase of swelling is due to the release of prostaglandin, bradykinin and lysozyme. The results of this study indicate that the leaf extract of *Spermacoe articularis* .L.f significantly reduced carrageenan induced paw edema in rats. Therefore, the mechanism of action may be by inhibition of histamine, serotonin or prostaglandin synthesis. COX and 5-LOX are two important enzymes

which catalyze the formation of mediators involved in the inflammatory process. Inhibitors of COXs are the main strays of current therapy aimed to modulate pain, inflammation and to control fever [27] many COX-2 or 5-LOX inhibitors have been developed as drugs to treat inflammation; however, some have been withdrawn from the market, indicating a need for inhibitors free of side effect. [28]The exact mechanism of suppression of inflammation by *Annona muricata* .L and *Spermacoe articularis*.L.f not known. However, it contains flavonoids other phenolic compounds that may have contributed to its anti-inflammatory actions. Aqueous

Leaf extract of *Annona muricata* .L and *Spermacoe articularis*.L.f inhibited inflammatory process but *Spermacoe articularis*.L.f showed better action compared to other plants. This explains the beneficial effects of the plant. The observed anti-inflammatory action of both plant extracts may also be due to its inhibitory action on cyclooxygenase which is involved in prostaglandin synthesis [29].

## 5. Conclusion

The present study shows that aqueous extract of *Annona muricata* .L and *Spermacoe articularis*.L.f leaf possess anti-inflammatory property at the tested doses. It may be due to its ability to neutralize free radicals which are the main players in inflammation. It may have also suppressed the activation of pro inflammatory cytokines including NF- $\kappa$ B, TNF $\alpha$ , IL- 1 $\beta$ , and IFN $\gamma$  and the activity of cyclooxygenase enzymes which are involved in inflammation. The anti-inflammatory activities of *Annona muricata* .L and *Spermacoe articularis*.L.f may be due to the presence of flavonoids and other polyphenols. However, aqueous *Spermacoe articularis* .L.f leaf extract at 400 mg/kg showed better action when compared to other extracts. This could provide a rationale for the use of this plant in inflammation as an herbal medicine however, further studies are required to understand molecular mechanisms of action against inflammation.

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