



## Evaluation of the analgesic and anti-inflammatory properties of methanol extract of *Artanema sesamoides* Benth roots in animal models

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### SUMMARY

The methanol extract of the root of *Artanema sesamoides* Family Scrophulariaceae (MEAS) was investigated for possible analgesic and anti-inflammatory effects in animals. Three models were used to study the extract effects on nociception, which were acetic acid-induced writhing response, hot-plate method and the tail flick test in mice. The antiinflammatory effects were evaluated using carrageenan, dextran, histamine and serotonin induced rat paw oedema (acute) and cotton pellet induced granuloma (chronic) models in rats. Results of the study revealed that the extract exhibited significant ( $P < 0.001$ ) analgesic effect at a dose of 50, 100 and 200 mg/kg b.w p.o in mice in all the models. In acute model, the MEAS also exhibited significant ( $P < 0.001$ ) antiinflammatory effect in all the above mentioned doses. In chronic model (cotton pellet induced granuloma) the MEAS 200 mg/kg and indomethacin 10 mg/kg showed that inhibition of granuloma formation 25.0% and 47.7% respectively ( $P < 0.001$ ). The MEAS and indomethacin were effectively preventing the transudation of the fluid. Thus, the present study revealed that the methanol extract of the root of *Artanema sesamoides* exhibited significant analgesic and antiinflammatory activity.

**Key words:** Antiinflammatory; Analgesic; *Artanema sesamoides*; Cotton pellet

### INTRODUCTION

Herbal medicines derived from natural plant extracts are being utilized for various clinical diseases, even though the modes of action of medicinal plants are yet to be established. There is growing interest in the pharmacological evaluation of various medicinal plants used in Indian traditional system such as ayurveda and sidda. The root part

of *Artanema sesamoides* Benth was selected to evaluate analgesic and anti-inflammatory activity for the present investigation.

Inflammation is fundamentally a protective response, the ultimate goal of which is to get rid of from both the initial cause of cell injury (eg. microbes) and consequence of cell injury (necrotic cell). However, in some circumstances this protective response may play against the tissue of body itself and may indeed constitute part of the disease process such as asthma, rheumatoid arthritis, atherosclerosis, diabetes and cancer etc.

*Artanema sesamoides* Benth. (Scrophulariaceae) is

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a stout herb, 60 - 90 cm heights with sparingly branched. This plant is distributed in the West peninsula, Srilanka, Malay, Sumatra, and Java, Philippines and in southern part of India. The root and seeds of this plant is reported to be medicinally important in traditional system of medicine. Decoction of root is given in rheumatism, diarrhoea, stone, syphilis and ophthalmia. Seeds are given for biliousness, improve vitality and favour conception. (Kritikar and Basu, 1975; Nadkarni, 1992) However, still there is no scientific report of this plant, this plant has been selected for its antiinflammatory effect because of its traditional use in tribal people of Tamilnadu. This plant extract is also formulated as tablets with combination of some other plant extracts (*Tinospora cordifolia*, *Boerhaavia diffusa*, *Gmelina arborea*, *Aegle marmalos*, *Stereospermum scolaris*, *Oroxylum indicum*, *Premna corymbosa*, *Solanum xanthocarpum*, *Solanum indicum*, *Desmodium gangeticum*, *Pseudarthria viscida* and *Commiphora mukul.*) for the treatment of rheumatoid arthritis, osteoarthritis and other inflammations of the skeletal-muscular system. The preliminary phytochemical studies of MEAS show the presence of alkaloids, flavonoids and saponins. Any of these phytoconstituents may be responsible for this pharmacological activity, however detail study about the phytoconstituents are to be undertaken in order to confirm the clear mode of the pharmacological action. The purpose of this work was thus to study analgesic and antiinflammatory activity of the methanol extract of root of *Artanema sesamoides* Benth in some animal model.

## MATERIALS AND METHODS

### Plant material

The roots of the plant *Artanema sesamoides* Benth. were collected from Kolli hills of Tamilnadu, India. The plant material was identified by the Botanical Survey of India, Kolkata, India and the voucher specimen (GMT-2) has been preserved in our research laboratory for future reference. The root

parts of the plant were dried under shade and powdered with a mechanical grinder. The powdered plant material was passed through sieve #40 and stored in an airtight container for future use.

### Extraction of the powdered plant material

The air-dried powdered plant material (2 kg) was defatted with petroleum ether (60 - 80°C) in a Soxhlet extraction apparatus. The defatted plant material was extracted with methanol. The solvents were completely removed under reduced pressure to obtain a dry mass. The yields of the petroleum ether and methanol extracts were found to be 7.20 and 14.00% w/w respectively. The extracts were stored in a vacuum dessicator for further use.

### Chemical reagents and drugs

Carrageenan (S.D. Fine Chemicals Limited, Bombay), 5-hydroxytryptamine hydrochloride (serotonin), histamine and dextran (sigma, USA) were used for the study and indomethacin, paracetamol (IPCA, Bombay) and morphine (M.M. Pharma, New Delhi) were used as standard drugs.

### Animals

Adult male Wister albino rats weighing 180 - 200 g and male Swiss albino mice weighing 20 - 25 g were used for the present investigation. They were housed in clean polypropylene cages and were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory condition for one week before start of experiment. All procedures described were reviewed and approved by the University animal ethical committee.

### Acute toxicity study

Acute toxicity study was performed as per OECD-423 guidelines (Ecobichon, 1997; Perianayagam *et al.*, 2006). Swiss Albino mice (20 - 25 g) of either sex were used. The animals were fasted for 4 h, but allowed free access to tap water. The fasted mice were divided into two groups of six animals each.

The group one received MEAS at the dose of 5 mg/kg b.w of orally. The control group (group two) received a similar volume of distilled water (5 ml/kg). Mortality in each group was observed for 3 days. If mortality was not observed, the procedure was repeated for higher doses such as 50, 300 and 2000 mg/kg b.w. p.o.

### **Antiinflammatory activity**

#### **Carrageenan-induced rat paw oedema**

The carrageenan induced rat paw oedema test was performed according to the method of Winter *et al.* (1962). Male rats of 180 - 200 g body weight were divided into five groups each group contains six animals. Group I served as control. Group II, III and IV were treated with MEAS (50, 100, 200 mg/kg b.w. p.o). Group V received Indomethacin (10 mg/kg. p.o) as a reference drug. Acute oedema induced was induced in the hind right paw of rats by injecting 0.1 ml of freshly prepared 1% carrageenan solution. The left paw served as control (non inflamed paw; 0.9%, 0.1 ml saline injected) for comparison. The carrageenan was injected under subplantar region of right hind paw and paw volume was measured by plethysmometer at 0 and 3 h after Carrageenan injection. Anti-inflammatory activity (%) =  $(1 - D/C) \times 100$ , Where D represents difference in paw volume and width after MEAS was administered to the rats and C represents difference in paw volume in control groups (Gupta *et al.*, 2005).

#### **Dextran-induced paw oedema**

The dextran induced rat paw oedema test was performed according to the method of Winter *et al.* (1962). This study was carried out in a similar manner of carrageenan induced paw oedema model; dextran (0.1 ml, 1% w/v in normal saline) was used in place of carrageenan.

#### **Histamine-and serotonin-induced inflammation**

Histamine and serotonin are phlogistic agents, which act as inflammatory mediators. These phlogistic

agents were used to induce the paw oedema in rats by subplantar injection of freshly prepared histamine (1 mg/kg b.w) and serotonin (1 mg/kg b.w) and paw oedema was measured as mentioned earlier (Winter *et al.*, 1962).

#### **Cotton pellets-induced granuloma**

The Cotton pellets-induced granuloma test was performed according to the method of Gupta *et al.* (2003). The rats were divided into five groups (n = 6). Adsorbent cotton wool cut into pieces, weighing 10 mg and made up a pellet. The pellets were sterilized in hot air oven at 120°C for two hrs and two pellets were inserted one in each axilla of the animal under light anesthesia. The MEAS (50, 100 and 200 mg/kg b.w. p.o), indomethacin (10 mg/kg. p.o) and vehicle control (normal saline; 5 ml/kg b.w. p.o) were administered once daily for a period of 7 days. On the 8<sup>th</sup> day, rats were anesthetized and cotton pellets were removed carefully from the surrounding tissue and weighed immediately for the wet weight then dried at 60°C for 24 h. The dried pellets weighed again and increments in the dry weight of the pellets were taken as measure of granuloma formation. The antiproliferative effect of MEAS was compared with the control.

#### **Analgesic activity**

This study was evaluated by monitoring the animals exposed to chemical and thermal stimuli.

#### **Acetic acid-induced writhing response in mice**

The test was performed as described by Koster *et al.* (1959). Male Swiss albino mice (n = 10) weighing 20 - 25 g were used. A writhing response was produced for this study by intraperitoneal injection of an aqueous solution of 1.0% acetic acid in a volume of 0.1 ml/10 g body weight of animals and the response consisting of contraction abdomen wall, pelvic rotation followed by hind limb extension. Mice were pretreated with MEAS (50,100 and 200 mg/kg b.w. p.o), 60 min before administration of acetic acid. A group of mice were treated with

indomethacin (10 mg/kg b.w. p.o) used as a reference drug. Control animals received similar volume of normal saline solution (5 ml/kg b.w. p.o). Wriths was counted after 10min from administration of acetic acid and until the 10 min period.

### Hot plate testing in mice

The Eddy's hot plate apparatus was used to measure response latencies and the method described by Eddy and Leimbach (1953). The animals were grouped into five groups and each group contains 10 animals. Group I received normal saline (5 ml/kg b.w. p.o) (control). Group II, III and IV received the MEAS (50,100 and 200 mg/kg b.w. p.o) respectively. Group V received morphine (5 mg/kg b.w. s.c.) as a reference drug. The mice were screened by placing them in Eddy's hot plate platform, maintain at  $55 \pm 1^\circ\text{C}$  temperature and the reaction time noted in seconds at the time of shaking or licking of paws or jumping. After 30 min, the reaction time was noted.

### Tail flick test in mice

The test was performed as described by Alviano *et al.* (2004); Matheus *et al.* (2005); Bem-Bassat *et al.* (1959). In this study, pain was induced in animals

by immersing two-third of the tail in water bath at  $50 \pm 1^\circ\text{C}$ . The animals were individually placed in restrainer, with the tail hanging freely, and the latency of tail flick was measured. The basal reaction time was obtained by three measurements, and the mean is called pre-drug latency time (cut off time = 10 s). The animals were treated with MEAS (50,100 and 200 mg/kg b.w. p.o) and morphine (5 mg/kg s.c.) (Standard) and other sets of measurements were taken 30, 60, 120 and 180 min afterwards.

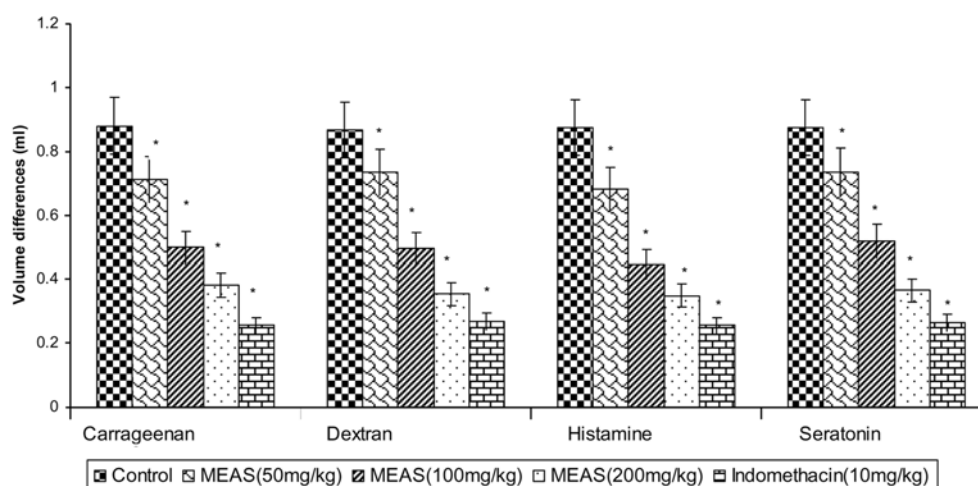
### Statistical analysis

The statistical significance of differences between the groups was assessed by means of variance followed by Dunnett's tests. Values are expressed as mean  $\pm$  S.E.M. and *P* values less than 0.05 were considered as significance.

## RESULTS

### Acute toxicity study

The methanol extract of *Artanema sesamoides* did not produced any mortality even at dose level of 2,000 mg/kg. Hence, the MEAS was considered as safe for administration up to 2,000 mg/kg.



**Fig. 1.** Effect of methanol extract of *Artanema sesamoides* root on carrageenan, dextran, histamine and serotonin induced paw oedema in rats. Each value is the mean  $\pm$  S.E.M. (n = 6) Statistical differences from the control were determined by ANOVA followed Dunnett's test. \**P* < 0.001.

**Table 1.** Effect of methanol extract of *Artanema sesamoides* root on cotton pellets-induced granuloma in rats

Group	Dose (mg/kg)	Weight of the cotton pellet (mg)(moist)	Percentage of inhibition	Weight of the cotton pellet (mg)(dried)	Percentage of inhibition
Control (0.9% NaCl)	5 ml/kg	225.40 ± 4.66	-	44.00 ± 1.52	-
MEAS	50	216.60 ± 2.96	3.9	40.00 ± 0.71*	9.0
	100	206.80 ± 2.42**	8.2	36.20 ± 0.80**	17.7
	200	202.20 ± 2.29**	10.2	33.00 ± 0.89**	25.0
Indomethacin	10	144.50 ± 1.39**	35.7	23.00 ± 1.10**	47.7

Each value is the mean ± S.E.M. (n = 6) Statistical differences from the control were determined by ANOVA followed Dunnett's test. \*\* $P < 0.001$ ; \* $P < 0.05$ .

### Anti-inflammatory studies

The anti-inflammatory activity of MEAS was evaluated by acute and chronic inflammatory model and results are shown in Fig. 1 and tabulated in Table 1, respectively. In acute paw oedema induced by carrageenan, dextran, histamine and serotonin, the anti-inflammatory activity of MEAS produced significant ( $P < 0.001$ ) as compare with control. MEAS at the doses of 50, 100, and 200 mg/kg showed an inhibition (19.00%, 43.00% and 56.50%), (15.43%, 42.85% and 59.21%), (21.96%, 48.97% and 59.95%), (15.78%, 40.73% and 58.12%) against acute paw oedema induced by carrageenan, dextran, histamine and serotonin respectively.

In chronic model (cotton pellet-induced granuloma), the antiinflammatory activity would be calculated by prevention of transudative components of chronic inflammation. MEAS (100 and 200 mg/kg) are significantly reducing wet cotton pellet as compare to control ( $P < 0.001$ ). According to the results the antiproliferative effects of MEAS (200 mg/kg b.w) and indomethacin were calculated as 10% and 35.7% ( $P < 0.001$ ) respectively. At low dose

(MEAS 50 mg/kg), it is not significant as compare to control. After dried the pellets, the antiproliferative effects of MEAS (200 mg/kg b.w) and indomethacin were calculated as 25% and 47.4% ( $P < 0.001$ ) respectively.

### Analgesic studies

#### Acetic acid-induced writhing response in mice

The effect of MEAS on writhing response in mice is summarized in Table 2. The methanol extract at 50, 100 and 200 mg/kg produced significant response ( $P < 0.001$ ) and the maximum inhibition of the nociceptive response (57.84%) achieved at 200 mg/kg. MEAS 50 and 100 mg/kg and indomethacin 10 mg/kg exhibited significant ( $P < 0.001$ ) inhibition of control writhes at the rate of 32.61%, 42.46% and 72.61% respectively.

#### Hot plate test in mice

The effect of MEAS on hot plate test is tabulated in Table 3. The methanol extract produced significant ( $P < 0.001$ ) analgesic activity at all doses. Morphine at a dose of 5 mg/kg significantly increases the

**Table 2.** Effect of methanol extract of *Artanema sesamoides* root in acetic acid induced writhing in mice

Group	Dose (mg/ kg)	Number of writhing	% inhibition
Control (0.9% NaCl)	5 ml/ kg	32.50 ± 0.43	-
MEAS	50	21.90 ± 0.41**	32.61
	100	18.70 ± 0.50**	42.46
	200	13.70 ± 0.45**	57.84
Indomethacin	10	8.90 ± 0.28**	72.61

Each value is the mean ± S.E.M. of results from 10 mice. Statistical differences from the control were determined by ANOVA followed Dunnett's test. \*\* $P < 0.001$ .

**Table 3.** Effect of methanol extract of *Artanema sesamoides* root against thermal pain induced in mice by hot plate method

Group	Dose (mg/kg)	Latency (s)
Control (0.9% NaCl)	5 ml/kg	9.10 ± 0.35
MEAS	50	12.20 ± 0.29**
	100	13.70 ± 0.26**
	200	15.70 ± 0.26**
Morphine	5	25.00 ± 0.33**

Each value is the mean ± S.E.M. of results from 10 mice. Statistical differences from the control were determined by ANOVA followed Dunnett's test. \*\* $P < 0.001$ .

pain latency.

#### Tail flick test in mice

The ability of MEAS to induce the central analgesia was evaluated by temperature based tests. As per the results (Table 4), the pretreated animals increased the basal reaction time. At high dose (200 mg/kg) of methanol extract and morphine (5 mg/kg) increase the percentage reaction time effectively.

### DISCUSSION

This study was to establish the scientific basis of one of the traditional use of *Artanema sesamoides* Benth against rheumatism. For that we have studied the effect of methanol extract of *Artanema sesamoides* root on different analgesic models (acetic acid-induced writhing, tail flick and hot plate method) and acute and chronic anti-inflammatory

models (acutely by carrageenan, dextran, and inflammatory mediators *viz.* histamine and serotonin induced paw oedema and chronically by cotton pellet induced granuloma). The results indicate that *Artanema sesamoides* possesses analgesic and anti-inflammatory activity.

There is no scientific information of this plant regarding therapeutic dose and LD<sub>50</sub>. From the acute toxicity studies the dose of the extract has been fixed. The LD<sub>50</sub> was determined to be higher than 2000 mg/kg body weight. In this study, neither deaths nor symptoms associated with toxicity such as convulsion, ataxy, diarrhoea or increased diuresis occurred during the 72 h observation period. These results indicate the effectiveness and relative safety of MEAS for the treatment of conditions associated with inflammation.

In acetic acid-induced writhing test, the MEAS significantly inhibited the abdominal constriction induced by acetic acid in mice, and exhibited peripheral analgesis activity. Acetic acid causes an increase in peritoneal fluid level of prostaglandins (PGE<sub>2</sub> and PGF<sub>2α</sub>) and pain inflammation induced by capillary permeability ((Deraedt, 1980; Amico-Roxas, 1984).

One possibility of the analgesic activity of MEAS could be that it blocks the stimulus propagation in the pain nervous fibre. Another possibility could be blockade of eicosanoid system, because it is well known that arachidonic acid liberates the several mediators, which stimulate the nociceptive pathway (*viz.* cytokinins, eicosanoids). Phospholipids are also

**Table 4.** Effect of methanol extract of *Artanema sesamoides* root against thermal pain induced in mice by Tail flick method

Group	Dose (mg/kg)	Increase in tail flick reaction time (%) in pre treated animals after different periods to heat stimulus <sup>a</sup>			
		30 min	60 min	120 min	180 min
MEAS	50	33	83	116.66	114.8
	100	65.56	111.65	165.64	179.75
	200	74.19	129.03	196.54	198.3
Morphine	5	87	232.8	251.6	276.5

<sup>a</sup>Results are from comparison between the animals that were treated with methanol extract of *Artanema sesamoides* or with morphine with control (untreated) animals.

breakdown into arachidonic acid by phospholipase A<sub>2</sub> and arachidonic acid goes to cyclooxygenase and lipoxygenase pathway. From this pathway it produces prostaglandins and leukotrienes (Martinez *et al.*, 1999). In this situation extracts could act inhibiting phospholipase A<sub>2</sub> or COX (COX1 and/or COX2).

By the tail flick and hot plate test, the central analgesic activities were evaluated. In the tail flick test, MEAS increases the baseline with dose dependent manner 50, 100, and 200 mg/kg. This result indicates that MEAS acts as spinal analgesia. The inhibitory effect could be due to prevention of C-fibres stimulation in the afferent nerves, which induced by the production of prostaglandin (Rossi *et al.*, 1993). The hot plate test has been commonly used to assess narcotic analgesia. The MEAS increases the base line of animals and leads us to think; the extract has supra-spinal activity.

In acute inflammatory model, histamine and serotonin are the important inflammatory mediators and act as vasodilator as well as increases the vascular permeability (Rand and Dale, 1999; Linari *et al.*, 2002). In this study, MEAS effectively inhibited the paw oedema induced by histamine and serotonin at the third hour of injection. From this, it may be suggested that the antiinflammatory activity of the extract could be due to inhibition of release action of inflammatory mediators *viz.* histamine, serotonin and kinins. Inflammation is a complex chronic process. In order to study its various test models has been developed, among them cotton pellet induced granuloma formation is a typical feature of chronic inflammatory reaction. This model has been employed to evaluate the transudative and proliferative components of chronic inflammation. In present investigation, MEAS (200 mg/kg) effectively inhibited the fluid adsorption in the pellet and greatly influences the wet weight of granuloma. The methanol extract at all doses and indomethacin effectively inhibited the granulomatous tissue formation in dried pellets. From a clinical point of view, Non steroidal anti inflammatory drugs have proven to be useful analgesic and anti

inflammatory drugs in the treatment of inflammation mediated joint damage such as rheumatoid arthritis, but also for other forms of joint damage in which inflammation is a secondary phenomenon, like in osteoarthritis. However the cause of rheumatism is autoimmune disease, the pathological progression carried by inflammatory mediators such as prostaglandins, leukotrienes, IL-1 and TNF- $\alpha$  and it leads to pain and inflammation. From the above mentioned analgesic and anti-inflammatory activity, it may be applicable to rheumatoid arthritis condition by inhibit the pathological progression of inflammation (Oddis CV, 1996).

Based on this present study it can be concluded that methanol extract of *Artanema sesamoides* root has potential antiinflammatory activity against acute and chronic phases of inflammation. The MEAS exhibit analgesic property, which is acting on central as well as peripheral analgesia.

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