

## Anti-inflammatory activity of *Syzygium cumini* leaf against experimentally induced acute and chronic inflammations in rodents

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### Abstract

*Syzygium cumini* (L.) Skeels (Myrtaceae), commonly known as *Jam* in Bengali, *Jamun* in Hindi and Black Plum or Black Berry in English, is a large size evergreen tree indigenous to India and is cultivated for its fruits. In the present study, the methanol extract of leaves from *S. cumini* (MESC) was evaluated for anti-inflammatory activity in experimental acute (carrageenan, histamine and serotonin induced rat paw oedema) and chronic models (cotton pellet induced rat granuloma). In all the models, the MESC (100 and 200 mg/kg body wt. p.o.) exhibited significant anti-inflammatory activity ( $P < 0.001$ ) in a dose dependent manner. These findings revealed that the *S. cumini* leaf had remarkable acute and chronic anti-inflammatory actions in the tested rodent models.

### Introduction

Traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their therapeutic principles. The rich wealth of plant kingdom can represent a novel source of new compounds with therapeutic activity. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

*Syzygium cumini* (L.) Skeels (Synonym: *Eugenia jambolana* Lam.), a member of the family Myrtaceae commonly known as *Jam* in Bengali, *Jamun* in Hindi and *Black Plum* or *Black Berry* in English, is a large size evergreen tree indigenous to India and is cultivated commercially for its consumable fruits. Besides India, it is also found in South-East Asian and Eastern African countries.<sup>1,2</sup> Traditionally the plant has been used in India for several important medicinal purposes. The

stem bark of the plant has been used as astringent, refrigerant, carminative, diuretic, digestive, antihelminthic, febrifuge, constipating, stomachic and antibacterial. The fruits and seeds are used in treatment of diabetes, pharyngitis, spleenopathy, urethrorrhea and ringworm infection. The leaves are antibacterial and used to strengthen the teeth and gums. The leaves have also been extensively used to treat diabetes, constipation, leucorrhoea, stomachalgia, fever, gastropathy, strangury, dermopathy and to inhibit blood discharge in the faeces.<sup>1-5</sup>

Several phytochemical and pharmacological investigations have been reported on this plant mainly on its stem bark, fruits and seeds. However, reports on the experimental biological studies on its leaf are scanty. The present work therefore, attempted to report the preliminary results of studies on anti-inflammatory effects of *S. cumini* leaf in experimentally induced inflammations in rodents to justify the traditional and folkloric beliefs.

### Materials and Methods

#### Plant material

The mature leaves of *Syzygium cumini* (L.) Skeels (Myrtaceae), were collected during November 2010 from Nadia, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The voucher specimen [CNH/I/(82)/2010/Tech.II/351] was maintained in our research laboratory for future reference. The plant material was shade-dried with occasional shifting and then powdered with mechanical grinder, passing through sieve no. 40, and stored in an air-tight container.

#### Extraction

The powdered plant material (400 g) was first defatted with petroleum ether and then macerated at room temperature (24-26°C) with methanol (850 mL) for 4 days with occasional shaking, followed by re-maceration with the same solvent for 3 more days. The macerates were combined, filtered and distilled off in reduced pressure. The resulting concentrate was vacuum dried at 40°C to yield the dry extract (MESC, yield: 10.45% w/w). The dry extract was kept in a vacuum desiccator until use. Preliminary phytochemical studies on MESC revealed the presence of alkaloids, triterpenoids, steroids and tannins.<sup>6</sup>

#### Drugs and chemicals

$\lambda$ -Carrageenan (type IV) was obtained from S. D. Fine Chemicals Ltd., Bombay; 5-hydroxytryptamine hydrochloride (serotonin), hista-

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mine sulphate, were from Sigma Chemical Co., USA; indomethacin was from Recon, Bangalore, India.

#### Experimental animals

Studies were carried out using adult male Wistar albino rats of weighing 150-180 g. The animals were grouped in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25±2°C, dark and light cycle 14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of experiment. All experimental methods were reviewed and approved by the Institutional Animal Ethical Committee.

#### Acute toxicity

The oral LD<sub>50</sub> value of MESC in male Swiss albino mice were determined as per reported method.<sup>7</sup>

#### Evaluation of anti-inflammatory activity

##### Carrageenan-induced rat paw oedema

The rats were divided into four groups ( $n=6$ ). The first group (which served as control) received normal saline (0.9 % w/v, 3 mL/kg body wt., p.o.). The second and third group received the test extract MESC (100 and 200 mg/kg body wt., p.o., respectively). The fourth group (which served as reference)

received indomethacin (10 mg/kg body wt., p.o.). After 30 mins, acute inflammation was produced by the sub-plantar administration of 0.1 mL of 1% (w/v) of freshly prepared suspension of carrageenan in the right hind paw of each rat. The paw volume was measured at 0 h and 4 h after carrageenan injection by using plethysmometer (Ugo Basile, Italy). The difference between the two readings was taken as the volume of oedema and the percentage of inhibition was calculated by using the following formula:<sup>8,9</sup>  $(\text{Control} - \text{Treated}/\text{Control}) \times 100\%$ .

#### Mediator-induced inflammation

The paw oedema was induced in rats by sub-plantar injection of 0.1 mL of freshly prepared histamine (1 mg/mL) and serotonin (1 mg/mL) solutions respectively.<sup>10,11</sup> Group division and treatment regime of the animals were same as the carrageenan induced rat paw oedema model and the paw oedema was measured and calculated as described above.

#### Cotton pellet-induced granuloma

The animals were divided into four groups ( $n=6$ ). The rats were anaesthetized and sterile cotton pellets weighing  $10 \pm 1$  mg were implanted subcutaneously into both sides of the groin region of each rat. The first group (which served as control) received normal saline (0.9% w/v, 3 mL/kg body wt., p.o.). The second and third group received the test extract MESC (100 and 200 mg/kg body wt., p.o., respectively). The fourth group (which served as reference) received indomethacin (10 mg/kg body wt., p.o.). All groups were treated in this way for seven consecutive days from the day of cotton pellet implantation.<sup>12</sup> On 8<sup>th</sup> day the animals were anaesthetized and the pellets together with the granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were then dried in an oven at 60°C for 24 h to constant weight. Increment in the dry weight of the pellets was taken as a measure of granuloma formation.<sup>13</sup> Percentage inhibition was calculated as per the formula stated above.

#### Statistical analysis

The values were expressed as mean  $\pm$  standard error of mean (SEM). Statistical significance was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test of significance. Values of  $P < 0.001$  were considered as statistically significant.

## Results

The MESC was found to be safe up to the dose of 3500 mg/kg body wt. in Swiss mice when administered orally.

Anti-inflammatory activity of *S. cumini* leaf extract (MESC) was evaluated against carrageenan induced acute paw oedema in rats and the results are summarized in Table 1. The MESC produced significant ( $P < 0.001$ ) anti-inflammatory activity in a dose dependent manner. The MESC showed maximum inhibition of 56.37% at the dose of 200 mg/kg body wt. after 4 h of treatment, whereas the reference drug indomethacin produced 72.06% of inhibition.

In case of histamine and serotonin induced rat paw oedema, MESC exhibited significant ( $P < 0.001$ ) and dose dependent protection from oedema (Tables 2 and 3). The MESC produced 51.01% inhibition in case of histamine and 58.75% of inhibition in case of serotonin at the dose of 200 mg/kg body wt.; while the reference drug, indomethacin produced 63.00 and 71.08 % of inhibition of rat paw oedema respectively in cases of above two mediators.

In the chronic inflammatory model (cotton

pellet induced granuloma), the MESC significantly ( $P < 0.001$ ) and dose dependently reduced the weight of cotton pellets as compared to the vehicle control (Table 4). The MESC produced the maximum inhibition of 55.40% at the dose of 200 mg/kg body wt. and the reference drug indomethacin produced 63.11% of inhibition of granuloma formation in rats.

## Discussion

The present study establishes the significant anti-inflammatory activity of the methanol extract from the leaf of *S. cumini* (MESC) in both experimentally induced acute and chronic inflammations in rodents. Carrageenan-induced oedema has been commonly used as an experimental animal model for acute inflammation and it is believed to be

**Table 1. Effect of MESC on carrageenan induced rat paw oedema.**

Treatment	Dose (mg/kg)	Increase in paw volume (mL)	Inhibition (%)
Control	-	0.784 $\pm$ 0.13	-
MESC	100	0.493 $\pm$ 0.08*	37.11
MESC	200	0.342 $\pm$ 0.04*	56.37
Indomethacin	10	0.219 $\pm$ 0.02*	72.06

Values are mean  $\pm$  SEM ( $n=6$ ). \* $P < 0.001$ , compared to control group.

**Table 2. Effect of MESC on histamine induced rat paw oedema.**

Treatment	Dose (mg/kg)	Increase in paw volume (mL)	Inhibition (%)
Control	-	0.592 $\pm$ 0.024	-
MESC	100	0.351 $\pm$ 0.008*	40.70
MESC	200	0.290 $\pm$ 0.015*	51.01
Indomethacin	10	0.219 $\pm$ 0.006*	63.00

Values are mean  $\pm$  SEM ( $n=6$ ). \* $P < 0.001$ , compared to control group.

**Table 3. Effect of MESC on serotonin induced rat paw oedema.**

Treatment	Dose (mg/kg)	Increase in paw volume (mL)	Inhibition (%)
Control	-	0.657 $\pm$ 0.007	-
MESC	100	0.399 $\pm$ 0.011*	39.26
MESC	200	0.271 $\pm$ 0.005*	58.75
Indomethacin	10	0.190 $\pm$ 0.003*	71.08

Values are mean  $\pm$  SEM ( $n=6$ ). \* $P < 0.001$ , compared to control group.

**Table 4. Effect of MESC on cotton pouch induced granuloma in rats.**

Treatment	Dose (mg/kg)	Increased wt of cotton pellet (mg) $\pm$ SEM	Inhibition (%)
Control	-	38.23 $\pm$ 0.18	-
MESC	100	24.72 $\pm$ 0.35*	35.33
MESC	200	17.05 $\pm$ 0.27*	55.40
Indomethacin	10	14.10 $\pm$ 0.38*	63.11

Values are mean  $\pm$  SEM ( $n=6$ ). \* $P < 0.001$ , compared to control group.

a biphasic response. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine and serotonin (5-HT). The late phase (2-4 h) is mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages.<sup>14</sup> The MESC produced dose dependent and significant ( $P < 0.001$ ) inhibition of carrageenan-induced paw oedema after a period of 4 h.

The MESC also significantly ( $P < 0.001$ ) suppressed the inflammation produced by the putative inflammatory mediators viz. histamine and serotonin in a dose related manner. It indicates that the MESC inhibited the inflammation caused by carrageenan and mediators. The cotton pellet method is widely used to evaluate the exudative and proliferative components of the chronic inflammation.<sup>15</sup> Chronic inflammation is a reaction arising when the acute response is insufficient to eliminate the pro-inflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and the infiltration of neutrophils and exudation. Chronic inflammation occurs by the development of proliferative cells. These cells can either spread or remain in granuloma form. The dry weight of the cotton pellets correlates with the amount of the granulomatous tissue formed.<sup>16</sup> The MESC showed significant ( $P < 0.001$ ) and dose dependent anti-inflammatory action in cotton pellet induced granuloma and hence found to be effective in chronic inflammatory conditions. Based on the present results it can be concluded that the MESC demonstrated anti-inflammatory potential in both acute and chronic phases of inflammation. The gotten dose-dependent effect is relative and it would be necessary to use several doses to confirm it and to get the real dose having the maximum effect.

It is pertinent to mention here that previous workers reported anti-inflammatory and anti-allergic activities of *S. cumini* stem bark.<sup>17,18</sup>

The results of the present study confirmed acute and chronic anti-inflammatory effects of its leaves, thereby indicating that likewise bark, the leaves are also effective in counter-acting inflammatory conditions.

The present preliminary investigation confirms that *S. cumini* leaf possessed remarkable acute and chronic anti-inflammatory properties in the experimented rodent models. The outcome of present study can substantiate its traditional usage in Indian subcontinent. Further studies are presently underway to confirm the molecular mechanisms and to identify the bioactive principles responsible for these actions.

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