Evaluation of anti-inflammatory potential of *Jussiaea suffruticosa* Linn. extract in albino rat

T. Murugesan M. Pal, Saha B. P. *

Department of Pharmaceutical Technology, Jadavpur University Calcutta 700 032 (India)
Email: drbpsaha@yahoo.com; goldmurugesan@yahoo.com

*Corresponding author

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Introduction

Inflammation is commonly divided into three phases; acute inflammation, the immune response and chronic inflammation. One of the most important condition is rheumatoid arthritis, in which chronic inflammation results in pain and destruction of bone and cartilage that can lead to severe disability and in which systemic changes occur that can result in shortening of life (Katzung, 1998).

In a review of plants exhibiting anti-inflammatory activity, (Handa *et al.*, 1992) cited that species of 96 genera belonging to 5 families are ascribed such activity. In addition to the wide range of plants involved there is a similar diversity in the chemical nature of the active constituents. Flavonoids are one important group of chemical constituents that are associated with anti-inflammatory activity (Alcaraz and Jimenez, 1998). *Jussiaea suffruticosa* Linn. (Onagraceae) is a well-known plant used in traditional medicine of India. The whole plant is reduced to pulp steeped in buttermilk and used in dysentery, fever, rheumatoid arthritis and diarrhea (Anonymous, 1986; Anonymous, 1966; Kiritikar, 1935; Nadkarni *et al.*, 1992). The present study was emphasized on the evaluation of anti-inflammatory potential of methanol extract of *Jussiaea suffruticosa* Linn. (MEJS) on several experimental animal models and reported hereunder.

Materials and methods

Plant material

The whole plants of *Jussiaea suffruticosa* Linn. (Onagraceae) were collected from Thanjavur district, Tamilnadu, India and the taxonomical identification was established by the Botanical survey of India, Shibpur, Howrah. The voucher specimen (JS-01) has been deposited in our research laboratory for future references.

Extraction procedure

The whole plants of *Jussiaea suffruticosa* were dried under shade, pulverized, passed through 40-mesh sieve and extracted with 90 % methanol in a soxhlet apparatus. The solvent was completely removed by vacuum distillation. A brown coloured dry mass was obtained (yield 12.5%w/w with respect to the powdered material) and stored in a refrigerator. The extract was examined for its chemical nature by preliminary phytochemical analysis. The extract showed positive answer for the presence of flavonoids, steroids and tannins and they were confirmed by High Performance Thin Layer Chromatography (HPTLC). A weighed amount of the extract (MEJS) was dissolved in propylene glycol for the present experiment.

Animal used

Wistar albino rats of either sex weighing between 180-200 gm were housed in standard metal cages provided with food and water ad libitum. The animals were fasted for 24 hours prior to the experiment.

Carrageenin induced rat paw oedema

Oedema was induced by subplanter injection of 0.1 ml of 1 % freshly prepared suspension of carrageenin (Sigma Chemical Co. USA) into the right hind paws of the rats of five groups (6 in each group). The volume of the injected paws and contra-lateral paws were measured at 1, 2, 3, 4 and 5 hours intervals using

Plethysmometer as per the method stated by Winter *et al.*, (1962). The MEJS was administered to three groups of animal at the dose levels of 100, 200 and 300 mg/kg, i.p. and remaining fourth and fifth group of animals received propylene glycol (Control 10 ml/kg) and Indomethacin 10 mg/kg (Standard) respectively for assessing comparative pharmacological significance.

Serotonin induced rat paw oedema

The paw oedema was induced in the right foot by subplanter injection of 0.05 ml of 1 % freshly prepared solution of serotonin (Maity *et al.*, 1998). Hind paw volumes were measured 30 minutes before and after serotonin injection and the animals were treated with MEJS, control and standard and the paw volumes were measured in the same manner of previous model.
The oedema volume and inhibition rates were calculated as follows (Lin *et al.*, 1994) :

$$\text{a. Oedema volume (E) \%} = \frac{Vr}{Vc} \times 100$$

Vr = Right hind paw volume
Vc = Contra-lateral paw volume

$$\text{b. Inhibition rate (I) \%} = \frac{Ec - Et}{Ec} \times 100$$

Ec = Paw volume of control
Et = Paw volume of treated group

Cotton pouch-induced granuloma

The rats were divided into five groups (6 in each group), anaesthetized and 10mg of sterile cotton pellets were inserted in each axilla of rats. MEJS at three different doses (100, 200 and 300 mg/kg,), Indomethacin 10 mg/kg (Standard) and propylene glycol 10 ml/kg (Control) were administered intraperitoneal route to the respective group of animals for seven consecutive days from the day of cotton pellet implantation. The animals were anaesthetized again on day 8 and cotton pellets were removed surgically, free from extraneous tissue; incubated at 370 C for 24 hours and dried at 600 C to constant weight. The increment in the dry weight of the pellets was taken as measure of granuloma formation (Winter and Porter, 1957).

Statistical analysis

The results were expressed as mean ± SEM and the significance was evaluated by Student's t-test versus control. $p<0.01$ implies significance (Woodson, 1987).

Results and discussion

The effects produced by the MEJS against various inflammation models have been furnished in Table 1, 2 and 3. The MEJS (100, 200 and 300 mg/kg) exhibited significant ($p < 0.01$) anti-inflammatory activity in the entire examined animal models such as carrageenin, serotonin induced paw oedema and cotton pouch granuloma.

The MEJS at 300 mg/kg demonstrated the maximum activity of 45.64 % inhibition in carrageenin induced paw oedema volume, while the standard drug (Indomethacin 10 mg/kg) exhibited 40.70 % inhibition after 3 hours of drug treatment. The MEJS at 300 mg/kg dose produced potential inhibition on serotonin induced paw oedema volume (46.75 %) whereas standard drug produced 40.97 % of inhibition. In chronic inflammation model (cotton pouch granuloma), the MEJS at 300 mg/kg produced maximum of 49.77 % inhibition in granuloma weight, while standard drug showed 47.77 % reduction in granuloma weight.

The present study establishes the anti-inflammatory potential of methanol extract of *Jussiaea suffruticosa*. Carrageenin induced paw oedema is commonly used as an experimental model for evaluating anti-inflammatory activity of natural products (Della Loggia *et al.*, 1986; Winter *et al.*, 1962; Alcaraz and Jimenez, 1988) and is believed to be biphasic. The first phase is due to release of histamine and serotonin; second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome (Castro *et al.*, 1968). It has been reported that the second phase of oedema is sensitive to most clinically effective anti-inflammatory agents (Smucker *et al.*, 1967). The effect of MEJS and the inflammation process induced by serotonin suggested that, they act by affecting a time-delayed system in a similar fashion of glucocorticoids.

From this investigation it is found that the MEJS exhibited potential anti-inflammatory activity in the examined dose levels and the potential may be due to the presence of flavonoid alone or may be the combined effect with steroids. The further establishment of the mechanism of action and isolation of bioactive molecules of the plant extract is under process in our research laboratory.

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Table I. Effect of MEJS on carageenin induced paw oedema in rats

Treatment and Dose	Oedema rate percentage (Mean ± SEM)				
	1h	2h	3h	4h	5h
Control 10ml/kg	28.2 ± 1.2	35.5 ± 1.1	43.2 ± 1.1	45.2 ± 1.2	49.5 ± 1.1
MEJS 100mg/kg	25.1 ± 1.1 (10.99)	30.5 ± 1.2* (14.08)	36.2 ± 1.2** (16.20)	37.1 ± 1.2** (17.92)	40.0 ± 1.2** (19.19)
MEJS 200mg/kg	24.2 ± 1.2 (14.18)	25.3 ± 1.1** (28.73)	29.0 ± 1.2** (32.87)	32.6 ± 1.1** (28.87)	36.0 ± 1.1** (27.07)
MEJS 300mg/kg	22.5 ± 1.1* (21.45)	21.5 ± 1.2** (39.43)	23.0 ± 1.2** (46.75)	25.5 ± 1.1** (43.58)	30.2 ± 1.1** (38.98)
Standard 10mg/kg	22.8 ± 1.2* (19.14)	22.4 ± 1.1** (39.90)	25.5 ± 1.1** (40.97)	27.1 ± 1.4** (40.04)	31.5 ± 1.2** (36.36)

Figure in the parenthesis indicates % of inhibition (n=6)
** p <0.001; * p <0.01 ; MEJS = Methanol extract of *Jussiaea suffruticosa* Linn.
Control = Propylene glycol. ; Standard = Indomethacin

Table II. Effect of MEJS on serotonin induced paw oedema

Treatment and Dose	Oedema rate percentage (Mean ± SEM)				
	1h	2h	3h	4h	5h
Control 10ml/kg	28.2 ± 1.2	35.5 ± 1.1	43.2 ± 1.1	45.2 ± 1.2	49.5 ± 1.1
MEJS 100mg/kg	25.1 ± 1.1 (10.99)	30.5 ± 1.2* (14.08)	36.2 ± 1.2** (16.20)	37.1 ± 1.2** (17.92)	40.0 ± 1.2** (19.19)
MEJS 200mg/kg	24.2 ± 1.2 (14.18)	25.3 ± 1.1** (28.73)	29.0 ± 1.2** (32.87)	32.6 ± 1.1** (28.87)	36.0 ± 1.1** (27.07)
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Standard 10mg/kg	22.8 ± 1.2* (19.14)	22.4 ± 1.1** (39.90)	25.5 ± 1.1** (40.97)	27.1 ± 1.4** (40.04)	31.5 ± 1.2** (36.36)

Figure in the parenthesis indicates % of inhibition (n=6)
** p <0.001, * p <0.01 ; MEJS = Methanol extract of *Jussiaea suffruticosa* Linn.
Control = Propylene glycol. ; Standard = Indomethacin

Table III. Effect of MEJS on weight of granuloma pouch in rats

Treatment and dose (Mean ± SEM)	Weight of granuloma pouch	% Inhibition
Control 10 ml/kg	45.0 ± 1.2	-
MEJS 100 mg/kg	34.2 ± 1.4*	24.00
MEJS 200 mg/kg	25.1 ± 1.1**	44.22
MEJS 300 mg/kg	22.6 ± 1.4**	49.77
Standard 10 mg/kg	23.5 ± 1.5**	47.77

** p <0.001, * p <0.01 (n=6)
MEJS = Methanol extract of *Jussiaea suffruticosa* Linn.
Control = Propylene glycol. ; Standard = Indomethacin