Anti-inflammatory and antinociceptive activity of
Justicia gendarussa Burm. f. leaves

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The ethanolic extract of *Justicia gendarussa* Burm.f. leaves was screened for its anti-inflammatory and antinociceptive activity in experimental animals. The extract showed significant inhibition of carrageenan and formalin-induced paw oedema and cotton pellet-induced granuloma formation compared to the standard anti-inflammatory drug, Indomethacin. The extract at 125, 250 and 500 mg/kg showed significant inhibition of pain in the acetic acid-induced writhing and hot plate models in mice when compared to the standard analgesic drug, acetyl salicylic acid.

Keywords: *Justicia gendarussa*, Anti-inflammatory, Analgesic, Indomethacin, Acetyl salicylic acid.

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Introduction

Plants are being used as a source of drug since time immemorial. The Indian systems of medicine such as Siddha, Unani and Ayurveda are all based on the knowledge of drugs from plant sources. Inflammation is the response of living tissues to injury and it involves a complex array of enzyme activation, mediator release and extravasations of fluid, cell breakdown and repair[1]. The non-steroidal anti-inflammatory drugs (NSAIDs) and opiates currently available in the market are not useful in all cases because of their unwanted side effects[2]. Hence, the search for other alternatives seems necessary and beneficial.

Justicia gendarussa Burm.f. (Family-Acanthaceae) is an evergreen scented shrub, up to 1.5 m in height, found throughout the greater part of India (Plate 1). The plant is considered as emetic, emmanogogue, febrifuge, diaphoretic, and leaves have traditionally been used in the treatment of arthritis, jaundice, cephalgia, hemiplegia, eczema, etc.[3-4]. The chemical constituents of the leaves include O-disubstituted aromatic amines, 2-aminobenzyl alcohol, 2-(2'aminobenzyl) amino benzyl alcohol and their respective O-methyl ethers, friedelin, lupeol and β-sitosterol[5]. The present study was undertaken to investigate the anti-inflammatory and analgesic activities of the leaves of *J. gendarussa.*

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Materials and Methods

Plant material

J. gendarussa was collected from Kannur, Kerala. It was identified and authenticated by Dr Mathew

Plate 1—*Justicia gendarussa* in flower

Dan, plant taxonomist of the Institute. A voucher specimen of the plant was deposited at the herbarium of the Institute (TBGT 57030 dated 22/02/2008).

Preparation of plant extract

The leaves were separated from the plant, washed, shade dried and powdered. The powder (100 g) was extracted with 1000 ml 95% ethanol by cold maceration. The filtrate was then concentrated and the solvent was evaporated under reduced pressure in a rotary evaporator. The crude extract was referred to as JG. For oral administration the drug was reconstituted in 0.5% Tween-80 to concentrations of 125 mg/kg, 250 mg/kg and 500 mg/kg.

Animals

Wistar albino rats (150-250 g) and Swiss albino mice (20-30 g), of either sex, obtained from the Institute's animal house were used for the studies. They were housed under standard laboratory conditions and were fed with commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water, *ad libitum*. All animal experiments were carried out according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee (Registration No. 25-1/99/ AWD 176/ CPCSEA dated 29/09/1999).

Anti-inflammatory Activity

Carrageenan-induced rat paw oedema

Oedema was induced in rats according to the method of Winter *et al* (1962)[6]. The animals were divided into five groups of six animals each and fasted overnight. Group 1, the control group was administered 1 ml 0.5% Tween-80, Group 2 received 1ml Indomethacin (10 mg/kg, standard) dissolved in normal saline, while Groups 3, 4 and 5 received JG at various concentrations (125, 250 and 500 mg/kg) prepared in 0.5% Tween-80. After 30 min of JG administration, 0.1 ml, 1% carrageenan (Sigma Chemicals Company, USA) dissolved in normal saline was injected into the right hind paw, under the plantar aponeurosis of all the animals. The hind paw volume was measured just before and 3 h after carrageenan injection using a plethysmometer. The difference in the paw volumes indicated the degree of inflammation. The anti-inflammatory activity of the JG was estimated as the degree of oedema inhibition.

Formalin-induced paw oedema

Oedema was induced in rats according to the method of Winter *et al* (1962)[6]. Overnight fasted rats were divided into five groups of six animals each. Group 1 received 0.5% Tween-80 (control), Group 2 received indomethacin (10 mg/kg, standard), while Groups 3, 4 and 5 received JG extract at various concentrations (125, 250 and 500 mg/kg), respectively. Formalin (20 µl of 1%) was injected into the right hind paw of all animals, under the plantar aponeurosis, 30 min after drug administration[7]. The hind paw volume was measured using a plethysmometer just before and 3 h after formalin injection. The difference in the paw volumes indicated the degree of inflammation. The anti-inflammatory activity of the JG was estimated as the degree of oedema inhibition.

Cotton pellet induced granuloma

The method as described by Ismail *et al* (1997)[8] was used with modifications. Sterile cotton pellets (20±1.5 mg) were implanted subcutaneously on the back of rats. The animals received 125, 250 and 500 mg/kg of JG, Indomethacin (10 mg/kg) and 0.5% Tween-80 orally, once a day for seven consecutive days. On the 8th day, the rats were sacrificed and the cotton pellet excised, weighed and dried overnight at 60°C. The dry weight was also estimated[9].

Analgesic activity

Acetic acid-induced writhing assay

Analgesic responses were assessed by the method of Koster *et al* (1959)[10]. Swiss albino mice were divided into 5 groups of six animals each. Control group (Group 1) received a single dose of 0.5% Tween-80 (0.5 ml) orally and the standard group (Group 2) a single dose of 0.5 ml acetyl salicylic acid (25 mg/kg) in distilled water orally. Groups 3, 4 and 5 received a single dose of 0.5 ml of JG at varying concentrations (125, 250 and 500 mg/kg). After 20 min, 0.5% acetic acid (0.25 ml) prepared in distilled water was administered intra-peritoneally to all the groups. The number of writhes per animal was counted for 30 min, starting 5 min after treatment with acetic acid.

Hot plate test in mice

Analgesia was assessed by the modified method of Turner (1965)[11] using Eddy's hot plate (thermal stimulus), at temperature 55 ± 1°C. Mice were divided into 5 groups of 6 animals each. Group 1, the control group received 0.5 ml of 0.5% Tween-80, p.o., Group 2, the standard group received 0.5 ml of Indomethacin (100 mg/kg) dissolved in distilled water. Groups 3, 4

and 5 received 0.5 ml of 125, 250, and 500 mg /kg JG, p.o. Mice were placed on hot plate maintained at 55 ± 1ºC for 0, 15, 30, 60, 90, 120 and 180 min after drug administration. The time taken by animals to lick the fore or hind paw or jump off the plate was taken as the reaction time (sec); 15 sec cut off was used to prevent tissue damage.

Anti-lipid peroxidation studies

Anti-lipid peroxidation effect of JG was studied *in vitro* following the modified method of Yoshiuki *et al* (1981)[12] and Masao *et al* (1993)[13]. Briefly, 2 g of rat liver tissue was sliced and homogenized with 150mM KCl-Tris HCl buffer (*p*H 7.2). The reaction mixture was composed of 0.25 ml liver homogenate, Tris-HCl buffer (*p*H 7.2), 0.1 mM ascorbic acid (A.A), 0 4 mM $FeCl_2$ and 0.05 ml of various concentrations of JG extract. The mixture was incubated at 37ºC for 1 h in capped tubes. Then, 0.05 ml of 0.1N HCl, 0.2 ml of 9.8% sodium dodecyl sulphate (SDS), 0.9 ml distilled water and 2 ml of 0.6% thiobarbituric acid (TBA) were added to each tube and the tubes were vigorously shaken. The tubes were placed in a boiling water bath at 100ºC for 30 min. After cooling, 5 ml of butanol was added and centrifuged at 1500 rpm for 20 min. The supernatant was collected and the absorbance was measured at 532 nm.

Behavioural and toxicological effects

Six groups of ten mice each were treated with graded doses of the JG (100, 500, 1000, 2500, and 5000 mg/kg, p.o.). One group was maintained as control and was given 0.5% Tween-80. They were observed continuously for 1 h for any gross behavioural changes and death, if any, and then, intermittently for the next 6 h, and then again at 24 h after dosing with JG.

Statistical analysis

The analysis was carried out using the Students't'-test (Snedecor and Cochran., 1980)[14]. Results were reported as mean+S.D and the 't' test was used to evaluate difference between groups with $P \leq 0.01$, considered as significant.

Results

Anti-inflammatory activity

Carrageenan–induced paw oedema

The three doses used in the study (125, 250 and 500 mg/kg) significantly inhibited carrageenan-induced paw oedema in rats. At 125 and 250 mg/kg doses, there was 56.92% and 61.53% inhibition and at

500 mg/kg dose, 75.38% inhibition was obtained, at 3 h after carrageenan injection. Indomethacin (10 mg/kg) produced 84.61% inhibition of oedema formation (Table 1).

Formalin-induced paw oedema

JG at the three doses used in the study (125, 250 and 500 mg/kg) significantly inhibited formalin-induced paw oedema in rats. At 125 and 250 mg/kg doses, there was 55.55% and 70.4% inhibition and at 500 mg/kg dose, 74.22% inhibition was obtained, at 3 h after formalin injection. Indomethacin (10 mg/kg) produced 86.66% inhibition of oedema formation (Table 2).

Cotton-pellet induced granuloma

JG at the three doses used in the study inhibited formation of granulamatous tissue. The amount of exudates released by the cells in response to the cotton-pellet was found to decrease with increase in JG concentration (Table 3).

Analgesic activity

Acetic acid induced writhing assay

JG at all the three doses used in the study significantly inhibited acetic acid-induced writhing

Table 1—Effect of *Justicia gendarussa* (JG) on carrageenan(CG)-induced paw oedema in rats

Treatment	Oral dose (mg/kg)	Difference in paw volume at 3 h(ml)	Percentage inhibition of oedema
CG control (0.5% Tween-80)	–	0.65 ± 0.03	–
CG + Indomethacin	10	0.10 ± 0.01**	84.61
CG + JG	125	0.28± 0.10**	56.92
CG + JG	250	0.25 ± 0.08**	61.53
CG + JG	500	0.16 ± 0.05**	75.38

Values are the mean ± SD, n=6; **$P \leq 0.01$ compared to CG control

Table 2—Effect of *Justicia gendarussa* (JG) on formalin(FN)-induced paw oedema in rats

Treatment	Oral dose (mg/kg)	Difference in paw volume at 3 h(ml)	Percentage inhibition of oedema
FN control (0.5% Tween-80)	–	0.45 ± 0.06	–
FN +Indomethacin	10	0.06 ± 0.01**	86.66
FN + JG	125	0.20± 0.06**	55.55
FN +JG	250	0.13 ± 0.05**	70.44
FN +JG	500	0.11 ± 0.03**	74.22

Values are the mean ± SD, n=6. **$P \leq 0.01$ compared to FN control

response in mice, which was dose dependent. Writhing response inhibition at the doses 125 and 250 mg/kg were 66.66% and 77.55%, respectively. The extent of writhing response inhibition at 500 mg/kg dose was 88.78%. Acetyl salicylic acid, the positive control used in the study, which is a known analgesic agent produced an inhibition of 92.75% (Table 4).

Hot plate test in mice

The JG produced a highly significant (*P*<0.001) increase in the latency response of mice to hot plate thermal stimulation. This effect started at 15 min and persisted for at least 120 min after administration of the plant extract (Table 4). Only a mild effect was seen with 125 mg/kg dose while significant antinociceptive effects were obtained with 250 and 500 mg/kg doses (Table 5).

In vitro **anti-lipid peroxidation effects**

JG at 50 µg/ml showed the highest percentage of inhibition of $FeCl_2$-AA stimulated rat liver lipid peroxidation *in vitro*. There was significant increase in malondialdehyde (MDA) in $FeCl_2$-AA treated rat liver homogenate, compared to the normal one without $FeCl_2$-AA (Table 6). JG was found to be effective in decreasing MDA production *in vitro* in rat liver homogenate treated with $FeCl_2$-AA treated mixture showing its anti-lipid peroxidant effects. There was decreased inhibition of MDA with increased concentrations of JG beyond 100 µg/ml.

Behavioural and toxicological effects

In the behavioural and toxicity study, the mice did not show any gross behavioural changes and no mortality occurred within 24 h with all the doses of JG tested. The LD_{50} was therefore, greater than 5000 mg/kg p.o, in mice (data not shown).

Discussion

The anti-inflammatory activity of JG was evaluated by using three screening protocols which are widely used for testing non-steroidal anti-inflammatory drugs namely carrageenan-induced paw oedema, formalin-induced paw oedema and cotton pellet induced granuloma formation[15],.

Carrageenan induced paw oedema is a standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility[16]. The first phase is mediated by the release of histamine and serotonin, followed by kinin release and then prostaglandins from the tissue arachidonic acid in the later stage[17]. JG at the three doses studied significantly reduced carrageenan-induced paw oedema in rats. The inhibitory effect of

Table 3—Effect of *Justicia gendarussa* (JG) on cotton pellet induced granuloma

Treatment	Oral dose (mg/kg)	Wet weight/100g bwt	Dry weight/100g bwt
Cotton pellet control	–	0.124 ± 0.01	0.023 ± 0.01
Indomethacin	10	0.083 ± 0.04	0.014 ± 0.01**
JG	125	0.118 ± 0.04	0.021 ± 0.01
JG	250	0.116 ± 0.03	0.020 ± 0.01 **
JG	500	0.102 ± 0.08	0.017 ± 0.01**

Values are the mean ± SD, n=6; **$P \le 0.01$ compared to cotton pellet control

Table 4—Effect of *Justicia gendarussa* (JG) on acetic acid (AA)-induced writhing response in mice

Treatment	Oral dose (mg/kg)	Mean number of writhes in 30 min	Percent inhibition of writhes
AA control (0.5%Tween -80)	–	73.5 ± 9.6	–
AA+ Acetyl salicylic acid	25	5.33 ± 3.3**	92.75
AA+ JG	125	24.50 ± 0.50**	66.66
AA+ JG	250	16.50 ± 1.8**	77.55
AA+ JG	500	8.25 ± 5.3**	88.78

Values are the mean ± SD, n=6; **$P \le 0.01$ compared to AA control

Table 5—Effect of Justicia gendarussa (JG) on hot plate test in mice

Treatment	Oral Dose (mg/kg)	Reaction time(sec)					
		0 min	15 min	30 min	60 min	120 min	180 min
Control (0.5%Tween-80)	–	2.25±0.20	5.20±0.32	5.05±1.36	3.99±0.82	3.42±0.34	1.79±0.52
Indomethacin	100	2.51±1.87	3.68±0.62	6.26±3.06**	4.12±3.39	3.00±1.66	1.95±0.52
JG	125	3.12±0.97	8.27±0.48**	9.82±2.28**	4.54±1.29	3.43±2.90	1.95±0.25
JG	250	2.74±0.56	3.75±0.73	6.68±0.83	7.65±1.86**	9.33±2.90**	4.61±3.7**
JG	500	3.63±0.24	5.55±0.46	7.86±2.03	8.13±1.46**	6.31±1.77	3.82±0.87

Values are the mean ± SD; n=6, **$P \le 0.01$ compared to control

Table 6—Inhibitory effect of *Justicia gendarussa* (JG) on FeCl$_2$-ascorbic acid (AA)-induced lipid peroxidation in rat liver homogenate *in vitro*

Group	Concentration (µg/ml)	MDA	MDA inhibition (%)
Normal control (without FeCl$_2$-AA)	–	0.782 ± 0.01	-
FeCl$_2$-AA control	-	2.420 ± 0.09	-
FeCl$_2$-AA + JG	50	0.632 ± 0.03**	73.88
FeCl$_2$-AA + JG	100	0.652 ± 0.05**	73.05
FeCl$_2$-AA + JG	150	0.692 ± 0.07	71.04

Values are the mean ± SD, n=3; **$P \leq 0.01$ compared to FeCl$_2$-AA control

JG may be due to the inhibition of the above mentioned mediators of inflammation. The percentage inhibition of oedema produced by 500 mg/kg concentration of JG was found to be significantly higher than that produced by the other two concentrations (125 and 250 mg/kg).

JG significantly inhibited the formation of paw oedema induced by formalin. The mechanism of anti-inflammatory effect of JG in formaldehyde induced oedema in rats could depend on the neutralization of active globulins which are non-steroidal anti-inflammatories as reported by Suleyman *et al* with *Rumex patentia* in a similar study[18]. It is well established that the non-steroidal anti-inflammatory drugs exert their effect by the inhibition of prostaglandin synthesis[19].

The cotton pellet granuloma method is widely employed to evaluate the transudative, exudative and proliferative components of chronic inflammation. The inflammatory granuloma is a typical feature of established chronic inflammatory reaction. Multiplications of small blood vessels as well as proliferation of fibroblasts are the characteristic features of the repair phase of inflammation. In the present study; JG efficiently reduced the cotton pellet-induced granuloma, suggesting its capacity to significantly reduce granular tissue formation during the proliferative phase of inflammation after pellet implantation[20,21]. Decrease in granulomatous tissue is an indicator of the anti-proliferative effect of non-steroidal anti-inflammatory agents[18].

The present results showed that JG, the ethanolic extract of *J. gendarussa* induced a dose dependent protective effect against both writhing syndrome and thermal stimuli indicating its nociceptive effects against the peripheral and central nervous system, respectively. The increased latency to response of mice

to thermal stimuli after administration of JG indicated its CNS effects.

Acetic acid-induced writhing response in mice is a simple and reliable model to rapidly evaluate peripheral type of analgesic action of herbal and other drugs. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics[22]. JG at the three doses used in the study significantly inhibited acetic acid-induced writhing response in mice, dose dependently. The abdominal constrictions are related to the sensitization of nociceptors by prostaglandins[18]. It is therefore, possible that JG exerts analgesic effect by inhibiting synthesis or action of prostaglandins.

The hot plate test in mice is commonly used for assays of narcotic analgesics[23]. The results suggests that JG extract has a central analgesic effect as evidenced by the increase in reaction time of mice in the hot plate test.

FeCl$_2$-ascorbic acid mixture is known to stimulate lipid peroxidation in microsomes and mitochondria of rat liver *in vitro*[13]. Numerous pathological events such as the inflammation process and ageing phenomenon are associated with the generation of reactive oxygen species and the induction of lipid peroxidation[24]. The antioxidant action of plant constituents has been found to be related to the polyphenolic compounds[25]. Lipid peroxidation proceeds in the presence of appropriate species of transitional elements such as iron and copper[26]. Lipid peroxidation process increases during inflammatory conditions and treatment with herbal products is found to inhibit lipid peroxidation process[27]. In the present study, JG prevented the rise in lipid peroxides (MDA production) showing its significant anti-lipid peroxidant effect up to 100 µg/ml dose, beyond which it declined.

J. gendarussa is known to contain the flavonoids, vitexin and apigenin besides alkaloids, sitosterols, saturated steroidal saponins and triterpenoidal saponins. The anti-inflammatory and analgesic effects exhibited by JG in the present study may be due to the presence of these compounds because they are known to cause significant anti-inflammatory and analgesic effects[28-31]. Further studies are on in our laboratory to decipher the exact nature of the phytocompounds responsible for the anti-inflammatory and analgesic effects of JG and its mechanism of action.

Conclusion

The present study indicated that *J. gendarussa* has potent anti-inflammatory and analgesic action and

therefore, it can be used for the development of an effective herbal drug for these conditions.

Acknowledgements

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