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Anti-Inflammatory and Analgesic Activities of Ethanol Extract of Aerial Parts of *Justicia gendarussa* Burm.

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Abstract: The aim of the present study was to evaluate the anti-inflammatory and analgesic activities of the ethanol extract of aerial parts of *Justicia gendarussa* (EJG) in animal models. The anti-inflammatory activity of the extract was evaluated by using carrageenan-induced rat paw edema and cotton pellet granuloma method. The analgesic activity of the extract was evaluated for its central and peripheral pharmacological actions by using Eddy's hot plate method and acetic acid-induced writhing, respectively. The study was carried out in two different dose levels of 250 and 500 mg kg^{-1} orally. The EJG did not produce any mortality up to 2000 mg kg^{-1}. EJG at the dose of 500 mg kg^{-1} showed maximum inhibition of 52% in carrageenan-induced paw edema and 45% inhibition in dry weight cotton pellet granuloma formation. Dose dependent increase in latency of response in the hot plate method and 33% inhibition in acetic acid induced writhings in mice were observed with EJG at the dose of 500 mg kg^{-1}. The pharmacological screening of the extract showed significant ($p<0.001$-0.01) dose-dependent anti-inflammatory activity with good analgesic profile when compared with reference standard. The presence of flavonoids might be responsible for these activities and which are probably mediated via inhibition of various autocoids formation and release.

Key words: Anti-inflammatory, analgesic, *Justicia gendarussa*, mouse, rats

INTRODUCTION

In Indian system of medicine, a large number of drugs of either herbal or mineral origin have been advocated for various types of diseases and other different unwanted conditions in humans (Brekhman and Dardimov, 1969). Ayurvedic medicines are largely based upon herbal and herbomineral preparations and have specific diagnostic and therapeutic principles (Patwardhan and Hopper, 1992). Inflammation is a disorder involving localized increase in the number of leukocytes and a variety of complex mediator molecules (Mantri and Witiak, 1994). Prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. Their biosynthesis has also been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas and Alzheimer's diseases (Smith and De Witt, 1995; Lipsky, 1999).

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Franthworth, 1988). The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore, be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs.

Justicia gendarussa Burm. an evergreen scandent shrub belonging to the family Acanthaceae is commonly known as Vadaikkuthi in Tamil and widely distributed throughout river beds of Southern India. In traditional medicinal system, different parts of *Justicia gendarussa* have been mentioned to be useful in a variety of diseases. The leaves and tender shoots are diaphoretic and used in chronic rheumatism. Fresh leaves are used to treat edema and earache. The plant has been used by the native medical practitioners and tribes to treat various ailments including liver disorders, tumours, inflammation and skin diseases (Kirtikar and Basu, 1993). Woradulayapinij *et al.* (2005) reported that *Justicia gendarussa* has *in vitro* HIV type 1 reverse transcriptase inhibitory activity. It has been very recently reported that ethanolic and aqueous extracts of leaves of *J. gendarussa* inhibits the angiogenesis *in vitro* in dose dependant manner (Umamaheswari *et al.*, 2009) and stem extract of *J. gendarussa* shows moderate hepatoprotective activity (Krishna *et al.*, 2010).

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However, to our knowledge, there is no scientific report on the verification of the use of this plant in the treatment of inflammation, fever, tumors and liver disorders. Therefore, the aim of this study is to evaluate the anti-inflammatory and analgesic potential of the ethanolic extract of aerial parts of *Justicia gendarussa* (EJG) in animal models.

MATERIALS AND METHODS

Collection and extraction: Areial parts of *Justicia gendarussa* were collected in and around Kutralam in Tirunelveli district, Tamil Nadu, India, during the month of May 2007 and authenticated by Botanical Survey of India, Coimbatore, Tamilnadu, India. The aerial parts were shade dried and pulverized. The coarse powder was treated with petroleum ether for dewaxing and removal of chlorophyll. Later, it was packed (250 g) in a soxhlet apparatus and subjected to continuous hot percolation for 8 h using 450 mL of ethanol (95% v/v) as solvent. The extract was concentrated under vacuum and dried in a dessicator. The percentage yield was found to be 4.5% w/w.

Preliminary phytochemical screening: The extract was screened for the presence of various phytochemical constituents employing standard screening test (Wagner *et al*., 1984). Conventional protocol for detecting the presence of steroids, alkaloids, tannins, flavonoids, glycosides, etc., was used.

Animals: Male Wistar albino rats (150-200 g) and Swiss albino mice (20-25 g) were procured from Venkateshwara Enterprises, Bangalore, Karnataka, India and used throughout the study. The animals were housed in microlon boxes in a controlled environment (temperature 25±2°C and 12 h dark/light cycle) with standard laboratory diet and water *ad libitum*. The experiments were performed in accordance with the guidelines established by the European community for the care and use of laboratory animals and were approved by Institutional Animal Ethical Committee (IAEC).

Chemicals: Pentazocin (Ranbaxy, India), Aceclofenac and Aspirin (Micro Lab., India), Carrageenan type III (Sigma, St. Louis, USA) and acetic acid (Merck Co.) were used in the pharmacological studies.

Acute toxicity studies (LD_{50}): Acute Oral Toxicity (AOT) of EJG was determined using Swiss albino mice. The animals were fasted for 3 h prior to the experiment and were administered with single dose of extracts dissolved in 5% gum acacia (doses ranges from 500-2000 mg kg^{-1} at various dose levels) and observed for mortality up to 48 h (short term toxicity). Based on the short-term toxicity, the dose of next animal was determined as per OECD guideline 425. All the animals were also observed for long-term toxicity (14 days). The LD_{50} of the test extract was calculated using AOT 425 software provided by Environmental Protection Agency, USA.

Anti-inflammatory activity

Carrageenan-induced rat paw edema: The rats were divided into 4 groups (n = 6). The different groups were treated orally with EJG (250 and 500 mg kg^{-1}), aceclofenac (10 mg kg^{-1}) and vehicle control (5% gum acacia, 1 mL 100 g^{-1}). The ethanol extract, standard drug and vehicle control was administered 1 h prior to injection of 0.1 mL of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw sub plantar of each rat. The paw volume was measured initially and then at 1, 2 and 3 h after the carrageenan injection by using plethysmometer. The anti-inflammatory effect of EJG was calculated by the following equation:

$$\text{Anti-inflammatory activity (\%)} = (1\text{-Vt/Vc}) \times 100$$

where, Vt represents the paw volume in drug treated animals and Vc represents the paw volume of control groups animals (Winter *et al*., 1962).

Cotton pellet-induced granuloma: The animals were divided into 4 groups of 6 animals in each group. The rats were anaesthetized and sterile cotton pellets weighing 10±1 mg were implanted subcutaneously into both sides of the groin region of each rat. Group I served as control and received the vehicle (5% gum acacia, 1 mL 100 g^{-1}). EJG at the concentration of 250 and 500 mg kg^{-1} was administered orally to groups II, III animals for 7 consecutive days from the day of cotton pellet implantation. Group IV animals received aceclofenac at a dose of 10 mg kg^{-1} for the same period. On 8th 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 weighed and then dried in an oven at 60°C for 24 h to constant weight, after that the dried pellets were weighed again. Increment in the dry weight of the pellets was taken as a measure of granuloma formation. The antiproliferative effect of EJG was compared with control (D'Arcy *et al*., 1960).

Analgesic activity

Hot plate method: The analgesic activity of EJG was assessed using as described by hot plate method of Eddy and Leimbach (1953). The evaluated parameters were the latency time for paw licking and jumping responses on exposure to the hot plate surface which is kept at 55±1°C. The animals were kept in the hot plate until it lifted one of its hind paws. For this method, the mice were divided into 4 groups of 6 animals each. Group I served as control (5% gum acacia, 1 mL 100 g^{-1}), group II and group III received EJG at a dose of 250 and 500 mg kg^{-1} orally. Group IV received pentazocin at a dose of 5 mg kg^{-1}. All the treatments were given 30 min before the thermal stimulus and the response was determined at 60, 120 and 180 min.

Acetic acid induced writhing test: The writhing test in mice was carried out using the method of Koster *et al.* (1959). The writhes were induced by intraperitoneal injection of 0.6% v/v acetic acid (80 mg kg^{-1}). Two different doses of EJG (250 and 500 mg kg^{-1}) were administered orally to the group II and group III of 6 animals each. Group I served as control (5% gum acacia, 1 mL 100 g^{-1}) and group IV animals received aspirin at a dose of 300 mg kg^{-1}. The extract and standard drug was administered 30 min before chemical stimulus. The number of muscular contractions was counted over a period of 20 min and is expressed as writhing numbers.

Statistical analysis: Values are expressed as Mean±SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's test. p-values<0.05 were considered to be statistically significant when compared to control.

RESULTS

Phytochemical screening: Preliminary phytochemical screening of the ethanolic extract revealed the presence of alkaloids, glycosides, triterpenes, flavonoids and phenolic compounds. Further separation of the specific phytochemical is in progress.

Acute toxicity studies (LD_{50}): The extract treated animals were observed for mortality up to 48 h (short term toxicity) and for long-term toxicity (14 days). The study indicated that the EJG did not produce any mortality up to 2000 mg kg^{-1}.

Effect of EJG on carrageenan induced rat paw edema: The result of EJG against carrageenan-induced paw edema is shown in Table 1. EJG (250 and 500 mg kg^{-1}) gave significant ($p<0.001$) reduction of rat paw edema at all assessment times in a dose dependent manner. The extract showed maximum inhibition of 52% at the dose of 500 mg kg^{-1} after 3 h of drug treatment in carrageenan-induced paw edema whereas the standard drug showed 58% of inhibition.

Effect of EJG on cotton pellet granuloma: The EJG at the dose of 500 mg kg^{-1} showed 52 and 45% inhibition in the wet and dry weight cotton pellet granuloma formation respectively. The effects of EJG and aceclofenac on the proliferative phase of inflammation are shown in Table 2. A significant ($p<0.01$) reduction in the weight of the cotton pellets was observed with EJG at the dose of 250 and 500 mg kg^{-1} compared with the vehicle control treated rats. However, the degree of reduction was less than the effect caused by aceclofenac.

Effect of EJG on hot plate method: The animals pretreated with EJG (250 and 500 mg kg^{-1}) showed a dose dependent increase in latency of response in the hot plate method. The increase in the latency responses were significant ($p<0.01$) when compared to control. At time interval of 3 h, 250 and 500 mg kg^{-1} EJG effect were found be decreased remarkably compared with standard drug. However, their effects are equal during the second hour of experiment. The results are shown in Table 3.

Table 1: Effect of ethanol extract of aerial parts of *Justicia gendarussa* on carrageenan induced rat paw edema

		Paw volume in (mL)			
Treatments	Dose	0 h	1 h	2 h	3 h
Control (Normal saline)	10 mL kg^{-1}	0.17±0.02	0.22±0.007	0.29±0.003	0.5±0.006
Aceclofenac	10 mg kg^{-1}	0.10±0.006	0.12±0.005 (97.36%)	0.14±0.008 (48.27%)	0.21±0.130*(58%)
EJG	250 mg kg^{-1}	0.16±0.001	0.22±0.007 (0%)	0.23±0.010 (20.68%)	0.26±0.006* (48%)
EJG	500 mg kg^{-1}	0.12±0.006	0.14±0.011 (36.36%)	0.17±0.008 (41.37%)	0.24±0.004* (52%)

N = 6. *p<0.001 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

Table 2: Effect of ethanol extract of aerial parts of *Justicia gendarussa* on cotton pellet granuloma

Treatments	Dose	Weight of cotton pellets (mg) (wet)	Percentage inhibition	Weight of cotton pellets (mg) (dry)	Percentage inhibition
Control (Normal saline)	10 mL kg^{-1}	183.17±14.3	-	48.62±3.6	-
Aceclofenac	10 mg kg^{-1}	78.25±6.3*	57.25	23.54±2.4*	51.57
EJG	250 mg kg^{-1}	121.16±12.1*	33.85	34.42±2.4*	29.20
EJG	500 mg kg^{-1}	87.487±7.4*	52.25	26.71±2.1*	45.06

N = 6. *p<0.01 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

Table 3: Effect of ethanol extract of aerial parts of *Justicia gendarussa* on thermic stimulus induced (Hot Plate) pain in rats

		Reaction time (sec)			
Treatments	Dose	0 h	1 h	2 h	3 h
Control (Normal saline)	10 mL kg^{-1}	2.4±0.15	2.32±0.40	2.45±0.16	2.36±0.14
Pentazocin	5 mg kg^{-1}	2.3±0.4	7.50±0.22*	9.72±1.10*	7.84±0.14*
EJG	250 mg kg^{-1}	2.5±0.22	5.30±0.23*	8.06±0.75*	6.90±1.10*
EJG	500 mg kg^{-1}	2.6±0.6	6.83±0.30*	8.76±0.36*	7.20±0.36*

N = 6. *p<0.01 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

Table 4: Effect of ethanol extract of aerial parts of *Justicia gendarussa* on chemical stimulus induced (writhing test) pain in rats

Treatments	Dose	No. of writhing (20 min)	Percentage inhibition
Control	10 mL kg^{-1}	79.8±2.45	-
Aspirin	300 mg kg^{-1}	26.5±1.72*	66.79
EJG	250 mg kg^{-1}	62.7±1.67*	21.42
EJG	500 mg kg^{-1}	53.5±1.58*	32.95

N = 6. *p<0.01 vs. control. Data were analyzed by one way ANOVA followed by Dunnett test

Effect of EJG on acetic acid induced writhing in mice: Administration of different doses of the extract (250 and 500 mg kg^{-1}) decreased the number of writhings in mice and the effect was found to be dose dependent. The reduction was statistically significant ($p<0.01$) when compared to control. Aspirin showed a 67% inhibition and EJG showed 33% inhibition at the dose of 500 mg kg^{-1} in acetic acid induced writhing in mice. The results are given in Table 4.

DISCUSSION

The most widely used primary test for screening of anti-inflammatory agents is Carrageenan induced rat paw edema (Winter *et al*., 1962). The development of edema in the paw of the rat after injection of Carrageenan is believed to be biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin; the second phase is due to the release of prostaglandin-like substances (Antonio and Brito, 1998). Based on this, it could be argued that the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin and the action in the second phase may be explained by an inhibition of cyclooxygenase (Olajide *et al*., 1999).

Ueno *et al*. (2002) found that the injection of carrageenan into the rat paw induces the liberation of bradykinin, which later induces the biosynthesis of prostaglandins and other autocoids, which are responsible for the formation of the inflammatory exudates. Besides, in the carrageenan-induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism (Nantel *et al*, 1999). The EJG extract produced minimum inhibition in the initial phase of development of inflammation and EJG extract showed maximum inhibition of 52% at the dose of 500 mg kg^{-1} in the second phase of development of inflammation. The standard drug, aceclofenac, showed 58% of inhibition in the second phase. Therefore, it is suggested that the mechanism of action of EJG may be related to prostaglandin synthesis inhibition.

The cotton pellet granuloma method has been widely employed to assess the transductive, exudative and proliferative components of chronic inflammation and is a typical feature of established chronic inflammatory reaction. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma and dry weight correlates well with the granuloma of the granulomatous tissue formed (Olajide *et al*., 1999, 2000). Administration of EJG at the doses of 250 and 500 mg kg^{-1} significantly reduced the granulomatous tissue formation when compared to control.

It is known that non-steroidal anti-inflammatory drugs usually do not increase the pain threshold in normal tissues, whereas, local anesthetics and narcotics do (Ferreira *et al*., 1978). However, the hot plate test was undertaken to verify if EJG would have any central analgesic effect. The results for the group treated with EJG showed significant activity when compared to control group and nearly equal to the group treated with pentazocin (5 mg kg^{-1}). Hence, it is assumed that EJG has significant central analgesic effect.

During the first 30 min of intraperitoneal injection of acetic acid in rats showed high levels of prostaglandins $PGE_{2\alpha}$ and $PGF_{2\alpha}$ in peritoneal exudates (Derardt *et al*., 1980). It is also proved that intraperitoneal administration of acetic acid liberates sympathetic nervous system mediators along with prostaglandins (Hokansan, 1978; Duarte *et al*., 1988). The EJG was effectively inhibiting the acetic acid induced writhings in mice in dose dependent manner. The results were comparable with the group treated with aspirin. Hence, we could assume that EJG may also involve in the peripheral analgesic activity.

Preliminary phytochemical screening indicated the presence of flavonoids in EJG. Selected phenolic compounds and flavonoids were shown to inhibit both the cyclooxygenase and 5-lipoxygenase pathways (Ferrandiz *et al*., 1990; Ferrandiz and Alcaraz, 1991). This

inhibition reduces the release of arachidonic acid (Yoshimoto *et al*., 1983). The exact mechanism by which flavonoids inhibit these enzymes is not clear. Quercetin, in particular, inhibits both cyclooxygenase and lipoxygenase activities, thus, diminishing the formation of these anti-inflammatory metabolites (Robak and Gryglewski, 1996).

The ability of flavonoids to inhibit eicosanoid biosynthesis has been documented (Damas *et al*., 1985; Hertog *et al*., 1995). Eicosanoids, such as prostaglandins, are involved in various immunological responses (Moroney *et al*., 1988) and are the end products of the cyclooxygenase and lipoxygenase pathways. Flavonoids also inhibit both cytosolic and membranal tyrosine kinases which play key roles in the signal transduction pathway that regulates cell proliferation (Formica and Regelson, 1995). Further, flavonoids are able to inhibit neutrophils degranulation and thereby decrease the release of arachidonic acid (Hoult *et al*., 1994; Tordera *et al*., 1994). Thus, the presence of flavonoids in the extract of EJG might be responsible for the anti-inflammatory and analgesic activity in Wister albino rats and mice.

CONCLUSION

Data obtained in this study indicated that the ethanol extract of aerial parts of *Justicia gendarussa* possess anti-inflammatory and analgesic effects. The presence of flavonoids might be responsible for these activities and which are probably mediated via inhibition of various autocoids formation and release. Further detailed investigation is underway to determine the exact phytoconstituents that are responsible for these activities.

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