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Research Article

SCREENING FOR ANALGESIC AND ANTI INFLAMMATORY ACTIVITIES OF ETHANOLIC EXTRACT OF *JUSTICIA GENDARUSSA* BURM LEAVES

Ulhas Shrimant Surwase*[1], Shivanand Malkappa Patil[2]

[1]*K.T. Patil College of Pharmacy, Osmanabad, Maharashtra, India*

[2]*Shree Dev Bhoomi Institute of Education, Science & Technology Department of Pharmaceutical Science, Poundha, Dehradun, Uttarakhand, India*

**Corresponding author: ulhaspharmacy22@gmail.com*

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ABSTRACT

Ethanol extract of the leaves of *Justicia gendarussa* Burm was subjected to analgesic and anti-inflammatory activities in animal models. Albino Wistar rats and mice were the experimental animals respectively. Different CNS depressant paradigms like analgesic (determined via Eddy's hot plate approach and acetic acid writhing approach) and anti-inflammatory activity (determined via carrageenan induced paw edema using plethysmometer in albino rats) were carried out, following the intra-peritoneal administration of ethanol extract of formulation *Justicia gendarussa* Burm leaves (EEJG) at the dose level of 250mg/kg and 500mg/kg. The analgesic and anti-inflammatory activities of ethanol extracts of EEJG showed significant results ($P < 0.001$). The maximum analgesic activity was observed at 120 min at the dose of 500mg/kg (i.p.) and analgesic and anti-inflammatory activity was compared with standard drug Diclofenac sodium (5 mg/kg). Ethanol extract of *Justicia gendarussa* Burm leaves has shown significant analgesic and anti-inflammatory activities at the dose of 500mg/kg and was comparable with corresponding standard drugs. The activity was attributed to the presence of phytoconstituents in the tested extract.

Keywords: *Justicia gendarussa* Burm, Analgesic, Anti-inflammatory.

1. INTRODUCTION

India is recognized for its agro-climatic range and additionally for its wealthy background of conventional structures of medicines. The Indian System of Medicine (ISM) contains of *Ayurveda*, *Siddha*, *Unani* and *Tibetan* machine of drugs at the side of the opposite peoples medicinal practices and age vintage domestic remedies. All those structures have positioned India, because the main kingdom in the usage of herbal medicinal healing field, that's primarily based totally at the conventional information of the usage of medicinal flowers. India possesses a wealthy treasure of biodiversity, which has been used for fitness care, for the closing extra than 4 thousand years. Medicinal flowers are the ones that comprise materials which can be used for healing functions or which can be precursors for the synthesis of beneficial drugs [1]. Pain has been described through International Association for the Study of Pain (IASP) as an ugly sensory and emotional revel in related to real or capacity tissue damage. Clinically, ache may be labeled "nociceptive" if its miles inferred that the ache is because of ongoing activation of the nociceptive machine through tissue injury. Although neuroplastic changes (which include the ones underlying tissue sensitization) are without a doubt involved, nociceptive ache is presumed to arise due to the regular activation of the sensory device through noxious stimuli, a manner that entails transduction, transmission and modulation [2]. Drugs which can be used presently for ache management & inflammatory situations are both narcotic analgesics or NSAID'S and steroids. All the above stated categories own unfavorable & poisonous consequences which include addiction, constipation and breathing melancholy in case of narcotic analgesics, peptic ulcers and kidney troubles in NSAID'S. So to keep away from unfavorable consequences from those medicines, strong and secure medicinal drug from plant starting place has been used considering the fact that

lengthy time. It is crucial that efforts need to be made to introduce new medicinal flora to broaden inexpensive pills. Plants constitute nonetheless a big untapped supply of structurally novel compounds that would function a beacon mild for the improvement of novel drugs [3].

Justicia gendarussa Burm F. (Family: *Acanthaceae*) is a shade-loving, quick-growing, evergreen plant usually discovered in wet areas. It is assumed to be local to China and is sent broadly throughout India, Sri Lanka, and Malaysia. In Indian and Chinese conventional medicine, the leaf of the plant is usually recommended to deal with illnesses including fever, hemiplegia, rheumatism, arthritis, headache, earache, muscle pain, respiration disorders, and digestive trouble. [4] However, to our knowledge, there are no published scientific studies on the anti-arthritic activities of the leaves of *J. gendarussa* or its potential toxicity. Therefore, the objective of this study is to examine the analgesic and anti-inflammatory activities of the Ethanolic leaf extract of this plant.

2. MATERIAL AND METHODS

2.1. Plant collection and authentication

Leaves of *Justicia gendarussa Burm* were obtained from the local places of Tirupati, AP. *Justicia gendarussa Burm* Plant was authenticated by Dr. K. Madhava Chetty, M.Sc., M.Ed., M.Phil., Ph.D., PG DPD., Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh.

2.2. Extraction by Maceration

Fresh leaves were washed with water to get rid of contaminants like dirt and other impurities and were shade-dried. These dried leaves were ground and sieved to get a uniform, coarse powder. Powdered plant material was weighed (1Kg) and was immersed in ethanol [5] and kept for maceration [6] for a period of 7 days with occasional stirring. On the 8th day, the solvent was filtered by pressing with a muslin cloth and was evaporated in a rotary evaporator at 40°C. The resultant extract was put in a desiccator to remove any ethanol left in it. The dried ethanolic extract of *Justicia gendarussa Burm*. (EEJG) was packed in an air-tight bottle and put in a dry place for further studies.

2.3. Qualitative evaluation of phytoconstituents

The EEJG was screened for the presence of various phytoconstituents like carbohydrates, flavonoids, poly-phenolic compounds, saponins, tannins, triterpenoids, etc [7].

2.4. Toxicity studies

Albino rats (200250 g) of both sexes were selected and separated into 8 groups of 6 animals each. Ethanolic extract of, starting from the minimum dose of 50 mg/kg up to 3000 mg/kg was administered orally. Animals treated with the drug were carefully observed for signs of toxicity and mortality. From the maximum dose, 1/5 and 1/10 of the concentration was considered a therapeutic dose for further studies.

2.5. Animals

Albino rats (175-225gm) of either sex and of approximate same age used in the present studies were procured from Central Animal facility, CMR college of Pharmacy, Hyderabad, India. The animal was fed with standard pellet diet and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours in darkness and light. The animals were acclimatized to the laboratory condition for a one week before starting the experiment. The experiment protocols were approved by Institutional Animal Ethics committee after securitization (IAEC No: CPCSEA/1657/IAEC/ CMRCP/COL-19/67). The animal received the drug treatment by oral gavage tube.

2.6. Acetic Acid Induced Writhing Method [7, 8]

This study was conducted using an acetic acid-induced writhing abdominal reflex pain model [9]. Twenty four mature mice were randomly divided into 4 groups of 6 mice per group, fasted for 12 hours and treated as follows, group 1 (control group) received 10 ml/kg of saline solution, the group 2 (standard) received 30 mg/kg diclofenac sodium; groups 3 and 4 received respectively 250 and 500 mg/kg of EEJG by gavage. One hour after drug and extract administration, all mice received intraperitoneal (IP) 0.6% glacial acetic acid (IP) to induce abdominal writhing or contortion. The analgesic effect was evaluated in each mouse for 30, 60, 120 minutes and recorded.

2.7. Eddy's Hot Plate Method: (Thermal stimulus) [10]

In the hot plate method, albino mice (18-28) were divided into four groups consisting of six animals each. All animals selected for the study underwent normal baseline reaction time and then separated into different groups such as group I served as control (received vehicle), group II served as standard (received

diclofenac sodium 30 mg/kg) while groups III and IV received EEJG (250 and 500 mg/kg, respectively). All animals were lowered to the surface of a hot plate (50±1.00°C) enclosed in cylindrical glass and the time taken for the animal to jump or lick the front paw was noted as reaction time (RT). The no-response time limit was 15 seconds to prevent the animals from being burned. Observations were made before and after administration of the respective drugs at 30 min, 60 min, and 120 min and at the end of 180 min.

2.8. Carrageenan induced hind paw edema [11, 12]

Albino rats of either sex weighing 150-200 grams were divided into four groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline 0.5 ml/kg), Group II - Diclofenac sodium (30 mg/kg, p. o.), Group - III and IV - EEJG (250 mg/kg and 500 mg/kg, p. o.). All the drugs were administered orally. Diclofenac sodium served as the reference standard anti inflammatory drug. After one hour of the administration of the drugs, 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the left hind paw of the rat and the right hind paw was served as the control. The paw volume of the rats were measured in the digital plethysmograph (Ugo basile, Italy), at the end of 0 min., 60min., 120min., 180min.and 240min. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the drugs was studied.

3. RESULTS AND DISCUSSION

The preliminary phytochemical screening showed the presence of various phytoconstituents like flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates in EEJG.

3.1. Acetic acid induced writhing test in mice

The EEJG with two selected doses i.e 250 and 500 mg/kg have exhibited significant increase percentage of inhibition in writhing method in mice at different time intervals. Results were shown in Table 2. Diclofenac sodium (30mg/kg) was used as standard and it has significantly increased the percentage inhibition of writhing by 90.7% at the end of 2hr which was found to be a time dependent effect. During first 30 min of the study, EEJG with medium and high dose the % of inhibition of writhing were 55.1, and 60.5%. During 1hr of the study EEJG with medium and high dose the % of inhibition of writhing was 67.1 and 73.5% respectively which was time dependent effect. During 2hr of the study EEJG with medium and high dose the % of inhibition of writhing was 79.8 and 86.7% and the results were tabulated.

Table 1: Results of Phytochemical screening

S. No	Name of the Phytochemical	EEJG
1.	Carbohydrates	+
2.	Amino acids	+
3.	Proteins	+
4.	Alkaloids	+
5.	Cardiac glycosides	+
6.	Triterpenoids	+
7.	Saponins	+
8.	Flavonoids	+
9.	Phenolic compounds	+
10.	Tannins	-
11.	Steroids	-
12.	Gums	-

Where, + means positive and - means negative.

Table 2: Acetic acid induced writhing test in mice:

Groups	Treatment	Dose	Number of writhing (Mean± SEM)			% inhibition of writhing		
			30 min	60 min.	120 min	30 min	60 min.	120 min
Group- I	Control	0.1ml	26.56±3.62	27.56±4.35	35.83±4.1	-	-	-
Group-II	Standard (Diclofenac sodium)	30 mg/kg	13.23±1.33**	8.46±1.02**	3.66±1.2**	64.7±3.2	76.8±2.6	90.7±4.3
Group- III	EEJG	250mg/kg	16.32±1.81**	12.24±1.87**	8.57±1.6**	55.1±4.3	67.1±4.5	79.8±3.6
Group-IV	EEJG	500mg/kg	14.34±0.99**	9.73±0.30**	5.83±1.4**	60.5±2.5	73.5±5.6	86.7±5.3

Values indicate mean± SEM (ANNOVA test followed by Dunnet's t- test), Control n =6: comparison with control group
Significant at P < 0.05, 0.01** and 0.001***, compared to control group*

3.2. Analgesic activity by Eddy's hot plate test in mice

The EEJG with two selected doses i.e. 250 and 500 mg /kg have exhibited significant increase in reaction time in Eddy's hot plate method in mice at different time intervals. Results were tabulated in Table 3. Diclofenac sodium (30mg/kg) was used as standard and it has significantly increased the reaction time at the end of 1st and 2nd h which was found to be a time dependent effect. During 30min of the study, EEJG with medium

and high dose the reaction time was 3.43±0.18, 3.51±0.17, seconds. During 60min of the study, EEJG with medium and high dose the reaction time was 4.36±0.21, 4.75±0.19 respectively which was time dependent effect. During 120min of the study, EEJG with medium and high dose the reaction time was 6.18±0.23, 7.12±0.10 respectively. During 180min of the study, EEJG with medium and high dose the reaction time was 7.16±0.19, 7.45±0.16 seconds. Diclofenac sodium showed a significant reaction time during 30min and 60min where as test groups have shown during 60 and 180min

3.3. Carrageenan induced paw oedema in rats

The EEJG in 250 mg/kg and 500mg/kg have exhibited a significant reduction in paw edema volume in Carrageenan induced paw oedema in rats at different time intervals. Results are tabulated in Table 4. Diclofenac sodium (30 mg/kg) was used as standard reference and it has significantly reduced paw oedema volume which was found to be a time dependent effect. Diclofenac showed maximum response at 4th hour which was found to be 71.43%. At 4th hour EEJG showed significant reduction in oedema volume, 400mg/kg showed the maximum reduction in oedema volume i.e. 60%.

Table 3: Analgesic effect of EEJG in Eddy's hot plate method in mice

Groups	Treatment	Hot plate reaction time			
		30 min	60 min	120 min	180 min
Control	Formalin(1%w/v)	2.51±0.318	2.39±0.38	2.15±0.36	2.17±0.35
Standard	Diclofenac sodium (30mg/kg)	3.57±0.47	6.05±1.14**	9.94±0.45**	7.95±0.32**
EEJG	250mg/kg	3.43±0.18	4.36±0.21	6.18±0.23**	7.16±0.19**
EEJG	500mg/kg	3.51±0.17	4.75±0.19*	7.12±0.10**	7.45±0.16**

Values are expressed in mean ± SEM, where n = 6, Significant at P < 0.05, 0.01** and 0.001***, compared to control group*

Table 4: Carrageenan induced paw oedema in rats

Groups	Oedema volume (ml)				
	0 hr	1hr	2hr	3hr	4hr
Control	0.18 ± 0.06	0.25 ± 0.09	0.30 ± 0.07	0.33 ± 0.07	0.36 ± 0.06
Diclofenac sodium (30mg/kg)	0.16 ± 0.03 (11.77)	0.17 ± 0.05* (33.33)	0.13± 0.04* (58.63)	0.11 ± 0.04* (68.75)	0.11 ± 0.03* (71.43)
EEJG 250mg	0.24±0.018 (6.66%)	0.28±0.039 (26.9%)	0.25±0.034** (44%)	0.22±0.04** (58.91%)	0.23±0.04 (59.81%)
EEJG 500mg	0.16 ± 0.04 (11.77)	0.17 ± 0.05* (33.33)	0.18 ± 0.04* (41.39)	0.17 ± 0.03* (50.00)	0.15 ± 0.06* (60.00)

*Data presented as mean ± S.E.M. n=6, *** P< 0.001, ** P< 0.01, * P< 0.05, Compared with control group, followed by ANOVA followed by Post hoc test (Dunnett's 't' test).*

4. DISCUSSION

Inflammation is a complex process triggered by several factors ranging from bacterial infection and chemical damage to environmental pollution that causes cell damage or death. Non steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs in the world today. Pain and fever are the most common conditions associated with inflammation. NSAIDs used in inflammatory conditions do not cure or eliminate the underlying cause of the disease, only modify the inflammatory response to the disease. Pain and fever are the most common conditions associated with inflammation. To assess anti-inflammatory activity, JGEE was assessed by two popular screening models widely used for NSAIDs, namely carrageenin-induced rat paw edema and Acetic acid induced writing method. Carrageenan-induced rat paw edema exhibits a biphasic effect. The first phase is due to the release of histamine and serotonin (5HT) (0 2 hours), the plateau phase is maintained by the quinine-like substance (3 h) and the second phase of accelerated swelling is attributed to the release of PG (4h). In our study, EEJG (250 and 500 mg/kg, p.o.) significantly reduced (P and <0.05) carrageenan-induced edema in all three phases.

5. CONCLUSION

Based on the study, the data showed that the ethanolic extract of *Justicia gendarussa Burm* possesses significant anti-inflammatory activity compared to standard diclofenac sodium. As phytochemical tests showed the presence of glycosides, carbohydrates, flavonoids, steroids and resin in the ethanolic extracts, they might suppress the formation of prostaglandins and bradykinins or antagonize their action and exert their activity. The formulation has been shown to be useful for the treatment of local inflammation and pain.

Conflict of interests

None declared

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