Original Research Article

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Analgesic and Anti-inflammatory Potential of *Ficus glomerata* Leaves Fractions in Experimental Animals

Vishal Gulecha*, Manoj Mahajan, Rakesh Khandare, Chandrashekhar Upasani

Department of Pharmacology, SNJB'S SSDJ College of Pharmacy, Neminagar, Chandwad. District, Nashik-423101, Maharashtra, India.

Abstract

The present study evaluated the analgesic and anti-inflammatory potential of fractions of *Ficus glomerata* using various well established experimental models. Analgesic activity of *F. glomerata* was screened using acetic acid-induced writhing in mice and tail flick test in rats. Anti-inflammatory activity was done by carrageenan-induced rat paw edema and cotton pellet-granuloma formation in rats. Ibuprofen was used as the reference standard in this study. Five fractions of *F. glomerata* (FGI, FGII, FGIII, FGIV and FGV) at the dose level of 20 and 40 mg/kg, p.o. were used. The fractions FGI (40 mg/kg, p.o.) and FGIII (40 mg/kg, p.o.) were found to be effective inhibitors of carrageenan induced rat paw edema, cotton pellet granuloma formation and acetic acid-induced writhing. The fractions FGI (20 mg/kg, p.o.) and FGIII (20 mg/kg, p.o.) were found to be more effective in increasing latency period in tail flick method. In conclusion, from the five different fractions of *F. glomerata* tested, FGI and FGIII possess potent analgesic and anti-inflammatory activities against different models of inflammation and pain.

Key words: *Ficus glomerata*, cotton pellet, carrageenan, tail flick, acetic acid, writhing

1. Introduction

World Health Organization (WHO) has defined the traditional system of medicine as "the sum total of all the knowledge and practices, whether explicable or not; used in diagnosis, preventions and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down by generation to generation whether verbally or in writing". In other words traditional system of medicine might also be considered as a solid amalgamation of dynamic medical know-how and experience [1].

Inflammation or phlogosis is a pathological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in inflammatory reaction can be induced, maintained or aggravated in many diseases. It is a complex phenomenon, comprising of biochemical as well as immunological factors. Inflammation is recognized by rubor (redness), tumor (Swelling), calor (heat), dolor (pain) and functio laesa (loss of functions) [2]. Currently, both steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs (NSAIDs) are used in the aid of inflammation. Steroids have an obvious role in the treatment of inflammatory diseases, but due to their toxicity, can only be used over short periods except in very serious cases where the risks are acceptable. Prolonged use of NSAIDs is also associated with severe side effects, notably gastrointestinal hemorrhage [3, 4].

Inflammatory diseases are among the most common health problems treated with traditional remedies. Therefore, it is crucial to evaluate the potential of herbal remedies that might serve as leads for the development of potent drugs. A large number of Indian medicinal plants are attributed with various pharmacological activities owing to different classes of phytochemicals present.

Ficus racemosa Linn (synonym Ficus glomerata, family - Moraceae) is a large deciduous tree distributed all over India especially in outer Himalayan ranges, Punjab, Khasia mountain, Chota Nagpur, Bihar, Orissa, West Bengal, Rajasthan Deccan and commonly in south India. It is commonly known as Gular fig or Cluster fig in English, Gular in Hindi and Udumbara in Sanskrit. Susruta has described the properties of the plant, such as astringent, promoter of callus healing in fractures (bhagna sandhaniya), reliever of rakta pitta, burning sensation and obesity, and useful in vaginal disorders. The roots, bark-skin, fruits, lattes and leaves of udumbara have great medicinal value. Udumbara is used both internally as well externally. Externally, the latex is applied on chronic infected wounds to alleviate edema, pain and to promote the healing. The

* Corresponding Author: Vishal S Gulecha, E-mail: vishalgulecha@rediffmail.com

tender leaf buds are applied on the skin, in the form of paste, to improve the complexion; the decoction of leaves is salutary in washing the wounds for better cleansing and healing [5]. According to Ayurveda, roots are useful in hydrophobia whereas bark is acrid, cooling, galactagogue and good for gynaecological disorders. Fruits are astringent to bowels, styptic, tonic and useful in the treatment of leucorrhoea, blood disorders, burning sensation, fatigue, urinary discharges, leprosy, menorrhagia, epistaxis and intestinal worms. According to Unani system of medicine, leaves are astringent to bowels and good in case of bronchitis whereas fruits are useful in treatment of dry cough, loss of voice, diseases of kidney and spleen. Bark is useful in Asthma and piles. Latex is applied externally on chronic infected wounds to alleviate edema, pain and to promote the healing. The tender leaf buds are applied on the skin, in the form of paste, to improve the complexion [6].

2. Materials and methods

2.1. Collection and authentication of plant materials

The leaves of *F. glomerata* were collected from local region of Nashik, Maharashtra, India in the month of July 2008. The plant material was identified and authenticated by Prof. P. G. Diwakar, Botanical survey of India, Pune and a voucher specimen was kept with voucher No. BSI/WC/Tech/08/340.

2.2. Preparation of F. glomerata fractions

The extraction was carried out using petroleum ether followed by alcohol, then it was allowed to evaporate slowly in shallow dish and resinous mass was discarded. For column chromatography, neutral alumina was first activated at 150 °C for 3 hr in an oven. After cooling, slurry was prepared in benzene; it was poured in glass column and set aside for 2 hr. The residue of petroleum ether extract was dissolved in minimum volume of benzene and it was mixed thoroughly with neutral alumina. It was air dried and charged into the column. The elution was carried out first with benzene and successively eluted with ethyl acetate to obtain fractions FGI and FGII respectively. The dried alcoholic extract was separated into water soluble and water insoluble portion. Water soluble portion was shaken vigorously with methanol to get a gelatinous precipitate fraction, FGIII. The water insoluble part was dissolved in minimum volume of absolute alcohol and column chromatography was carried out with benzene FGIV and ethyl acetate to obtain fractions FGIV and FGV respectively.

2.3. Experimental animals

Albino rats of Wistar strain (150-200 g) and Swiss albino mice (25-30 g) of either sex were used in the entire study and were procured from Yash farm, Pvt. Ltd, Pune, Maharashtra, India. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2 °C with relative humidity 60 - 70% in a 12 hr light-dark cycle. The animals were fed with standard laboratory diet and water ad libitum. Food was withdrawn 12 hr before and during the experimental hours. The experimental protocol was approved by Institutional Animal Ethics Committee.

2.4. Tail flick latency period in rats

Male rats were divided into six groups comprising five animals per group. A tail flick response test was performed with analgesiometer (Space Scientific, Nashik, India) and was evoked by placing each rat tail over the wire heated electrically. The intensity of heat was adjusted so that the baseline tail flick latency averaged 3 - 4 sec in all animals. The cut off time was maintained at 15 sec in order to avoid injury to tail. The fractions FGI, FGIII and reference standard ibuprofen were administered orally in their respective doses 1 hr prior to the test [7].

2.5. Acetic acid-induced writhing in mice

The writhing syndrome was elicited by intraperitoneal injection of acetic acid (0.1 ml of 0.6% solution) and number of writhes displayed from 5 to 20 min were recorded [8]. The fractions FGI, FGIII and reference standard ibuprofen were administered orally in their respective doses 30 min prior to the test.

2.6. Carrageenan-induced rat paw edema

The anti-inflammatory activity of all fractions (FGI, FGII, FGIII, FGIV and FGV) was assessed using carrageenan-induced rat paw edema. The fractions were suspended in gum acacia (0.5% w/v) and were administered in doses of 20 and 40 mg/kg, p.o. respectively 1 hr prior to carrageenan injection (0.1 ml of 1% solution). The paw volume was measured at an interval of 0, 1, 2, 3 hr using Plethysmometer (UGO Basil, Italy. Model no.7130) [9].

2.7. Granuloma formation induced by cotton pellet in rats

Male rats were divided into seven groups comprising of five animals in each group. The cotton pellet weighing 50 ± 1 mg was sterilized in an autoclave (Lab hosp, Mumbai, India) and subsequently handled with sterile instrument. The pellet was inserted in each animal on the back. Control group (Group I) received vehicle. Group II, III, IV and V were treated with FGI (20 and 40 mg/kg, p.o.) and FGIII (20 and 40 mg/kg, p.o.) respectively. The groups VI and VII were treated with reference standards hydrocortisone (30 mg/kg, p.o.) and ibuprofen (40 mg/kg, p.o.) respectively for six days consecutively [10, 11]. The animals were sacrificed on seventh day and cotton pellet along with granuloma mass were collected, weighed and dried at 60 °C. Results of the assay were calculated as % inhibition of dry weight of granuloma formation by using the formula: 100 (A - B)/A, where, A = gain in dry weight of control pellet (mg), B = gain in dry weight of drug treated (mg).

2.8. Statistical analysis

Results of all the above estimations have been expressed in terms of mean ± S.E.M. Difference between the groups

was statistically determined by one-way analysis of variance (ANOVA) followed by Dunnett's test as the posthoc test using GraphPad InStat version 5.00, GraphPad Software, CA, USA. The level of significance was set at $p < 0.05$.

3. Results

3.1. Effect of F. glomerata fractions on tail flick latency period in rats

Treatment of FGI and FGIII (20 mg/kg, p.o.) significantly inhibited nociception in rats by 20.22 % and 20.96 % respectively. Whereas, FGI and FGIII (40 mg/kg, p.o.) significantly inhibited pain perception by 18.20% and 16.40% respectively. Ibuprofen treatment (40 mg/kg, p.o.) significantly inhibited pain perception by 27.92% (Table 1).

Table 1. Effect of *F. glomerata* fractions on tail flick latency period. Data were analyzed using one-way analysis of variance (ANOVA) and expressed as mean ± S.E.M. (n = 5) followed by Dunnett's test and differences between means were considered as significant at *$p < 0.05$ and **$p < 0.01$.

Treatment (mg/kg)	Tail flick latency in sec	% Analgesia
Control	4.15 ± 0.44	--
FGI (20)	6.35 ± 0.04*	20.22
FGI (40)	6.13 ± 0.04*	18.20
FGIII (20)	6.43±0.04**	20.96
FGIII (40)	5.93 ± 0.10*	16.40
Ibuprofen (40)	7.18 ± 0.88**	27.92

3.2. Effect of F. glomerata fractions in acetic acid-induced writhing in mice

It was observed that mice treated with FGI 20 (36.21 %) and FGIII 20 (34.21 %) showed significant ($p < 0.01$) protection compared to control group. Furthermore, FGI 40 (47.50 %) and FGIII 40 (51.16 %) were found to have very significant ($p < 0.01$) effect in protecting acetic acid-induced writhing compared to control group. Ibuprofen showed 56.14 % protection against acetic acid-induced writhing in mice (Table 2).

Table 2. Effect of *F. glomerata* fractions in acetic acid-induced writhing in mice. Data were analyzed using one-way analysis of variance (ANOVA) and expressed as mean ± S.E.M. (n = 5) followed by Dunnett's test and differences between the means were considered as significant at *$p < 0.05$ and **$p < 0.01$.

Treatment (mg/kg)	Number of writhings	% Inhibition
Control	60.2 ± 2.49	--
FGI (20)	38.4 ± 3.82**	36.21
FGI (40)	31.6 ± 2.37**	47.50
FGIII (20)	39.6 ± 2.92**	34.21
FGIII (40)	29.4 ± 3.41**	51.16
Ibuprofen (40)	26.4 ± 0.92**	56.14

3.3. Effect of F. glomerata fractions on carrageenan-induced rat paw edema

Out of all the fractions (FGI, FGII, FGIII, FGIV and FGV) tested, FGI and FGIII of *F. glomerata* significantly ($p < 0.01$ and $p < 0.05$) reduced the rat paw edema induced by carrageenan respectively. FGI and FGIII (40 mg/kg, p.o.) reduced inflammation by 38.12% and 44.15% respectively. However, other fractions (FGII, FGIV and FGV) showed mild inhibition to the paw edema induced by carrageenan during the three time points from 1 to 3 hr (Table 3).

Table 3. Effect of *F. glomerata* fractions on carrageenan induced rat paw edema. Data were analyzed using one-way analysis of variance (ANOVA) and expressed as mean ± S.E.M. (n = 5) followed by Dunnett's test and differences between means were considered as significant at *$p < 0.05$ and **$p < 0.01$.

Treatment (mg/kg)	Mean increase in paw volume (mL)				% Decrease in paw volume at 3 hr
	0 hr	1 hr	2 hr	3 hr	
Control	0.91 ±0.007	1.56 ±0.007	1.95 ± 0.01	2.51 ± 0.011	-
IBU (40)	0.91 ±0.008	1.00 ±0.01**	1.30 ± 0.003**	1.62 ± 0.001**	52.34
FGI (20)	0.90±0.026	1.37 ±0.012**	1.67 ± 0.04**	1.95 ± 0.027**	34.37
FGI (40)	0.87 ±0.036	1.30 ±0.028**	1.49 ± 0.016**	1.86 ± 0.023**	38.12
FGIII (20)	0.90± 0.042	1.32 ± 0.033**	1.52 ± 0.025**	1.91± 0.042**	36.87
FGIII (40)	0.97± 0.018	1.22 ± 0.051**	1.44 ± 0.029**	1.86 ± 0.046**	44.15

3.4. Effect of F. glomerata fractions on cotton pellet granuloma formation in rats

Treatment with FGI and FGIII (40 mg/kg, p.o.) to rats showed significant ($p < 0.01$) inhibition in the weight of cotton pellet in rats and the percentage inhibition was found to be 30 and 42 % respectively. Treatment with the reference standard ibuprofen (40 mg/kg, p.o.) showed significant inhibition in cotton pellets granuloma formation as compared to control group (Table 4).

4. Discussion

Inflammation is a necessary function of the human immune system but may become problematic and potentially lethal when acute inflammation becomes chronic inflammation. The selection of *Ficus glomerata* for the present investigation was on the basis of utilization in Ayurvedic preparations and recommendation of this plant for treatment of inflammation.

Table 4. Effect of *F. glomerata* fractions on cotton pellet granuloma formation in rats. Data were analyzed using one-way analysis of variance (ANOVA) and expressed as mean ± S.E.M. (n = 5) followed by Dunnett's test and differences between means were considered as significant at $*p < 0.05$ and $**p < 0.01$.

Treatment (mg/kg)	Average weight of cotton pellet	Average weight of cotton pellet with granuloma	% Inhibition
Control	50 ± 0.01	128 ± 4.99	-
Hydrocortisone (30)	50 ± 0.01	75 ± 2**	41.40
Ibuprofen (40)	50 ± 0.01	66.2 ± 6.87**	48.28
FGI (40)	50 ± 0.01	74.2 ± 3.98**	42.20
FGIII (40)	50 ± 0.01	77.4 ± 5.94**	42.45

In the present study analgesic and anti-inflammatory effects of different fractions of *F. glomerata* were tested in different experimental models of pain and inflammation. The non-narcotic model such as acetic acid-induced writhing and narcotic model such as tail flick method were used as the animal models for assessing analgesic activity. In acetic acid-induced abdominal constriction model, acetic acid causes inflammatory pain by inducing capillary permeability and release of arachidonic acid via cyclooxygenase pathway leading to prostaglandin biosynthesis which plays a significant role in the nociceptive mechanism [12-14]. Results from the study revealed that the intensity of antinociception of fractions of *F. glomerata* treated groups was higher than the control group in acetic acid-induced writhing in mice. The mechanism of analgesia by fractions of *F. glomerata* could probably be due to blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of ibuprofen and other NSAID's.

The most common test to evaluate narcotic drugs is the tail flick method. This test is based on phasic stimulus of high intensity. The nociceptive experience is short lasting and it is well accepted that μ opioid receptor agonists produce analgesia in acute pain models [15]. Therefore, it is believed that substances effective in tail flick model exert their effects predominantly through μ opioid receptors. The fractions of *F. glomerata* produced increased latency for tail flick which suggests that the analgesic activity may in part be mediated by opioid receptors. Collectively, fractions of *F. glomerata* exhibited analgesic activity in all the animal models of nociceptions used in this study and possibly exerted their effect through diverse mechanisms that may involve both central and peripheral pathways.

It is well known that carrageenan-induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. Serotonin, histamine, bradykinin and prostaglandins have been identified as mediators for carrageenan induced rat paw edema. The initial phase of carrageenan paw edema is mediated by histamine and serotonin, while the mediators in the later phase were suggested to be arachidonate metabolites (prostaglandins, leukotrienes) producing an edema dependent on mobilization of neutrophils [16]. In our experiments, the edematous response was only significantly suppressed in rats pretreated with the test extracts and their fractions on the first phase of the edema, suggesting an inhibitory effect on the release of histamine and/or serotonin. The edematous response was also significantly suppressed in rats pretreated with the test extracts and their fractions in the second and third phases of edema, suggesting an inhibition of 5-lipoxygenase and/or cyclooxygenase enzymes which are involved in the formation of prostaglandins and leukotrienes. This edematous response was also significantly reduced in rats pretreated with ibuprofen, a known cyclooxygenase inhibitor [17]. These effects were obtained due to presences of diversity of phytochemicals like tannins, steroids glauanol, glauanol acetate, β-sitosterol, lupeol acetate [18]. Analgesic and anti-inflammatory effects of flavonoids, steroids and tannins have been reported earlier [19, 20]. Deleterious effects of excessive release of nitric oxide (NO) have been implicated in tissue damage and inflammation. Tannic acid and polyphenols have been shown to be potent inhibitors of NO synthase activity and NO production, independent of their antioxidant activity [20]. Therefore, the analgesic and anti-inflammatory properties observed might thus be related in part to the tannins content of this plant. Hence, the analgesic and anti-inflammatory effects produced by these fractions may be attributed individually or collectively to the tannins and steroids.

5. Conclusion

The results presented here demonstrate that *F. glomerata* leaf fractions are capable of inhibiting pain and inflammation in rodents with analgesic and anti-inflammatory activity profile similar to previously described compounds. All results confirm that *F. glomerata* has great value as a source of tannins and polyphenol compounds with analgesic and anti-inflammatory properties.

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