Ficus Religiosa and *Ficus Glomerata* Extracts Exhibits Antiinflammatory Activity in the Rat

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Summary

The aim of the present study was to explore the probable antiinflammatory activity of different extracts of *Ficus religiosa and Ficus glomerata* leaves using carrageenan induced inflammation in the rat. Male Wistar rats were treated orally with normal saline [as control group] and *Ficus religiosa and Ficus glomerata* extracts [100, 200, 400, and 600 mg/kg], 60 min before 0.1 mL 1% carrageenan injection. Paw volume was measured before and 1, 2, and 3 h after the injection of carrageenan. The results were expressed as the Mean ±SEM and the statistical significance of differences between groups was analyzed by One Way Analysis of Variance [ANOVA] followed by Dunnett's test. The subplantar injection of carrageenan caused a time-dependent paw edema in the rat. Oral administration of *Ficus religiosa and Ficus glomerata* extracts [100, 200, 400, and 600 mg/kg] inhibited paw swelling dose-dependently at 1, 2, and 3, h after carrageenan injection. We can conclude from the outcome of the present work that *Ficus religiosa and Ficus glomerata* extracts exert an excellent antiinflammatory effect in rats.

Key Words: *Ficus religiosa*, *Ficus glomerata*, steroids, triterpenoids, Carrageenan,

Introduction

The use of foods and medicinal plants to improve health is nearly as old as humanity. Among such, none may be older than the fig, which recent investigations have indicated has been cultivated for over 11,000 years, possibly predating cereal grains [1]. Though this finding has recently been challenged [2], it has also been supported [3]. A number of *Ficus* species are used as food and for medicinal properties in Ayurvedic and Traditional Chinese Medicine [TCM] especially amongst people where these species grow. These uses, however, originated and are most widely found in the Middle East.

Ficus glomerata Roxb. syn. F. racemosa L. [Family: Moraceae], commonly known as Gular in Hindi and Cluster fig in English. It is medium sized to large evergreen or occasionally deciduous tree and found all over India and Southeast Asia. Its fruits are mixed with rice for making bread and used in several dishes. It has been reported to have many medicinal properties [4]. Traditionally the bark, fruits and latex are used to treat anemia and gastrointestinal disorders like constipation, dysentery [5]. The alcoholic extract of the fruit also possessed anti-filarial activity against Setaria cervi [6]. Fruits of F. glomerata contain glauanol, glauanol acetate, b-sitosterol, lupeol acetate [7-8]. The aerial part of plant contains b-sitosterol, lupeol and quercetin as major active con-64stituents [9]. Fruits of F. glomerata showed significant gastroprotective activity on physically and chemically induced gastric ulceration in rats [10].

Ficus *Linn* being the largest genus of the family Moraceae comprises about 755 fig tree species worldwide [11].According to the reports of traditional healers, Ficus species like *Ficus schimperiana* and *Ficus sycomorus* has been used for the treatment of epilepsy from ancient time [12]. *Ficus sycomorus* had shown anticonvulsant and sedative activity in animal models [13].*Ficus religiosa* [Moraceae] commonly known as Bodhi tree is regarded as a sacred tree to both Hindus as well as Buddhists; it is used for medicinal as well as religious purposes in India [14]. *Ficus religiosa* is reported to have numerous therapeutic uses in folk medicine viz.: leaf juice has been used for the treatment of asthma, cough, sexual disorders, diarrhea, haematuria, ear-ache and toothache, migraine, eye troubles, gastric problems and scabies;leaf decoction has been used as an analgesic for toothache; fruits for the treatment of asthma, other respiratory disorders and scabies; stem bark is used in gonorrhea, bleeding, paralysis, diabetes, diarrhea, bone fracture, antiseptic, astringent and antidote [15]. In Ayurveda it is claimed that *Ficus religiosa* possesses anticonvulsant activity [16].Many such reports had been validated pharmacologically for its actions on CNS viz.: different parts of *Ficus religiosa* showed acetylcholinesterase inhibitory activity [17] and Antianxiety activity [18]. Figs [fruits] of this plant contain numerous amino acids like asparagine and tyrosine in fruitedible part, alanine, threonine, tyrosine, and valine in seeds, alanineand valine in proteins [19].

Materials and Methods

Plant Material

The leaves of the plant *Ficus religiosa and Ficus glomerata* were collected from local region in July 2008. The plant material was identified and authenticated by P.G.Diwakar Botanical survey of India, Pune [Voucher No. BSI/WC/Tech/08/340]

Preparation of Extract:

The leaves were cleaned, dried under shade and powdered by a mechanical grinder. 500g of the powder was extracted with Petroleum ether, chloroform, methanol and water in order of their increasing polarity using soxhlet apparatus. The yield of extracts for *Ficus religiosa* was 4.2%, 8.26%, 13.2% and 4.9%, respectively and the yield for *Ficus glomerata* 4.8%, 7.56%, 14% and 5.86% respectively. Methanolic and aqueous extract were dissolved in normal saline whereas Petroleum ether and chloroform extracts were prepared in 2% gum acacia prior to oral administration.

Phytochemical screening
On preliminary screening the extracts showed the positive Liebermann–Burchard reaction for steroids [20] and a positive Noller test for triterpenoids. [21]

Animals:
Albino rats of Wistar strain [150-200 g] and Swiss albino mice [25-30 g] of either sex were procured from Yash farm, Pune. They were housed in standard polypropylene cages and kept under controlled room temperature [24 ± 2 ^{0}C; relative humidity 60-70%] in a 12 h light-dark cycle. The rats were given a standard laboratory diet and water ad libitum. Food was withdrawn 12 h before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee [IAEC].

Drugs:
The following chemicals and drugs were used: carrageenan [Sigma-Aldrich], Methanol [Qualigens, Mumbai], Petroleum ether [60-80°C, Qualigens, Mumbai], Chloroform [Qualigens, Mumbai], and Ibuprofen [Vikash Pharma, Mumbai] were used during experimental protocol

Anti-Inflammatory Activity:

Carrageenan induced hind paw edema
The effect of oral administration of 100, 200, 400 and 600 mg/kg of all the extract of *Ficus religiosa and Ficus glomerata*, 40 mg/kg ibuprofen or vehicle [Saline, 10ml/kg] on the hind-paw oedema induced by sub plantar injection of 0.1ml carrageenan [1% w/v] was evaluated according to the method described by Winter *et al*., [1962] [22]. In short, 0.1 mL of 1 % w/v carrageenan was injected into the sub plantar tissue of left hind paw of each rat. Swelling of carrageenan injected foot was measured at 0, 1, 2, 3 h using Plethysmometer [UGO Basile, Italy]. Animals were treated with test extract 1hour before the carrageenan injection. Measurement was carried out immediately before and 3hrs following carrageenan injection. Percent inhibition of test drugs was calculated in comparison with vehicle control [100%].

Statistical analysis
Results were analyzed using One way analysis of variance [ANOVA] and expressed as Mean ± SEM. Data was further subjected to Dunnett's test and differences between means were regarded significant at $P<0.01$ and $P<0.05$.

Results
The anti-inflammatory activity of the extract *Ficus religiosa and Ficus glomerata* leaves against acute pedal oedema has been shown in Tables 1 and Table 2 which showed significant anti-inflammatory activity and the results were comparable to that of control. It was observed that the methanolic extracts of both *Ficus religiosa and Ficus glomerata* [600 mg:kg, p.o.] exhibits maximum antiinflammatory activity against carrageenan induced hind paw edema. The inhibition obtained with *F religiosa and F glomerata* was 71.81 and 69.79 % respectively.

Table 1: Effect of varying doses of Ficus glomerata on Carrageenan induced rat paw edema

Treatment [mg/kg]	Mean increase in paw volume [mL]				% Decrease in paw volume at 3 h
	0 h	1 h	2 h	3 h	
Control	0.96 ± 0.01	1.52 ± 0.008	1.91 ± 0.004	2.45 ± 0.009	-
IBU [40]	0.91 ± 0.008	1.00 ± 0.01*	130± 0.003*	1.62 ± 0.001*	52.34
P.E[100]	0.96 ± 0.011	1.34 ± 0.022*	1.65± 0.03**	2.00 ± 0.042 **	30.20
PE [200]	0.97 ± 0.016	1.26 ± 0.029**	1.59± 0.035**	1.82 ± 0.043**	42.95
PE [400]	0.90 ± 0.02	1.21 ± 0.044**	1.49± 0.067**	1.70 ± 0.05**	46.30
PE [600]	0.90 ±0.014	1.24 ± 0.048**	1.40± 0.075**	1.61 ± 0.028**	52.34
CH [100]	0.90 ± 0.023	1.49 ± 0.021	2 ± 0.035	2.20 ± 0.09*	12.75
CH [200]	0.92 ± 0.025	1.41 ± 0.019	1.89 ± 0.031	1.99 ± 0.04**	28.18
CH [400]	0.95 ± 0.014	1.35 ± 0.035*	1.67 ± 0.024**	1.86 ± 0.035**	38.92
CH [600]	0.93 ± 0.016	1.26 ± 0.035**	1.56 ± 0.028**	1.75 ± 0.035**	42.28
ME [100]	0.98 ± 0.038	1.29 ± 0.036**	1.52 ± 0.022**	1.82 ± 0.041**	43.62
ME [200]	0.96 ± 0.037	1.23 ± 0.032**	1.43 ± 0.025**	1.65 ± 0.05**	53.69
ME [400]	0.93 ± 0.03	1.19 ± 0.061**	1.39 ± 0.034**	1.51 ± 0.082**	61.07
ME [600]	0.92 ± 0.025	1.14 ± 0.041**	1.22 ± 0.035**	1.34 ± 0.037**	71.81
AQ [100]	0.93 ± 0.019	1.43 ± 0.034	1.92 ± 0.062	2.29 ± 0.091	8.72
AQ [200]	0.95 ± 0.024	1.40 ± 0.043	1.81 ± 0.046	2.16 ± 0.068**	18.79
AQ [400]	0.94 ± 0.037	1.39 ± 0.052	1.75 ± 0.031	2.10 ± 0.053**	22.14
AQ [600]	0.96 ± 0.018	1.30 ± 0.059**	1.70 ± 0.037**	2.00 ± 0.041**	28.85

N= 5, treatment, mg/kg, data were analyzed using ANOVA and expressed as Mean ± SEM followed by Dunnett's test and differences between means were regarded significant at * [P<0.05], ** P<0.01

IBU – Ibuprofen, PE- Pet-ether extract, CH- Chloroform extract, ME-Methanolic extract, AQ- Aqueous extract

Table 2: Effect of varying doses of Ficus glomerata on Carrageenan induced rat paw edema

Treatment [mg/kg]	Mean increase in paw volume [mL]				% Decrease in paw volume at 3 h
	0 h	1 h	2 h	3 h	
Control	0.96 ± 0.01	1.52 ± 0.008	1.91 ± 0.004	2.45 ± 0.009	-
IBU [40]	0.91 ± 0.008	1.00 ± 0.01*	130 ± 0.003*	1.62 ± 0.001*	52.34
P.E[100]	0.93 ± 0.051	1.31 ± 0.027**	1.89 ± 0.035	2.1 ± 0.052**	21.47
PE [200]	0.94 ± 0.032	1.25 ± 0.029**	1.72 ± 0.051*	1.96 ± 0.54**	31.54
PE [400]	0.92 ± 0.02	1.21 ± 0.04**	1.61 ± 0.035**	1.8 ± 0.046**	39.59
PE [600]	0.93 ± 0.027	1.19 ± 0.034**	1.45 ± 0.036**	1.69 ± 0.036**	48.99
CH [100]	0.96 ± 0.048	1.47 ± 0.041	1.89 ± 0.041	2.24 ±0.035	14.09
CH [200]	0.93 ± 0.02	1.44 ± 0.031	1.75 ± 0.041	2.01 ± 0.048**	27.51
CH [400]	0.93 ± 0.025	1.4 ± 0.032	1.69 ± 0.037**	1.8 ± 0.04**	41.61
CH [600]	0.94± 0.025	1.35 ± 0.051	1.65 ± 0.036**	1.74 ± 0.035**	46.30
ME [100]	0.98 ± 0.038	1.29 ± 0.036**	1.61 ± 0.035**	1.86 ± 0.043**	40.93
ME [200]	0.96 ± 0.037	1.24 ± 0.035**	1.53 ± 0.034**	1.7 ± 0.049**	50.33
ME [400]	095 ± 0.046	1.19 ± 0.061**	1.33 ± 0.037**	1.45 ± 0.036**	66.44
ME [600]	0.94 ± 0.023	1.15 ± 0.036**	1.29 ± 0.034**	1.39 ± 0.035**	69.79
AQ [100]	0.92 ± 0.017	1.5 ± 0.053	1.92 ± 0.062	2.27 ± 0.1	9.39
AQ [200]	0.93 ± 0.034	1.49 ± 0.05	1.84 ± 0.054	2.12 ± 0.075**	20.13
AQ [400]	0.93 ± 0.038	1.47 ± 0.035	1.75 ± 0.031	1.96 ± 0.048**	30.84
AQ [600]	0.92 ± 0.028	1.44 ± 0.038	1.7 ± 0.037**	1.89 ± 0.045**	34.89

N= 5, treatment, mg/kg, data were analyzed using ANOVA and expressed as Mean ± SEM followed by Dunnett's test and differences between means were regarded significant at * [P<0.05], ** P<0.01

IBU – Ibuprofen, PE- Pet-ether extract, CH- Chloroform extract, ME-Methanolic extract, AQ- Aqueous extract

Discussion

In order to provide a scientific explanation fort he folk use of *Ficus religiosa and Ficus glomerata*, we have investigated the biological effects of its extracts, mainly the ones related to the inflammatory process. The present data clearly showed that extracts of dried leaves of *Ficus religiosa and Ficus glomerata* have antiinflammatory activity by the highly significant responses of some extracts on inhibiting the edema formation after carrageenan subplantar injection.

The extracts which showed the highest antiinflammatory activity, presented also highly significant statistic values [$P<0.01$] for carrageenan induced edema inhibition: 71.81 and 69.79 % respectively, 1, 2 and 3 h after the treatment with the phlogistic agent.

The present study establishes the anti-inflammatory activity of extracts of *Ficus religiosa and Ficus glomerata*. It is evident that carrageenan is a sulphated polysaccharide obtained from sea weed [Rhodophyceae] and is commonly used to induce acute inflammation and is believed to be bi-phasic.

The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome. Based on this, it would be argued that suppression of Ist phase may be due to inhibition of release of early mediators, such a histamine, serotonin and action in IInd phase may be explained by an inhibition of cyclo-oxygenase. These mediators take part in inflammatory response and are able to stimulate nociceptive and thus reduce pain. It has been reported that second phase of oedema is sensitive to most clinically effective anti inflammatory drugs, which has been frequently used to access the anti-edematous effect of natural products [23]. Based on these reports, it can be inferred that the inhibition effect of the extract of *Ficus religiosa and Ficus glomerata* on carrageenan induced inflammation in rats may by due to inhibition of the mediators responsible for inflammation.

In the present study, the phytochemical analysis for both the plants revealed that the plant had the triterpenes glutinol phytosterols stigmasterol, sitosterol and campesterol, already known to possess anti-inflammatory properties or related biological activities [24-26]. Therefore, these compounds appear to be among the constituents implicated in some of the pharmacological activities displayed by extracts of *Ficus religiosa and Ficus glomerata*.

So we can conclude that the present study supports the claims by traditional medicine practitioners about the usefulness of *F. racemosa* and *Ficus glomerata* in inflammatory diseases.

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