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Phytochemical and analgesic, anti-inflammatory screening of methanolic extract of *Ficus religiosa* fruits: An *in vivo* design

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Abstract

Background: Chronic inflammatory diseases remain one of the world's major health problems. Currently, both steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs (NSAIDs) are used in the aid of inflammation.

Objective: In the present research, we evaluated the analgesic and anti-inflammatory effect of methanolic extract of *Ficus religiosa* Linn. fruits on animal models.

Methods: The methanol extract of the dried fruits of *Ficus religiosa* Linn. (Peepal tree) was screened for its anti-inflammatory activity in Wistar albino rats and analgesic effects in Swiss albino mice.

Results: A significant inhibition of carrageenan-induced rat paw oedema, comparable to that produced by ibuprofen (40mg/kg i.v.), the standard anti-inflammatory drug, was obtained with all the two doses (200 and 400mg/kg) of the extract, tested in the present study. A significant inhibition of acetic acid-induced writhing in mice was observed with two doses of the extract (200 and 400mg/kg). The analgesic effect was comparable to that caused by the standard drug, Diclofenac (5mg/kg i.p.). The effect was more pronounced with high dose (400mg/kg) than low dose (200mg/kg).

Conclusion: The study has shown that the methanol extract of *Ficus religiosa* fruits does possess significant anti nociceptive and anti-inflammatory effects in laboratory animals at the doses investigated. Therefore the fruits part of the plant could be used in the management of pain and inflammatory conditions.

Keywords: *Ficus religiosa*, anti inflammatory, methanol, ibuprofen, Diclofenac, analgesic activity

Introduction

Despite progress within medical research during the past decades, the treatment of many serious diseases remains problematic. Chronic inflammatory diseases remain one of the world's major health problems. Currently, both steroidal anti-inflammatory drugs and non-steroidal anti-inflammatory drugs (NSAIDs) are used in the aid of inflammation^[1, 2]. Steroids have an obvious role in the treatment of inflammatory diseases, but due to their toxicity, they 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].

Ficus religiosa is a variety of fig tree that was already known as the bodhi tree, even before Gautama Buddha sat under its branches meditating and achieved enlightenment. It is the oldest depicted tree in Indian art and literature and it can be said that this is the mythical 'World Tree' or the 'Tree of Life' of the Indian subcontinent. This plant is considered sacred by the followers of Hinduism, Jainism and Buddhism, and hence the name 'Sacred Fig' was given to it. *Ficus religiosa* L. (Moraceae) commonly known as 'Peepal' is a variety of fig and sacred tree native to India^[5-7]. It is reported to have numerous therapeutic uses in folk medicine viz. leaf juice has been used for the treatment of asthma, cough, sexual disorders, diarrhoea, hematuria, 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 gonorrhoea, bleeding, paralysis, diabetes, diarrhoea, bone fracture, antiseptic, astringent, and antidote. In Ayurveda, it is claimed that *Ficus religiosa* possesses anti convulsant activity. It also showed acetyl cholinesterase inhibitory activity and anti anxiety activity. Fruits of this plant contain numerous amino acids whereas the fig of this plant has been reported to contain highest amount of serotonin [5-HT] as compared to figs of other *Ficus* species^[8, 9].

The tree fruits in May/June and bears a small flat-topped figs (12-13 mm or ½ inch in diameter), which appears in pairs in the angles of the leaves on the twigs (or above the scars in the bark left by fallen leaves). They have 3 basal bracts, are green at first and ripen to a blackish purple (may have reddish dots). The fruiting tree becomes a treat for many different birds and animals [10-13]. Although its fruit is extensively used in traditional medicine as analgesic and anti-inflammatory, no scientific evaluation of fruits is available. A study has, therefore, been carried out to investigate the analgesic and anti-inflammatory activity of extract of *Ficus religiosa* fruits.

Taxonomy / botanical classification: [14]

Domain: Eukaryota
 Kingdom: Plantae
 Subkingdom: Viridiplantae
 Phylum: Tracheophyta
 Subphylum: Spermatophytina
 Infraphylum: Angiospermae
 Class: Magnoliopsida Brongniart
 Subclass: Dilleniidae
 Super order: Urticanae
 Order: Urticales
 Family: Moraceae
 Division: Magnoliophyta
 Tribe: Ficeae
 Genus: *Ficus* (FY-kus) Linnaeus
 Specific epithet: *religiosa* L.



Fig 1: *Ficus religiosa* plant



Fig 2: *Ficus religiosa* fruits

The objective of this research is to screen the analgesic and anti-inflammatory activity of FRFME (*Ficus religiosa* fruit methanolic extract) in mice and rat.

Materials and Methods

Plant material and extraction

The fresh ripen fruits of *Ficus religiosa* were collected locally, identified and authenticated by Mr. G. Baba Shankar Rao, Department of Phamacognosy. In this process, the completely dried and coarse powder of crude drug (1000g) was obtained. Place about 350g of powdered crude drug in a muslin cloth of soxhlet apparatus with the 100ml methanol solvent. The drug from the powdered fruits was collected into 300ml of methanol.

The above obtained extract was kept under sunlight for about two weeks to obtain a semisolid extract. This semisolid extract was then administered orally into the experimental animals using water as a solvent.

Preliminary phytochemical screening [15-17]

The different chemical tests were performed for establishing profile of the fruits extract for its chemical composition; the following chemical tests for various phytoconstituents in the methanolic extract was carried out as described below.

(A) Test for alkaloids

i) Dragendroff's Test: In a test tube containing 1ml of extract, few drops of Dragendroff's reagent was added and the colour developed was noticed. Appearance of orange colour indicates the presence of alkaloids.

ii) Wagner's Test: To the extract, 2 ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicates the presence of alkaloids.

iii) Mayer's Test: To the extract, 2 ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

iv) Hager's Test: To the extract, 2 ml of Hager's reagent was added; the formation of yellow precipitate confirmed the presence of alkaloids.

(B) Test for Terpenoids

i) Salkowski test: To 1 ml of extract, tin (one bit) and thionyl chloride were added. Appearance of pink colour indicates the presence of terpenoids.

ii) Hirshonn reaction: When the substance was heated with trichloroacetic acid, red to purple colour was observed.

(C) Test for Steroids

i) Liebermann-Burchard Test: To 1ml of extract, 1ml of glacial acetic acid and 1ml of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution become red, then blue and finally bluish green indicates the presence of steroids.

(D) Test for Coumarins

i) To 1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.

(E) Test for Tannins

- i) To few mg of extract, ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.
- ii) The extract was mixed with basic lead acetate solution; formation of white precipitate indicated the presence of tannins.

(F) Test for Saponins

- i) To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

(G) Test for Flavones

- i) **Shinoda Test:** To the extract, a few magnesium turnings and 2 drops of concentrated hydrochloric acid were added, formation of red colour showed the presence of flavones.
- ii) To the extract, 10% sodium hydroxide or ammonia was added; dark yellow colour shows the presence of flavones.

(H) Test for Quinones

- i) To 1 ml of the extract 1 ml of concentrated sulphuric acid was added. Formation of red colour shows the presence of quinones.

(I) Test for Flavanones

- i) To the extract, 10% sodium hydroxide was added and the colour changes from yellow to orange, which indicates the presence of flavanones.
- ii) To the extract, conc. sulphuric acid was added, and the colour changes from orange to crimson red, which indicates the presence of flavanones.

(J) Test for Anthocyanins

- i) To the extract, 10% sodium hydroxide was added, and the blue color shows the presence of anthocyanins.
- ii) To the extract, conc. sulphuric acid was added, and the yellowish orange colour confirms the presence of anthocyanins.

(K) Test for Anthraquinones

- i) **Borntrager's test:** The extract was macerated with ether and after filtration; aqueous ammonia or caustic soda was added. Pink red or violet colour in the aqueous layer after shaking indicates the presence of anthraquinones.

(L) Test for Phenols

- i) **Ferric chloride test:** To the extract, few drops of 10 % aqueous ferric chloride were added. Appearance of blue or green colour indicates the presence of phenols.

(M) Test for Proteins

- i) **Biuret Test:** To the extract, 1 ml of 40% sodium hydroxide solution and two drops of one percent copper sulphate solution were added. Formation of violet colour indicates the presence of proteins.

- ii) **Xanthoprotein Test:** To the extract, 1 ml of concentrated nitric acid was added. A white precipitate was formed; it is then boiled and cooled. Then 20% sodium hydroxide or ammonia was added. Orange colour indicates the presence of aromatic amino acids.

- iii) **Tannic Acid Test:** To the extract, 10% tannic acid was added. Formation of white precipitate indicates the presence of proteins.

(N) Test for Carbohydrates

- i) **Molisch's Test:** To the extract, 1 ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

- ii) **Fehling's Test:** To the extract, equal quantities of fehling's solution A and B were added and on heating, formation of a brick red precipitate indicates the presence of carbohydrates.

- iii) **Benedict's Test:** To 5 ml of Benedict's reagent, extract was added and boiled for two minutes and cooled. Formation of red precipitate showed the presence of carbohydrates.

(O) Test for amino acids

- i) **Ninhydrin test:** Two drops of ninhydrin solution were added to the extract, a characteristic purple colour indicates the presence of amino acids.

(P) Test for fixed oils and fats

- i) **Spot Test:** A small quantity of extract was pressed between two filter papers. Oil stains on the paper indicates the presence of fixed oils and fats.

(Q) Test for volatile oils

- i) To the section of drug, add alcoholic solution of Sudan III. Formation of red colour obtained by globules indicates the presence of volatile oils.
- ii) To the thin section of drug, add a drop of tincture alkaline. Formation of red colour indicates the presence of volatile oils.
- iii) To the test sample, add 1% Osmic acid. Formation of black colour indicates the presence of volatile oils.

Animals

24 Albino mice weighing 20-30g were housed at 24±1 °C on a 12:12 h light dark cycle (lights on at 6a.m), and with free access to food and tap water. 8 hrs before the experiment, only tap water was available to the mice. All experiments were performed between 10a.m and 5p.m. The animals were randomly allocated to different experimental groups.

24 Wister rats weighing 200-300g were housed at 24±1 °C on a 12:12 hr light dark cycle (lights on at 6a.m), and with free access to food and tap water. 8hrs before the experiment, only tap water was available to the mice. All experiments were performed between 10a.m and 5p.m. The animals were randomly allocated to different experimental groups. The experiment protocol was approved by an Institutional Animal Ethics Committee of School of Pharmacy, Anurag Group Institutions and care of the animals was taken as per guidance of the Committee for the Process of Control and Supervision of Experiments on Animals (Reg no: 1412/a/11/CPCSEA).

Chemicals and Drugs

Table 1: List of chemicals

Water	Diclofenac (5mg/kg)
Methanol (700ml)	Ibuprofen (40mg/kg)
Acetic acid (0.1% v/v)	Carrageenan (0.1ml for each rat)

Analgesic evaluation methods

- 1. Writhing tests:** Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhing. The test is suitable to detect analgesic activity although some psychoactive agents also show activity. An irritating agent such as phenylquinone or acetic acid is injected intraperitoneally to mice and the stretching reaction is evaluated. The reaction is not specific for the irritant.
- 2. Procedure:** Mice of either sex with a weight between 20 and 25 g are used. 0.1ml of acetic acid is injected intraperitoneally. Groups of 6 animals are used for controls and treated mice. Preferably, two groups of 6 mice are used as controls. Test animals are administered the drug or the standard at various pre-treatment times prior to phenylquinone administration. The mice are placed individually into glass beakers and five min are allowed to elapse. The mice are then observed for a period of ten min and the number of writhes is recorded for each animal. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. The formula for computing percent inhibition is: average writhes in the control group minus writhes in the drug group divided by writhes in the control group times 100%. The time period with the greatest percent of inhibition is considered the peak time.
- 3. Evaluation:** A dose range is run in the same fashion as the time response except 6 animals/ groups are tested at the peak time of drug activity. Four drug groups and a vehicle control group are employed. Animals are dosed and tested in a randomized manner.

Table 2: Doses of control and standard for analgesic activity

Groups	Name	Treatment
1.	Control	Saline (0.09%, orally)
2.	Standard (Positive Control)	Diclofenac Sodium (5mg/kg, i.p.)
3.	Low Dose	FRFME (200mg/kg, o.p.)
4.	High Dose	FRFME (400mg/kg, o.p.)

Anti-inflammatory evaluation methods

Inflammatory paw edema test in mice

In this part of the experiment, the anti-inflammatory activity of the methanolic extract was investigated on carrageenan-induced inflammatory paw edema.

Procedure: The methanolic extracts of fruits of *Ficus religiosa* was dissolved and dispersed in physiological saline (0.09%) and administrated by orally for pre-treated group of mice at 200 mg/kg dosage. Physiological saline (0.09%) was given to the control group at the same volume as vehicle. One hour after administration, 0.1 ml of 0.5% carrageen solution

was injected into the footpad of the hind paws of each mouse in all groups. Prior to carrageenan injection, the mice paw volume was measured with a plethysmometer. Increasing of carrageenan induced inflammatory paw volume was measured at 1, 3, and 6 h over the injection. The anti-inflammatory activity of *Ficus religiosa* extracts was compared with that of 40 mg/kg ibuprofen.

Evaluation: The percentage inhibition of the inflammation was calculated from the formula:

$$\text{Inhibition \%} = (D - Dt) / Do * 100$$

where,

D is the diameter of injected paw,

Do is the average inflammation (hind paw edema) of the control group of mice at a given time 0; and

Dt is the average of diameters of hind paw edema of the drug treated (i.e. extract or reference ibuprofen) mice at the same time.

Table 3: Doses of control and standard for anti-inflammatory activity

Groups	Name	Treatment
1.	Control	Saline (0.09%, orally)
2.	Standard (Positive Control)	Ibuprofen (40mg/kg, i.v.)
3.	Low Dose	FRFME (200mg/kg,)
4.	High Dose	FRFME (400mg/kg,)

Results and Discussion

Preliminary Phytochemical Screening:

Our observation revealed that in the preliminary phytochemical screening was found that the methanolic fruit extract of *Ficus religiosa* contain terpenoids, flavonoids, tannins, glycosides and phenols. The preliminary phytochemical screening results are shown in Table 4.

Table 4: Preliminary phytochemical screening of *Ficus religiosa* fruits

Constituents	Methanolic extract
Terpenoids	+
Saponins	-
Steroids	-
Carbohydrates	-
Flavonoids	+
Alkaloids	-
Quinones	-
Tannins	+
Fixed oils and fats	-
Phenols	+
Glycosides	+
Amino acids	-
Anthraquinones	-
Volatile oils	-

(+) Present, (-) Absent

Table 5: Anti-inflammatory effect of methanolic extract of *Ficus religiosa* on Carrageenan induced oedema in rats

Sample	DOSE (mg/kg)	PAW Oedema (Mean ± SEM)				Oedema (%)			
		30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min
Control	2 ml Saline	0.3700 ± 0.0108	0.4750 ± 0.0086	0.6475 ± 0.013	0.7950 ± 0.013	-	-	-	-
Test-1	200	0.2825 ± 0.0125*	0.3550 ± 0.0064*	0.4825 ± 0.018*	0.5625 ± 0.013*	23.25	25.27	25.49	29.25
Test-2	400	0.2100 ± 0.0070*	0.2425 ± 0.011*	0.3600 ± 0.018*	0.4000 ± 0.010*	43.25	48.95	49	49.64
Standard	40	0.1780 ± 0.0064*	0.1975 ± 0.063*	0.2650 ± 0.0104*	0.2850 ± 0.012*	52.71	58.43	59.08	64.16

Values are expressed as mean±SEM

*p<0.05 as compared to control group

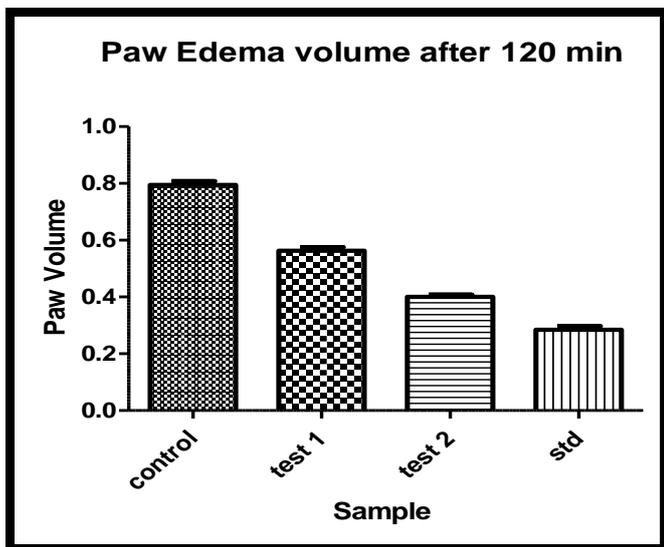


Fig 3: Paw edema volume after 120 minutes

Table 6: Analgesic effect of methanolic extract of *Ficus religiosa* on acetic acid algisia in rats

Sample	Dose (mg/kg)	Writhing (Mean ± SEM)	Writhing Inhibition (%)
Control	2ml Saline	0.4025±0.0047	-
Test-1	200	0.2325±0.0854*	42.23
Test-2	400	0.1700±0.00408*	57.76
Standard	5	0.1025±0.00479*	74.53

Values are expressed as mean±SEM

*p<0.05 as compared to control group

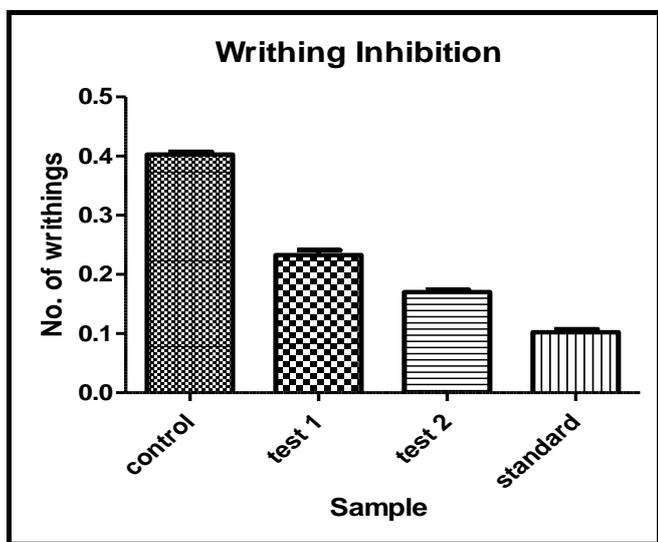


Fig 4: Writhing inhibition

The phytochemical screening revealed the presence of flavonoids, phenols, terpenoids and glycosides. The relatively high LD50, value 400mg/kg obtained in this study for *Ficus religiosa* methanolic fruit extract suggests that the plant extract is relatively safe to mice. The methanol fruit extract showed analgesic activity in acetic acid induced writhing test. The peripheral analgesic effect of the plant extract may be mediated via inhibition of cyclo-oxygenases. Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception. The ability of the fruits extract to prolong the reaction latency to pain thermally induced in mice suggests that the fruits extract has some central analgesic activity. In the anti-inflammatory studies, the percentage

inhibition obtained showed that the fruits extract exhibited significantly reduction in the oedema in the hind paw of the rats.

The analgesic and anti-inflammatory effects of flavonoids, phenols, terpenoids and glycosides have been reported. Based on this finding it seems that the analgesic and anti-inflammatory effects produced by the extract of *Ficus religiosa* fruits may be attributed individually or collectively to the flavonoids, phenols, terpenoids and glycosides present.

Conclusion

The study has shown that the methanol extract of *Ficus religiosa* fruits does possess significant anti nociceptive and anti-inflammatory effects in laboratory animals at the doses investigated. Therefore the fruits part of the plant could be used in the management of pain and inflammatory conditions. It also contains some biologically active constituents worthy of further investigations. Further work can be carried out to isolate the compounds and screen for their biological activities. Further studies (including the analysis and identification of the specific active compounds, toxicological and haematological studies) with this plant extract should be carried out using higher animal models, in order to authenticate it as a potent analgesic and anti-inflammatory effects. These local ethno medical preparations of plant sources should be scientifically evaluated and then disseminated properly. This knowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology. A large scale isolation and further spectral techniques are required to isolate and identify a particular compound responsible for anti-inflammatory activity.

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Conflict of interest

There is no conflict of interest.

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