Evaluation of Antinociceptive Activity of *Ficus Religiosa* Root Extract in Swiss Albino Mice

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ABSTRACT

Background

Ficus religiosa, commonly known as peepal, is widely distributed in Indian subcontinent. It has been used as an antiepileptic, aphrodisiac, analgesic, anti-inflammatory and laxative in traditional medicine.

Objective

To explore the analgesic effect of aqueous root extract of Ficus religiosa using thermal and chemical models of pain in swiss albino mice.

Method

The aqueous aerial root extract of *Ficus religiosa* was prepared using soxhlet apparatus. The anti-nociceptive effect of the extract at doses of 50 and 100 mg/kg was evaluated using peripheral (acetic acid-induced abdominal writhing), spinal (tail flick) and supra-spinal (hot plate) behavioral models of pain. All data were presented as Mean ± SEM. Statistical differences between *Ficus religiosa* (50 and 100 mg/kg) and standard control groups were evaluated using Mann-Whitney U test.

Result

There was significant dose dependent increase in the mean reaction time compared to the vehicle control in hot plate and tail- flick test. In acetic acid induced writhing test, mice treated with *Ficus religiosa* (50 and 100 mg/kg) exhibited significant dose-dependent decrease in the mean number of writhes (57.45% and 79.20% respectively) compared to the vehicle control. The activity of *Ficus religiosa* extract at doses of 50 and 100 mg/kg was equipotent to Standard control (Morphine and Indomethacin) used in different test models.

Conclusion

The extract of *Ficus religiosa* possesses both central and peripheral analgesic activity thus validating the traditional use of this plant in the management of pain.

KEY WORDS

Antinociceptive, Acetic acid induced writhing, Ficus religiosa, Hot plate method, Tail-flick method

INTRODUCTION

Drugs that are basically used for the management of pain are Non-Steroidal Antiinflammatory Drugs (NSAIDs) and opioid analgesics. Unfortunately, these drugs have many side effects and are costly too.[1,2] The World Health Organization is encouragingly supporting herbal medicines in developing countries as allopathic medicines are expensive and beyond reach of common people.[3] *Ficus religiosa (FR)* is widely distributed in the Indian subcontinent. Its seeds, barks, leaves, fruits, latexes and roots have been used in traditional medicine for the treatment of different diseases such as epilepsy, elephantiasis, diabetes, toothache, migraine, earache, skin and sexual problems.[4] Its root has been used in the treatment of gout, stomatitis, gingivitis and back pain.[5,6]

The analgesic property of bark and leaf of *FR* have been studied. The aqueous extract of bark of *FR* has anti-inflammatory effect in both acute and chronic models of inflammation and protected mast cell degranulation showing its beneficial role in kumkum dermatitis and various inflammatory conditions.[7,8] The methanol extract of leaf of *FR* inhibited production of nitric oxide and pro-inflammatory cytokines in Lipopolysaccharide-stimulated microglia.[9] Gulecha et al. had reported that different fractions of leaves of *FR* had both analgesic and anti-inflammatory activities.[10] However, till date to our best knowledge there are no studies done to delineate the analgesic property of root of *FR* despite of its wide ethno-pharmacological uses. The aim of this study was to evaluate the antinociceptive activity of the aqueous root extract of *FR* in experimental models of pain in mice.

METHODS

It was a quantitative experimental study in mice conducted in laboratory of Department of Clinical Pharmacology and Therapeutics, B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal during March-August 2014.

The aerial roots of *FR* were collected from the garden of BPKIH, Dharan with latitude and longitude 26° 49′ 0″ N and 87° 17′ 0″ E respectively. Specimen of the test drug was authenticated by a pharmacogonist, Dr. Shadmawahab and it was also deposited in National Herbarium and Plant Laboratories, Kathmandu, Nepal (voucher number 5021). The material was washed, shade dried for seven days and then grinded to fine powder. About ten gram of the fine powder was taken in clean sterile Soxhlet apparatus (Jain Scientific Glass Works Ambala Cantt; Extraction Pot: 250 ml; Soxhlet chamber size: 100 ml; Heater: DICA India) and extracted with 150 ml of distilled water continuously for six hours. The extract obtained was filtered with Whatman filter paper 1. The filtrate was evaporated at 50°C for a brief time interval, stopped just before the apparently saturated solution precipitated and left in room temperature till the moisture dried. The final percentage yield was 20% (w/w) which was used in the experiments. On the day of experiment, 5 mg/ml and 10 mg/ml solution of the extract were prepared in distilled water and used.[11]

All oral drugs were carefully administered with the help of oro-gastric tube 30 minutes prior to the experiment. All parenteral drugs were given through intraperitoneal (IP) injection route.

The study was conducted in Swiss albino mice and weighing 20-30 g and apparently free of any disease or handicap. Seventy-two healthy Swiss albino mice of either sex were used. The mice were twelve weeks old and bred in the breeding house of BPKIHS (Figure 1).

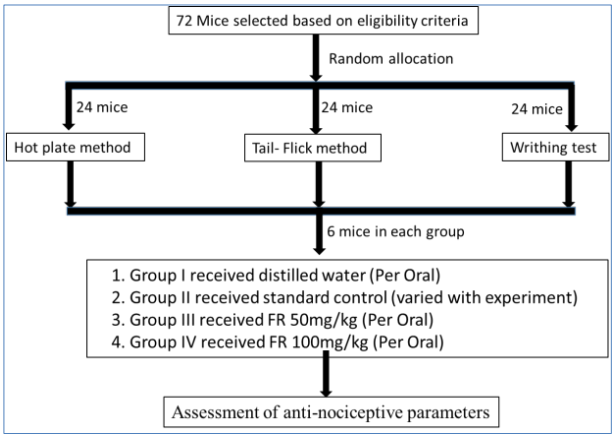

Figure 1. Study flowchart

Study setting: It was conducted in Laboratory of Department of Clinical Pharmacology and Therapeutics, BPKIHS between 8:00 and 16:00 hours. The average temperature was between 11 to 21°C. The animals were maintained under controlled room temperature (22±3°C) and light and dark (12:12 hours) conditions. They were given food pellets and water ad libitum; however, they were fasted overnight before the experiment. The animals were randomly selected and six mice were used per group in the experiments (Figure 1). The study was approved by the Institutional Ethical Review Board of BPKIHS (IERB number: 898/070/071).

Drugs and chemicals used in experimental models

1. Test drug: Aqueous solutions of aerial root extracts of *FR* 50 mg/kg and 100 mg/kg were given PO

2. Morphine (Martindale Pharmaceuticals, UK) 5 mg/kg

3. Acetic acid (Qualigens fine chemicals, India) at 60 mg/kg

4. Indomethacin (Indocap, Jagsonpal Pharmaceuticals, India) 20 mg/kg

5. Distilled water (Hindustan medicines, India)

Experimental designs

A total of 24 mice were used in a single experiment and each group consisted of six mice. The experimental mice

given various drugs as following:

Group I: Distilled water per oral

Group II: The standard control varied with the experiment

Group III: FR extract 50 mg/kg per oral

Group IV: FR extract 100 mg/kg per oral

Experimental methods

(i) Hot plate method: This test was performed according to the method described by Woolfe and MacDonald.[12] The thermal noxious stimuli was induced to the mice by placing them in a hot plate (UGO Basile, Italy) maintained at 55°C, ten minutes prior to the experiment. The reaction time was recorded. Reaction time was taken as the period between placing the mice in hot plate and the time when they jumped or licked their paws which ever occurred first. A cut off time of 30 seconds was used to minimize injury to the paws of mice. Morphine (5 mg/kg intraperitoneal injection) was the standard control which was administered 15 minutes prior to the test. The FR extracts were given 45 minutes prior to the experiment.

(ii) Tail–flick technique: Pain was induced by directing infrared light (Tail-Flick Unit, UGO Basile, Italy) 5 cm away from the tip of the tail. Response was achieved by observing the interval between placing the tail on the infrared light and the withdrawal of the tail. The cut-off time was set to 15 seconds to avoid damage to the tail of the mice. Morphine (5 mg/kg intraperitoneal injection) was the standard control, administered 15 minutes prior to the test.13 The FR extracts were given 45 minutes prior to the experiment.

(iii) Writhing test: This test is used for the evaluation of peripheral analgesic activity. At 30 minutes post-dosing with the drugs, 0.6 % acetic acid at a dose of 60 mg/kg was injected through intraperitoneal injection to the mice. The number of abdominal constrictions (writhes) produced were counted for ten minutes. Antinociception was expressed as the difference in the number of writhes between distilled water and the test drug group. Standard control for this test was Indomethacin (20 mg/kg per oral).[14] The percentage inhibition of writhing was calculated by using formula:- [(C-D)/C] x 100 where C is the average number of writhing for control group of mice and D is the average writhing of the test drug-treated mice.

Acute toxicity study: Organisation for Economic Co-operation and Development guideline number 425 using Swiss albino mice (25-30 g) was used to perform acute toxicity study of the aqueous extract of *FR*. The aqueous extract of *FR* up to dose limit of 2000 mg/kg was serially administered in six mice. Then each animal was observed every hour for signs of toxicity and abnormality in behavior up to 48 hours. Subsequently daily observations were made for toxicity and mortality up to 14 days.[11] Based on this toxicity test, two doses of *FR* extract, 50 mg/kg and 100 mg/kg, were selected for administration in the mice.

Statistical analysis: The data were compiled and entered into Microsoft Excel 2010. Descriptive statistics like Mean ± Standard Error of Mean (SEM) were calculated. The data were presented as tables. Statistical differences between the test drug and control groups were calculated using Mann–Whitney U test. Results were considered to be statistically significant at p value less than 0.05. The data were analyzed using SPSS version 11.

RESULTS

Effect of *FR* on nociception in Hot plate test: Pretreatment with *FR* (50 and 100 mg/kg) demonstrated a dose-dependent increase in the mean reaction time in Hot plate test. There was statistically significant ($p < 0.05$) difference between *FR* (50 and 100 mg/kg) and control group (Group I) whereas no statistically ($p > 0.05$) difference was found between Group II (Standard control) and *FR* (50 and 100 mg/kg). Thus, the antinociceptive effect of *FR* at doses of 50 and 100 mg/kg was similar to that of Morphine (Table 1).

Table 1. Hot plate test

Groups	Drug	Mean reaction time in sec (Mean± SEM)
Group I	Distilled water	11.45± 1.09
Group II	Morphine	19.51±1.97[a]
Group III	FR 50 mg/kg	16.58±1.72[ab]
Group IV	FR 100 mg/kg	16.98 ± 0.97[ab]

[a]P value < 0.05 vs Control; [b]P-value > 0.05 vs Group II

Effect of *FR* on nociception in Tail- flick test: The pretreatment of mice with doses 50 and 100 mg/kg *FR* extract significantly ($p < 0.05$) increased the mean tail-flick latency time compared to the control group. There was no statistical ($p > 0.05$) difference between Group II (Standard control) and *FR* (50 and 100 mg/kg) (Table 2).

Table 2. Tail- flick test

Groups	Drug	Mean latency time in seconds (Mean± SEM)
Group I	Distilled water	1.90±0.17
Group II	Morphine	10.95±1.11[a]
Group III	FR 50 mg/kg	7.83±1.37[ab]
Group IV	FR 100 mg/kg	8.45±1.11[ab]

[a]P value < 0.05 vs Control; [b]P-value > 0.05 vs Group II

Effect of *FR* on nociception in Acetic acid induced Writhing test: *FR* extract at 50 and 100 mg/kg produced a dose dependent inhibition of writhing by 57.45% and 79.20% respectively. The decrease in number of writhes between vehicle control and *FR* (50 and 100 mg/kg) was significant ($p < 0.05$). The effect of *FR* (50 and 100 mg/kg) in decreasing the number of writhing was similar to that of Indomethacin ($p > 0.05$) (Table 3).

Table 3. Acetic acid induced Writhing test

Groups	Drug	Mean number of writhing in 10 seconds (Mean± SEM)	% inhibition of writhing
Group I	Distilled water	16.83± 1.44	---
Group II	Indomethacin	2.5±1.14[a]	85
Group III	FR 50 mg/kg	7.16±1.74[ab]	57.45
Group IV	FR 100 mg/kg	3.50±1.11[ab]	79.20

[a]P value < 0.05 vs Control; [b]P-value > 0.05 vs Group II

DISCUSSION

The present study is the first report demonstrating anti-nociceptive effect of aqueous root extract of *FR* at oral doses of 50 and 100 mg/kg. The antinociceptive tests used for the present study were namely thermal (hot plate test), radiant (tail flick test) and chemical (acetic acid). More than one test was used for the confirmation of the antinociceptive effect as 'false positivity' can be observed with agents that aren't normally considered as analgesic.[15]

The tail-flick and hot plate test are used for screening compounds which have central analgesic activity. The tail-flick response is spinally mediated reflex whereas the hot plate response is supra-spinally organized behavior.[16] In both the test models, the present study showed that *FR* extract increased the pain threshold in dose-dependent manner and the effect was similar to that of the standard control (Morphine) which acts on μ receptor. On one hand, the activation of μ receptor leads to pain relief whereas on the other side leads to respiratory depression, constipation and physical dependence which is a limiting factor for it therapeutic considerations.[2] So, the *FR* root extract could be a better substitute for the opioid drugs which exerted its analgesic effect most likely by spinal and supra-spinal pathway; however, the definitive mechanism of action of *FR* extract can only be ascertained after binding studies.

Acetic acid injected in the intra-peritoneum produces abdominal constrictions or feelings of pain by inducing capillary permeability as well as by liberating prostaglandins such as PGE2 and PGF2 from arachidonic acid.[17] Vanilloid receptor (VR1) is also involved in the acid-induced writhing responses in mice.[18] Therefore, this test is used for screening compounds which have peripheral analgesic activity. In the present study, *FR* extract showed dose dependent and significant inhibition of acetic acid induced writhes in mice. The analgesic effect could probably be due to blockade of the effect or the release of endogenous substances that excite pain nerve endings similar to that of Indomethacin and other NSAIDs.[19]

Phytochemical analysis of the root of *FR* has revealed the presence of flavonoids, glycosides, steroid, tannin and saponin. Several studies have shown that the presence of phytochemical constituents such as tannin, saponin and glycoside have analgesic and anti-inflammatory activities through inhibition of cyclo-oxygenase pathway.[20] Different flavonoid molecules possess analgesic activity by inhibiting phospholipase A2 and thus they reduce production of arachidonic acid metabolites i.e. prostaglandins and leukotrienes. They also exhibits activity like inhibition of enzymes such as protein kinase C, protein tyrosine kinases, phosphodiesterases etc.[21]

The present study has some limitations. Sub-acute and chronic toxicity of the extract of *FR* could not been explored and hence we could not reveal about the long-term effects of the extract. Phytochemical studies were not done. Further studies with isolated fractions are needed to explore the antinociceptive effect of the *FR* extract. Despite of all these shortcomings, we consider that the present study has high internal and external validity and the findings can be replicated elsewhere as well.

CONCLUSION

On the basis of these findings it can be concluded that oral *FR* root extract has analgesic effect through both central and peripheral mechanism justifying the folkloric use of this plant. However, further studies with isolated fractions of *FR* are needed to delineate the mechanism responsible for the central and peripheral analgesic effect.

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