Original Article

Effect of root-extracts of *Ficus benghalensis* (Banyan) in pain in animal models

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ABSTRACT

Introduction: According to the WHO, 70–80% population in developing countries still relies on nonconventional medicine mainly of herbal origin. Even in developed countries, use of herbal medicine is growing each year. Pain is an unpleasant feeling often caused by intense or damaging stimuli. Traditionally, different plant parts of *Ficus benghalensis* are claimed to have several analgesic properties. Few scientific evidences support these uses. Interestingly, still others contradict these uses. It was shocking to find very scarce scientific studies trying to solve the mystery. **Materials and Methods:** It was a quantitative experimental study in Swiss albino mice of either sex. Sample size was calculated using free sample size calculating software G*Power version 3.1.9.2. Hot-plate test and tail-flick test were central antinociceptive paradigms. Writhing test was peripheral model for pain. Test drugs were aqueous root extracts of *F. benghalensis* at 100 mg/kg and 200 mg/kg mouse weight prepared by Soxhlet method. Suitable negative and positive controls were used. The experimental results were represented as mean ± standard deviation statistical level of significance was set at $P < 0.05$. For calculation, parametric test - one-way analysis of variance (ANOVA) or nonparametric test - Mann–Whitney U-test was appropriately used. **Results:** Hot-plate reaction time at 100 mg/kg (13.64 ± 1.30 s) and 200 mg/kg (10.32 ± 2.23 s) were nonsignificant ($P = 0.425$ and $P = 0.498$, respectively) compared to negative control (11.87 ± 1.92 s). One-way ANOVA revealed nonsignificant ($P = 0.178$) between-group comparison in mean tail-flick reaction time. Test drug at 200 mg/kg produced statistically significant more writhing (36.00 ± 14.85 in 10 min) than negative control, normal saline (11.83 ± 12.43 in 10 min) or the positive control, Indomethacin (3.50 ± 5.21 in 10 min), P value being 0.031 and 0.003, respectively. **Conclusion:** Aqueous root extracts of *F. benghalensis* at 200 mg/kg produces statistically significant writhing.

Key words: Analgesic, animal models, *Ficus*, pain

Introduction

The use of plant parts as medicine is as old as human civilization itself.[1] Even today, our existence cannot be imagined without plants serving different medicinal purposes. According to the WHO, 70–80% population in developing countries still relies on nonconventional medicine mainly of herbal origin. Even in developed countries, use of herbal medicine is growing each year.

In allopathy, pharmacological screening of plants often provides basis for developing new lead molecules.[2,3]

Pain is an unpleasant feeling often caused by intense or damaging stimuli such as pinching the skin, burning a finger, or putting alcohol on a cut.[4] Subjective sensation of pain can be blocked centrally (opioid-like) or peripherally (nonsteroidal anti-inflammatory drug [NSAID] - like).[5]

Traditionally, different plant parts of *Ficus benghalensis* are claimed to have several analgesic properties. Nadkarni mentioned its use in menorrhagia. An ancient Indian mythological book writes "A decoction of leaf

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How to cite this article: Panday DR, Rauniar GP. Effect of root-extracts of *Ficus benghalensis* (Banyan) in pain in animal models. J Neurosci Rural Pract 2016;7:210-5.

buds and aerial roots of *F. benghalensis,* mixed with honey, can be given for any burning sensation."[6] It has also been recommended for rheumatism and skin disorders such as sores.[7] The bark is considered useful in burning sensation, ulcers, and painful skin diseases.[8] It can be used in inflammation and toothache.[8]

Few scientific evidences also throw positive evidence. Garg and Paliwal concluded that extracts of *F. benghalensis* did have significant analgesic and antipyretic activities from hot-plate, writhing, and yeast-induced hyperthermia models.[9] Leaf extracts have shown remarkable benefits on freunds adjuvant-induced arthritis in rats.[10]

Again, a recent study by Thakare *et al.* concluded that methanolic extracts of *F. benghalensis* bark did have anti-inflammatory and analgesic properties in animal models.[11] Mahajan *et al.* verified significant analgesic activities of methanolic extract of leaves of *F. benghalensis*.[3]

On the other hand, there are some negative scientific evidences. Deraniyagala *et al.* studied the effects of aqueous leaf extract of *F. benghalensis* on nociception in rats. The results showed that the aqueous leaf extract of the of *F. benghalensis* had no analgesic effects but marked and significant hyperalgesic effect in male rats.[12]

Although there are several articles claiming usefulness of *F. benghalensis* parts in pain and inflammation, it was shocking to find very scarce scientific studies trying to scrutinize these claims, mostly anecdotal. Again scientific studies done elsewhere may not be the answer to different climatic perspective of Nepal.

This study was undertaken to answer whether aqueous roots of *F. benghalensis* have any effect in pain in animal models or not.

Materials and Methods

Experiments [Figure 1] were conducted in the Laboratory of Department of Clinical Pharmacology and Therapeutics, B.P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal.

Geographical coordinates of the studied plant are 26° 49′ 0″ North, 87° 17′ 0″ East. Annual average temperature varies from 5% to 21% centigrade. Average rainfall varies from 0 mm/day in January to 128 mm/day in July.

Sample size was calculated using free sample size calculating software G*Power version 3.1.9.2 (Franz, Universitat Kiel, Germany). With power of 80%, 0.05 level of statistical significance and effect size of 0.8, sample

size for each test was calculated to be 24. Seventy-two mice were randomly assigned into one of the three experimental groups. It was a quantitative experimental study in mice.

Inclusion criteria
- Swiss albino mice of either sex
- Weighing 20–30 g.

Exclusion criteria
- Any apparent disease or handicap.

Test drug preparation
A *F. benghalensis* tree was authenticated by a botanist. About 2.5 kg aerial roots of the tree was carefully collected, thoroughly washed with tap-water, shade-dried for several days, and pulverized to fine powder in a mixer. About 2 kg of resulting crude root-powder was extracted in several batches using soxhlet apparatus [Figure 2] (Jain Scientific Glass Works Ambala Cantt; Extraction Pot: 250 ml; Soxhlet chamber size: 100 ml; Heater: DICA India). Distilled water was used for extraction. Each batch was extracted for an approximately 24 h. Thus produced aqueous root extract was heated in 50°C for a brief time interval, stopped just before the apparently saturated solution precipitated and left in room temperature until the moisture dried. 2 kg crude root-powder yielded 102.68 g extract (5.13%) by soxhlet method.[13] Thus resulted dried powder extract was safely stored in a dry air-tight plastic container until the day of the experiment.[14] On the experiment day, 20 mg/ml and 10 mg/ml solutions in distilled water were prepared by serial dilution such that 1 ml/100 g mouse body weight could be injected into the mice in test-drug group for the desired test dose of 200 mg/kg and 100 mg/kg, respectively. On the day of experiment, the previous day solutions were discarded and fresh solutions prepared.

Timing of the drug administration
All *per os* (PO) drugs were carefully administered with the help of orogastric tube approximately 60 min before the intended test. All parenteral drugs were given intraperitoneal (IP).[15-17]

Hot-plate test
The method validated by Williamson *et al.,* and Eddy and Leimback was used.[18] The thermal noxious stimuli were given to a mouse by placing it on a thermostatically controlled hot-plate [Figure 3] (UGO Basile, Italy) at 55–56°C and the reaction time recorded. Reaction time was taken as the period between placing the mouse in the hot-plate and the time when it either jumped or licked its paws, whichever occurred first. A cutoff time of 15

s was used to minimize injury to paws. Morphine at 5 mg/kg IP was the positive control for the test and was administered approximately 15 min before the test.[17]

Tail-flick test
Radiant noxious stimuli were given to the proximal one-third of the mouse-tail by directing an infrared light of 50 unit intensity [Figure 4] (Tail-Flick Unit, UGO Basile, Italy). Response was obtained by observing the interval between the stimuli exposure and withdrawal of the tail (reaction time). A maximum radiation exposure period (cutoff time) was 6 s. Morphine at 5 mg/kg IP was

the positive control for this test and was administered approximately 15 min before the test.[17]

Writing test
Approximately, 1 h after the oral drug administration, 0.6% acetic acid at 60 mg/kg[17] was injected IP to every mouse. Then, the number of writhes (stretching of abdomen with simultaneous extension of one of the hind limbs) was counted for ten immediate minutes. Antinociception would be expressed as the difference in the number of writhes between normal saline and test drug group. Positive control was indomethacin 20 mg/kg, PO.[19]

Drugs and chemicals used in experimental models
- Test drug-aqueous solution of aerial root extracts of *F. benghalensis* 100 mg/kg and 200 mg/kg PO
- Negative control in all three models: Normal saline (0.90% w/v of NaCl) PO
- Positive control
  - Hot-plate and tail-flick method →morphine 5 mg/kg IP[17]
  - Writhing test→indomethacin 20 mg/kg PO[17,19,20]
- Inducing agents
  - Writhing inducing agent: 0.6% acetic acid at 60 mg/kg IP.[17]
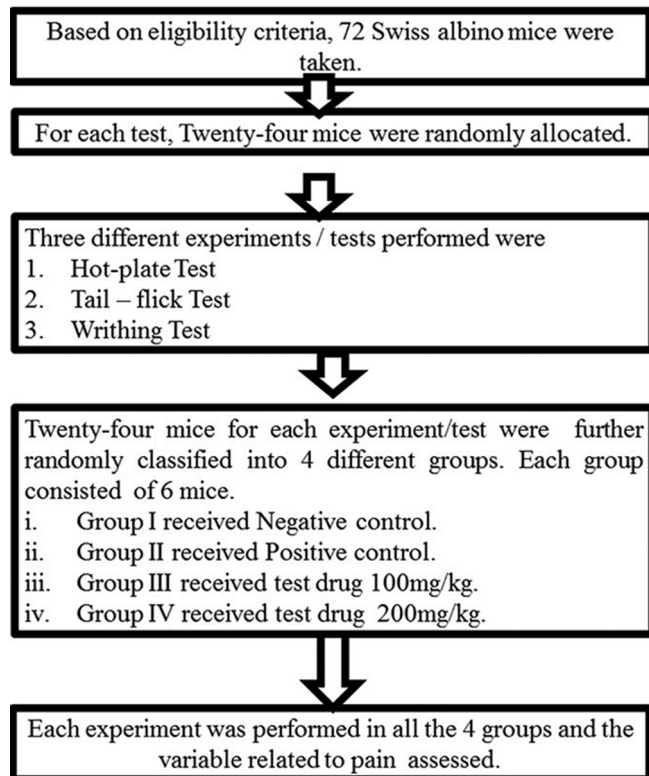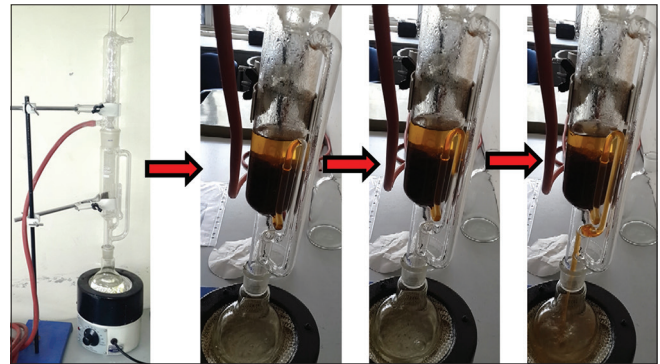

Figure 1: Study flow chart
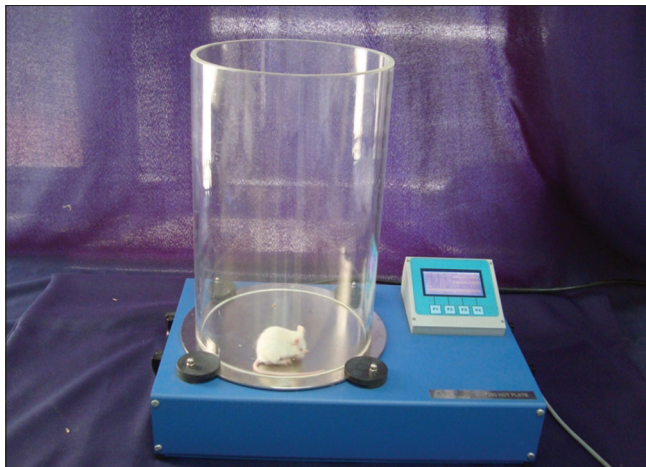

Figure 2: Soxhlet apparatus in use

Figure 3: Mouse licking forefoot in hot-plate test

Figure 4: Tail-flick test

All drugs and chemicals were diluted in distilled water such that 1 ml is for 100 g mouse weight, i.e., 25 g mouse receives 0.25 ml volume dose with 1 ml insulin syringe divided into 100 equal parts.

Statistical analysis

The experimental results were represented as mean ± standard deviation SPSS version 21 (IBM) was used. For normally distributed dependent variable, parametric test - one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison was applied. For those, not normally distributed, nonparametric test - Mann–Whitney U-test was employed for comparison. Statistical level of significance was set at $P < 0.05$.

Ethical considerations

Before the conduction of the experiment, ethical clearance was obtained from the Ethical Committee on Animal Research of BPKIHS. Maximum precaution was taken to reduce the pain and injury to the animals in the course of the experiment, yet not compromising the standard of the experiment. Appropriate help was procured from expert experimental animal handlers during the course of study.

Results

Hot-plate test [Table 1]

One-way ANOVA [Table 2] revealed a significant ($P = 0.000$) influence of aqueous extracts of aerial roots of *F. benghalensis* on mean hot-plate reaction time among Swiss albino mice. Multiple Comparisons [Table 3] by Tukey honestly significant difference (HSD) *post hoc* tests ($\alpha = 0.05$) revealed nonsignificant ($P_2 = 0.425$) effect of 100 mg/kg of aqueous extracts of aerial roots of *F. benghalensis* compared to normal saline. Again, there was nonsignificant ($P_3 = 0.498$) effect of aqueous extracts of aerial roots of *F. benghalensis* at 200 mg/kg compared to normal saline. However, group receiving 100 mg/kg of aqueous extracts of aerial roots of *F. benghalensis* had significantly ($P_4 = 0.048$) shorter mean hot-plate reaction time (13.64 s ± 1.30 s) compared to Morphine-group (16.87 s ± 1.75 s). Similarly, group receiving 200 mg/kg of aqueous extracts of aerial roots of *F. benghalensis* had significantly ($P_5 = 0.000$) shorter mean hot-plate reaction time (10.32 s ± 2.23 s) compared to morphine-group (16.87 s ± 1.75 s).

Tail-flick test [Table 4]

One-way ANOVA [Table 5] revealed nonsignificant ($P = 0.178$) influence of aqueous extracts of aerial roots of *F. benghalensis* on tail-flick antinociceptive test among Swiss albino mice.

Writhing test [Table 6 and Figure 5]

One-way ANOVA [Table 7] revealed a significant ($P = 0.003$) influence of aqueous extracts of aerial roots of *F. benghalensis* on acetic acid-induced writhing test among Swiss albino mice.

Multiple comparisons [Table 8] by Tukey HSD *post hoc* tests ($\alpha = 0.05$) showed 100 mg/kg of aqueous extracts

Table 1: Comparison of mean hot-plate reaction-time among four different experimental groups

Experimental groups	Mean reaction time in hot-plate±SD (s)
Negative control (normal saline PO)	11.87±1.92
Positive control (morphine) 5 mg/kg (IP)	16.87±1.75
Test drug 100 mg/kg (PO)	13.64±1.30
Test drug 200 mg/kg (PO)	10.32±2.23

SD: Standard deviation, PO: *Per os*, IP: Intraperitoneal

Table 2: One-way analysis of variance test - mean hot-plate reaction time (s)

	Sum of squares	df	Mean square	F	P*
Between-group	142.13	3	47.38	13.41	0.000
Within group	67.11	19	3.532		
Total	209.24	22			

*$P<0.05$ was considered statistically significant

Table 3: Tukey highly significant difference *post hoc* tests comparing mean hot-plate reaction time among experimental groups

Experimental group	Versus experimental group	P*
Negative control (normal saline)	Positive control (morphine)	P_1=0.001
	Test drug 100 mg/kg	P_2=0.425
	Test drug 200 mg/kg	P_3=0.498
Positive control (morphine)	Test drug 100 mg/kg	P_4=0.048
	Test drug 200 mg/kg	P_5=0.000
Test drug 100 mg/kg	Test drug 200 mg/kg	P_6=0.040

*$P<0.05$ was considered statistically significant

Table 4: Comparison of mean tail-flick reaction time among four different experimental groups

Experimental groups	Mean reaction time in tail-flick±SD (s)
Negative control (normal saline PO)	2.03±0.72
Positive control (morphine) 5 mg/kg (IP)	2.30±0.80
Test drug 100 mg/kg (PO)	3.16±0.80
Test drug 200 mg/kg (PO)	2.78±1.05

SD: Standard deviation, PO: *Per os*, IP: Intraperitoneal

Table 5: One-way analysis of variance test-mean tail-flick reaction-time (s)

	Sum of squares	df	Mean square	F	P*
Between-group	4.16	3	1.39	1.82	0.178
Within group	140.49	19	0.76		
Total	180.66	22			

*$P<0.05$ was considered statistically significant

of aerial roots of *F. benghalensis* had nonsignificant effect (P_2=0.463) compared to negative control (normal saline). However, group receiving 200 mg/kg of aqueous extracts of aerial roots of *F. benghalensis* significantly (P_3=0.031) produced more writhing in 10 min duration (36.00 ± 14.85) compared to normal saline group (11.83 ± 12.43). Again, 100 mg/kg of aqueous extracts of aerial roots of *F. benghalensis* had nonsignificant effect

Figure 5: Comparison of number of writhing in 10 min among four different experimental groups

Table 6: Comparison of number of writhing in 10 min among four different experimental groups

Experimental groups	Mean number of writhes in 10 min±SD
Negative control (normal saline PO)	11.83±12.43
Positive control (indomethacin 20 mg/kg PO)	3.50±5.21
Test drug 100 mg/kg (PO)	24.17±17.68
Test drug 200 mg/kg (PO)	36.00±14.85

SD: Standard deviation, PO: *Per os*

Table 7: One-way analysis of variance test - mean number of writhes in 10 min duration

	Sum of squares	df	Mean square	F	P*
Between-group	36,160.08	3	12,050.36	60.41	0.003
Within group	35,730.13	19	1880.06		
Total	71,890.21	22			

*$P<0.05$ was considered statistically significant

Table 8: Tukey analysis of variance *post hoc* tests - comparing mean number of writhes in 10 min among experimental groups

Experimental group	Versus experimental group	P*
Negative control (normal saline)	Positive control (indomethacin)	P_1=0.721
	Test drug 100 mg/kg	P_2=0.463
	Test drug 200 mg/kg	P_3=0.031
Positive control (indomethacin)	Test drug 100 mg/kg	P_4=0.093
	Test drug 200 mg/kg	P_5=0.003
Test drug 100 mg/kg	Test drug 200 mg/kg	P_6=0.502

*$P<0.05$ was considered statistically significant

(P_4=0.093) compared to Indomethacin. However, group receiving 200 mg/kg of aqueous extracts of aerial roots of *F. benghalensis* significantly (P_5=0.003) produced more writhing compared to indomethacin-group (3.50 ± 5.21).

Discussion

To the best of our knowledge, this is the first study on effect of root extracts of *F. benghalensis* in pain in animal models from Nepal. Preliminary experiments demonstrated that the high doses (2000 mg/kg) were tolerated without any acute signs of toxicity or mortality. Therefore, one tenth of this dose[10] (i.e., 200 mg/kg) was considered as the highest evaluation dose for pharmacological studies.

Subjective sensation of pain can be blocked either at the central or peripheral level.[5] Peripherally, the formation of pain mediators, i.e., prostaglandins can be blocked either by inhibiting cyclooxygenase (NSAID-like) or phospholipase A_2 (steroid-like). Centrally, pain sensation can be blocked by stimulating μ receptor (opioid-like).[21]

In our experiments, test drugs at both doses did not produce statistically significant analgesia in the central antinociceptive paradigms (hot-plate and tail-flick).

Unexpectedly, the test drug produced statistically significant writhing similar to known peripheral irritant acetic acid at higher dose (200 mg/kg). Thus, the aqueous extracts of aerial roots of *F. benghalensis* in our experiments pointed toward acetic acid such as peripheral hyperalgesic effect rather than analgesic effect as believed earlier. Our results, therefore, do not validate the usefulness of *F. benghalensis* aerial roots in painful conditions. Deraniyagala *et al*. also reported marked and significant hyperalgesic effect of aqueous leaf extract of *F. benghalensis* in male rats.[12]

Most past studies have, however, shown that different plant-parts, namely bark[9] and leaves[3,9] did have analgesic and anti-inflammatory properties.

The bark extracts of other taxonomically close species of *Ficus*, namely *Ficus racemosa*, *Ficus religiosa*, *Ficus insipida*, *Ficus elastica*, *Ficus indica*, and *Ficus carica* were also found to have analgesic and anti-inflammatory activities.[6]

Limitations and scope for further study
Although our test result has started a new argument, we assume certain shortcomings in our endeavor.
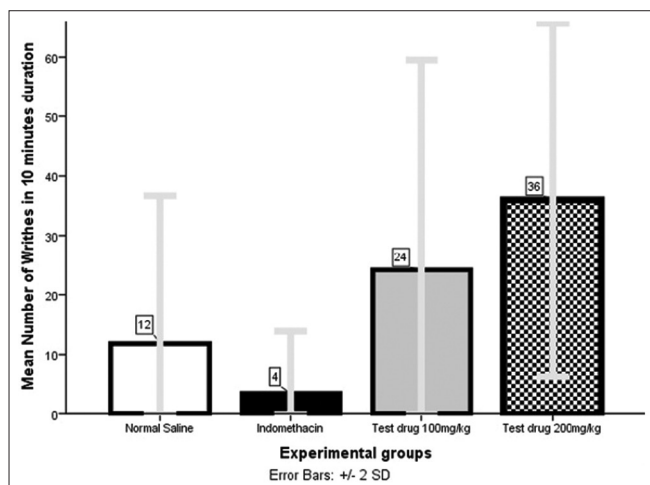• We did not have much past evidences to compare it with

- Distinct climate, soil, and environment of Eastern Nepal might have produced different properties that may reduce the global applicability of our findings
- In lack of phytochemical analysis accompanied by cause-effect establishment of individual phytoconstituents, we remain skeptical about extrapolating our report to other plant parts than aerial roots. Further, in-depth research disclosing the phytoconstituents responsible for each neuropharmacological activity along with their correct mode of action is desperately needed
- Individual differences in anatomy, organ function, drug absorption, and metabolism among animals are among the myriad of other differences that could have given us inadequate and distinct information
- Single acute dose-effect was studied. Subacute and chronic dose-effect could not be extrapolated from these data
- We studied only two most relevant doses of test drug. More doses are needed to establish statistically significant dose-effect relationship
- In spite of all these, we consider our study has high internal and external validity, and our findings can be replicated elsewhere.

Recommendation

Although technically more demanding, phytomolecular identification followed by *in vitro* receptor-ligand binding studies may be the answer to all the unanswered questions even without sacrificing the animals.

Conclusion

Aqueous root extracts of *F. benghalensis* at 200 mg/kg produces statistically significant writhing.

Acknowledgment

Financial support and sponsorship

Conflicts of interest
There are no conflicts of interest.

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