

**Original Article**

**ANALGESIC ACTIVITY OF BARK AND LEAVES OF *FICUS RELIGIOSA* L. FROM NEPAL**

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**ABSTRACT**

**Objective:** Because of adverse side effects, caused by NSAIDs, tolerance, and dependence induced by opiates, the use of these analgesic agents has not been successful in all cases. Therefore, alternative analgesic drugs from plant sources are the new target now days. The objective of this study was to evaluate the analgesic activity of ethanolic extracts of stem barks and leaves of *Ficus religiosa*.

**Methods:** The analgesic activity of ethanolic extract of stem barks and leaves was evaluated in the Swiss albino mice model using acetic acid-induced writhing response and Eddy's hot plate method. Analgesic activity was demonstrated with the percentage inhibition of acetic acid induced writhings and the percentage increased in latency time of paw licking. The potency of test extracts was compared with standard drug, Diclofenac.

**Results:** Ethanolic extract of leaves and bark of *F. religiosa* showed potential analgesic activity from both methods. From Eddy's hot plate model, it was observed that the percentage of increased latency time at 90 min by ethanolic extract of leaves and stem bark was found to be 70.81 % (8.54 min) and 70.78 % (8.53 min) respectively at a dose of 400 mg/kg. Both of these results are statistically significant ( $p < 0.05$ ) as compared to the test group. Furthermore, both of these extracts showed the dose-dependent and time-dependent increased in latency time and these results are compared to that of standard drug Diclofenac. Similarly, ethanolic extract of leaves and stem at 400 mg/kg significantly inhibited the number of writhings induced by acetic acid. The percentage inhibition of writhings by ethanolic extract of leaves at a dose of 400 mg/kg was 68.47 % which was similar to that of standard drug Diclofenac (68.47 %). However, ethanolic extract of bark showed relatively lower percentage inhibition (60.79 %) as compared to leaf extract and standard, but the result was significant as compared to that of the test group ( $p < 0.05$ ).

**Conclusion:** Ethanolic extracts of *F. religiosa* stem bark and leaf possess both central and peripheral analgesic properties and these effects may be beneficial for the management of pain.

**Keywords:** Analgesic, *Ficus religiosa*, NSAIDs

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**INTRODUCTION**

Pain can be characterized as a protective mechanism for the body. Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce pain and edema by suppressing the formation of prostaglandins, by inhibiting the activity of the enzyme Cyclooxygenase (COX-1 and COX-2). Pain occurs whenever any tissues are being damaged, and it causes the individual to react to remove the pain stimulus [1]. Several prostaglandins act as key mediators for several components of GI mucosal defense by the inflammatory response as well as stimulate tissue repair. Thus suppression of their synthesis by NSAIDs effectively reduces the resistance of the mucosa to injury as well as interfering with repair processes [2]. Prolonged use of NSAIDs cause several adverse side effects, like gastric lesions, tolerance and dependence are major problem associated with opiates medicines. Therefore, analgesic drugs devoid of those unwanted effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in traditional medicine has been paid great attention because of the cheap cost, which has little side effects, and according to WHO still about 80% of the world population relies mainly on plant-based drugs [3].

*Ficus religiosa* is a large deciduous tree belonging to family Moraceae. It grows up to 30 meters and popular with common name peepal in Nepal and India. *F. religiosa* is considered as a sacred plant, has golden colored exudates with or without aerial roots [4]. *F. religiosa* is a well-known ethnomedicinal tree used in Ayurveda. All parts of the plant (leaves, barks, leaves, latex, and sap of the roots) are medicinally

important in the traditional system of medicine. Leaves are a good remedy for visceral obstruction and also useful in regular diarrhea, asthma, toothache scabies, astringent and, constipation [5]. The astringent nature of the bark has been employed as a mouth wash in spongy gum and also internally in dysentery, menorrhagia, and hemoptysis. The bark is antiseptic, antipyretic, analgesic, and vermicide the decoction of the bark is used in the treatment of various skin diseases, ulcers, and diabetes [5]. Apart from the usage in traditional medicine, scientific studies indicate *F. religiosa* possess various biological effects such as anti-diabetic [6], anti-inflammatory, analgesic [7], anticonvulsant [8], anti-microbial [9], Wound healing [10], anti-acetyl cholinesterase activity [11] and proteolytic activity [12]. Decoction of bark as Jivaniya (invigorating), in hemorrhages; leaves for covering wounds; paste of roots and bark for skin infections were prescribed by Charaka and Sushruta [13, 14]. There is no significant scientific study for the analgesic property of *F. religiosa* of Nepalese origin. Therefore, this study aimed to evaluate the analgesic property ethanolic extract of *F. religiosa*.

**MATERIALS AND METHODS**

**Chemicals**

Diclofenac sodium was used as standard drug. It was purchased from Asian Pharmaceutical, Rupandehi, Nepal), Ethanol (Hangshu Kangyuan Chemical, China) and Acetic acid (Thermo Fisher Scientific, India Pvt. Ltd., Mumbai). Water was prepared in the laboratory with Distilled water plant, Normal saline, (Aishwarya Health Care, India Pvt. Ltd., Himachal Pradesh).

### Instruments and glass wares

Digital balance (ATX224, SHIMADZU Corporation, Philippines), Rotary evaporator (R-210/215, BUCHI Labor technok AG, Switzerland), Refrigerator (LG), Grinder and Distilled Water (DW) plant, Sonicator (INDOSATI Scientific Lab Equipments), Hot air oven (S. M. Scientific Instruments (P) Ltd., Delhi), Analgesiometer (Eddy's Hot Plate) and Rota Rod (Three Compartment) (INDOSATI Scientific Lab Equipments), Beakers, volumetric flasks, pipettes, round bottom flask, cotton, plastic bottle, aluminum foils, butter paper, conical flasks, measuring cylinders, spatulas (stainless steel), plant cutter, paper sheets, scissors, glass rods, washing brush, funnels, filter paper (Watman's no. 1), scale, map, marker, stopwatch, feeding tube, injection, gloves, mask, cage, mortar and pestle were used in this experiment.

### Plant materials

The plant parts were collected from Tamnagar, Rupandehi (350 m elevation from sea level) and Arkhale, Gulmi (1784 m elevation from sea level) districts, Lumbini Zone, western Nepal on the month of August. The selection of the species used in this study was mainly based on their ethnomedicinal evidences (literature) of use for conditions such as diabetes, diarrhea, aphrodisiac, menorrhagia, fever, pain and healing of wounds. The plant materials were identified and authenticated by botanist Mr. Hom Nath Pathak, Prithivi Narayan Campus, Tribhuvan University, Pokhara, Nepal (Identification letter-number FR-2015/26) and also compared with the literature. The voucher specimen of collected plants was preserved in the Pharmacognosy Laboratory of the Crimson College of Technology, Pokhara University, Nepal (voucher specimen number: CCT-HRB-2018/154).

**Table 1: Details of the plant collection site, part of plants utilized and its scientific and local names**

| S. No. | Plant                  | Local name | Collected parts | Collection site     | Collection date |
|--------|------------------------|------------|-----------------|---------------------|-----------------|
| 1.     | <i>Ficus religiosa</i> | Peepal     | Leaves          | Tamnagar, Rupandehi | August, 2018    |
| 2.     | <i>Ficus religiosa</i> | Peepal     | Stem bark       | Arkhale, Gulmi      | August, 2018    |

### Drying

Collected plant materials were cleaned and cut into small pieces to fasten the drying process. They were then naturally dried in shade at room temperature in a well-ventilated environment. The drying was carried out for 30 d.

### Comminution of dried plants

The dried plants were ground to a fine powder using a portable grinding machine. The reduced powder mass was then passed through the sieve of mesh size 40. The sieved powder was put into the airtight plastic bottle, sealed to prevent contamination and stored at room temperature in a dark place until use.

### Extraction procedure

Dried leaves and stem bark of *F. religiosa*, (540 g) each were subjected for cold maceration using 3000 ml of ethanol with occasional shaking and stirring for 7 d and the mixture was filtered by a piece of clean, white cotton material, marc pressed and then finally filtered with Whatman no.1 filter paper.

### Evaporation of extracts

The filtrates obtained from the extraction process were then evaporated to dryness using a rotary vacuum evaporator. Ethanolic extract of stem bark and leaves were evaporated at 40 °C. The dried extracts were kept in glass vials and the percentage yields of the extract were calculated. Then, the dried extracts kept in vials were covered with aluminum foil and stored in the refrigerator at a temperature of 4 °C until use.

### Animals and ethical approval

Swiss albino mice of either sex, weighing 20–40 g were used in the study. Mice were regularly supplied with standard feed and water *ad libitum*. They were kept in clean polypropylene cages with wooden dust (replaced every two days) under controlled temperature (22±1°C), 12/12 h light/dark cycle was maintained continuously throughout the study. Before experimental study, animals were allowed for acclimatization for laboratory conditions. For animal care, handling, and experimental protocols, all steps were carried out following the official ethical guidelines of Nepal [15, 16]. After completion of the study, every animal was submitted to 35% CO<sub>2</sub> euthanasia. All experimental procedures were approved by the Institutional Review Committee of, Pokhara University (Ref: CCT/IRC/058/18).

### Acute toxicity studies

To carry out acute toxicity studies, guidelines of the Organization of Economic Cooperation and Development (OECD) were adopted [17]. Swiss Albino mice were divided into six groups having six mice in each group (n = 6): five test groups and a control group. The test groups were fed with plant extract (500, 1000, and 2000 mg/kg, p. o.) at 10 ml/kg and a control with only saline. Any sign of toxic

effects and mortality were observed every 1 h for the next 6 h and body weight was measured on days 1, 7 and, 14 after treatments.

### Analgesic activity

*Ficus religiosa* stem bark and leaf extracts were evaluated for analgesic activity in mice using a hot plate and acetic-acid induced writhing method.

### Hot plate method

Experimental animals of either sex were randomly selected and divided into six groups consisting of six mice in each group for control, positive control and test sample group (200 mg/kg and 400 mg/kg). Group-I received a particular treatment i.e. control (Normal saline, 10 ml/kg, p. o.). Group-II received a solution of diclofenac sodium (9 mg/kg i. p.). Group-III and IV are fed with ethanolic extract of bark (EEB) at the dose of 200 mg/kg and 400 mg/kg, p. o. respectively whereas ethanolic extract of leaf (EEL) with dose 200 mg/kg and 400 mg/kg, p. o. respectively is given for Group V and VI. The animals were fed slowly and continuously using tube feeding and were positioned on Eddy's hot plate having a constant temperature of 55±0.5 °C. A cut off period of 15 sec was observed to prevent paw damage of the mice. Reaction time was recorded when animals licked their hind paws or fore, or jump prior to and after 0, 30, 60, and 90 of the sample administration [18, 19].

### Acetic acid induced writhing method

As above mice were grouped in 6 groups (group-I, group-II, group-III, group-IV, group-V and group-VI) consisting 6 healthy mice each of either sex. Group-I served as control group which received normal saline (10 ml/kg) 15 min prior to intraperitoneal administration of 0.5% w/v acetic acid solution. Group-II served as positive control group which received solution of diclofenac sodium (10 mg/kg) 15 min prior to intraperitoneal administration of 0.5% w/v acetic acid solution. Group-III and IV received EEB of dose 200 mg/kg and 400 mg/kg, p. o. respectively. Similarly Group V and VI received EEL of dose 200 mg/kg and 400 mg/kg, p. o. respectively. The test sample was administered orally 30 min prior to intra peritoneal administration of 0.5% w/v acetic acid solution. Finally, the number of writhes were noted for twenty minutes [20].

### Statistical analysis

Results are expressed as mean±SD. The significant difference between the control group and test group were evaluated by two-tailed student's t-test with the help of Microsoft excel. The statistical results with p<0.05 are considered as statistically significant.

## RESULTS

### Phytochemical analysis

The ethanolic extractive yield of the stem bark and leaf of *Ficus religiosa* were found to be 2.7 % and 2.9 %, respectively, as shown in table

Table 2: Extractive yield of stem bark and leaves

| Extract yield (%) |                                 |         |
|-------------------|---------------------------------|---------|
| S. No.            | Plants                          | Ethanol |
| 1.                | <i>Ficus religiosa</i> (Bark)   | 2.7     |
| 2.                | <i>Ficus religiosa</i> (Leaves) | 2.9     |

### Analgesic activity test

Table 3: Effects of ethanolic extracts of *F. religiosa* (stem bark and leaf extract) on hot plate test in mice

| Groups | Treatments        | Mean latency time after drug/extract administration (secs) |             |             |             |
|--------|-------------------|------------------------------------------------------------|-------------|-------------|-------------|
|        |                   | 0 min                                                      | 30 min      | 60 min      | 90 min      |
| I      | Normal saline     | 3.31±0.021                                                 | 3.20±0.015  | 3.11±0.024  | 3.52±0.019  |
| II     | Diclofenac sodium | 3.32±0.031                                                 | 12.14±0.019 | 13.12±0.019 | 13.71±0.017 |
| III    | EEB 200 mg/kg     | 3.43±0.038                                                 | 8.54±0.046  | 9.95±0.036  | 11.96±0.029 |
| IV     | EEB 400 mg/kg     | 3.22±0.027                                                 | 9.35±0.040  | 10.27±0.026 | 12.05±0.032 |
| V      | EEL 200 mg/kg     | 3.30±0.034                                                 | 8.82±0.017  | 10.26±0.026 | 11.61±0.017 |
| VI     | EEL 400 mg/kg     | 3.61±0.017                                                 | 9.30±0.017  | 10.67±0.029 | 12.06±0.033 |

Results are presented as mean±SD (n=6), the mean latency time after drug/extract administration at 30, 60 and 90 min are statistically significant (p<0.05) as compared to control. EEL (ethanolic extracts of leaves), EEB (ethanolic extracts of bark)

Table 4: Effects of ethanolic extracts of *F. religiosa* (stem bark and leaf extract) on acetic acid induced writhing in mice

| Groups | Treatments    | Dose, Route     | No. of writhings | Percentage inhibition |
|--------|---------------|-----------------|------------------|-----------------------|
| I      | Normal saline | 10 ml/kg p. o.  | 5.29±0.70        | -                     |
| II     | Diclofenac Na | 10 mg/kg i. p.  | 3.14±0.83        | 68.47 %               |
| III    | EEB 200 mg/kg | 200 mg/kg p. o. | 4.14±0.83        | 27.14 %               |
| IV     | EEB 400 mg/kg | 400 mg/kg p. o. | 3.29±0.70        | 60.79 %               |
| V      | EEL 200 mg/kg | 200 mg/kg p. o. | 3.42±0.90        | 54.67 %               |
| VI     | EEL 400 mg/kg | 400 mg/kg p. o. | 3.14±0.83        | 68.47 %               |

Results are presented as mean±SD (n=6), the mean latency time after drug/extract administration at 30, 60 and 90 min are statistically significant (p<0.05) as compared to control. EEL (ethanolic extracts of leaves), EEB (ethanolic extracts of bark).

### DISCUSSION

There are several traditional claims regarding the use of *F. religiosa* in pain and inflammation but have been limited only in literature. Hence, the results of present investigations might give scientific endorsement to the traditional claims [21]. Any injury or tissue damage is associated with pain and inflammations. Analgesic shows their action by acting on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptor sites of pain whereas centrally acting analgesics not only raise the threshold for pain but also suppress the anxiety [22].

The present study is focused on the analgesic activity of stem bark and leaves of *F. religiosa*. Experimental animal models were grouped into six groups containing six mice in each group i. e, control, positive control and test extract of stem bark and leaves (200 and 400 mg/kg), and were placed in Eddy's hot plate [18]. The extract of *F. religiosa* and diclofenac sodium presented longer latency time than the control group in the hot plate test in dose-related manner and latency time was found to increase as time increase. The percentage of increased latency time of two different doses (200 and 400 mg/kg) at 90 min. were found to be 69.68 (8.09 min) and 70.81(8.54 min) % for leaf and 70.56 (8.44 min) and 70.78 (8.53 min)% for bark extract respectively and it is very much comparable to the standard which have increased latency time by 74.32% (10.19 min). Ethanolic extract of leaves at a dose of 400 mg/kg was found to exhibit higher analgesic activity than the ethanolic extract of bark at the same dose which might be due to the presence of significant phytochemicals such as Flavonoids, Tannins, and Alkaloids in leaf as compare to bark. The results were found to be statistically significant (p<0.05). The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and one of the models normally used for studying central nociceptive activity. Flavonoids, Alkaloids, and Tannins are reported to inhibit Prostaglandins synthesis [23]. It is a fact that any agent that causes prolongation of hot plate latency time must be acting centrally [24]. Therefore, the ethanolic extract of this plant must have a central

activity. Again, narcotic analgesics inhibit both centrally and peripherally, while NSAIDs inhibit only peripheral pain [25].

Acetic acid-induced writhing inhibition test is a simple and effective scientific tool method for the assessment of peripheral analgesic activity. In our study, the writhing inhibition is directly proportional to the dose administered. The standard drug showed inhibition of 68.47% whereas the leaves extracts has 54.67 and 68.47% of writhing inhibition and that of stem bark was 27.14 and 60.79% in two different dose levels i. e, 200 and 400 mg/kg respectively. The percentage of writhing inhibition by leaves extract at a dose of 400 mg/kg is the same as the inhibition percentage caused by positive control and it is dose-dependent. The results were found to be statistically significant (p<0.05). The decrease of writhing which was induced by acetic acid in mice was probably due to the analgesic activity of ethanolic extract of the plant and the standard drug Acetic acid-induced writhing in mice is associated with visceral pain, hence plant extract act as peripheral analgesic drugs [26]. A previous study reported that the bark extract of *F. religiosa* showed dropping in number of writhing of 71.56 % and 65.93 % respectively at a dose of 250 mg/kg and 500 mg/kg [7]. But, our study reveals that, there is a maximum dropping in the number of writhing of 68.47 % by the leaves extracts (400 mg/kg). The analgesic effect of plants in both models suggests that they have been acting through central and peripheral mechanisms [27, 19]. In comparison to Hot plate test model, the Acetic acid-induced writhing model is more valuable and informative in our study. The activity is equally effective as standard drugs Diclofenac sodium at a dose of 400 mg/kg leaves extract of *F. religiosa*. Further research should be conducted to make it more conclusive that which phytochemicals are much pronounced to exhibit such potency.

### CONCLUSION

Our study suggests that central and peripheral analgesic properties of ethanolic extracts of *F. religiosa* stem bark and leaf are significant, thus can be used for the management of pain. But further study is

necessary for the investigation of the precise mechanism at the molecular level. For the identification of principal active analgesic compounds, isolation and fractionation of the active phytochemical constituents from the extracts of stem bark and leaf of the plant *F. religiosa* crucial. In the current scenario, evidence-based research for commercial herbal drugs or phytochemical entities is in the infant stage in Nepal. Therefore scientific efforts are necessary to develop and validate evidence regarding long term safety and effectiveness of plant-based medicines. Our result is scientific justification regarding the rational use of these plant parts in traditional medicine for the treatment of various diseases especially as analgesics.

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#### AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

#### CONFLICT OF INTERESTS

Declared none

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