

ANALGESIC AND ANTI-PYRETIC ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF *FICUS BENGHALENSIS*

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ABSTRACT

Objective: *Ficus benghalensis* Linn. (Family: Moraceae) is a reputed plant in ayurvedic medicine and commonly known as "banayan tree" in ayurvedic literature. Traditionally, *Ficus benghalensis* is used for pain and fever. Since no detailed scientific data is available regarding the analgesic and antipyretic activities of *Ficus benghalensis*, the present study was designed to explore the same.

Methods: In the present study, analgesic and anti-pyretic activities of ethanolic and aqueous extracts of *Ficus benghalensis* at dose levels of 100 mg/kg, 200mg/kg and 400 mg/kg was studied using hot plate, acetic acid induced writhing and yeast induced hyperthermia method. Student's t test was used to analyze the results obtained from the present study and $p < 0.05$ was considered significant.

Results: *Ficus benghalensis* showed significant analgesic and anti-pyretic activities in all models studied. Results support the traditional use of the plant in the treatment of pain and fever.

Conclusion: The present study has demonstrated the analgesic and antipyretic potential of the ethanolic and aqueous extracts of *Ficus benghalensis*. Therefore the present study had verified the traditional use of *Ficus benghalensis* in pain and fever. As the phytochemical screening has shown the presence of flavonoids and glycosides in both ethanolic and aqueous extracts, the potent activity may be attributed to the presence of these phytoconstituents.

Keywords: *Ficus benghalensis*, Analgesic, Anti-pyretic, Hot plate, Acetic acid induced writhing.

INTRODUCTION

Ficus benghalensis Linn. (Family: Moraceae) is a reputed plant in ayurvedic medicine and commonly known as "banayan tree" in ayurvedic literature. Milky juice from stem, seeds, or fruits of the plant is applied externally in rheumatism and to the soles of feet when inflamed, internally used in dysentery and diarrhea. All the parts of the plant have astringent, anti-inflammatory, antiarthritic, and antidiarrheal activities. The latex is useful in hemorrhage, diarrhea, and dysentery, as well as in hemorrhoid and inflammation [1].

Traditionally it is used for wounds, fever, swollen joints, inflammations and ulcers [2].

Various scientific studies have been carried out on *Ficus benghalensis* and various pharmacological activities have been reported. It has been reported to possess immunomodulatory [3], hypoglycemic [4], antioxidant [5], antistress and antiallergic [6], anthelmintic [7] activities. A glucoside, bengalenoside was isolated from *Ficus benghalensis* and evaluated for hypoglycemic activity [8]. Efforts are being made all over the world to discover agents that can reduce pain and fever and thereby reduce the cost of hospitalization and save the patient from severe complications. The need for safer and effective analgesic and antipyretic agents and the lack of enough scientific data to support the claims made in ancient literature prompted the present study.

MATERIALS AND METHODS

The bark of *Ficus benghalensis* was collected in the month of August 2006 from the local area of Meerut district and identified and authenticated by Dr. Anjula Pandey, Taxonomist, National Herbarium of Cultivated Plants, New Delhi. Voucher specimens (No. NHCP/NBPGR/2006/94/51/8929) have been kept in National Herbarium of Cultivated Plants, New Delhi and Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology (MIET), Meerut for future reference.

Extraction

The bark of *Ficus benghalensis* was dried under shade, crushed into small pieces and powdered. The powder was loaded into Soxhlet extractor and was subjected to successive extraction with petroleum ether, benzene, chloroform, ethanol and water to get different extracts. The ethanolic and aqueous extracts were concentrated to dryness using Rotary evaporator, giving yield as 4.10% w/v and 4.42% respectively and preserved in a refrigerator. Aliquot portions of the ethanolic and aqueous extracts of *Ficus benghalensis* were weighed and suspended in an appropriate volume of Tween 80 (2% v/v) for use on each day.

Acute toxicity study of the extract

Female Albino Wistar rats weighing 200-220 g were used in the study. Acute oral toxicity was performed as per OECD-423 guidelines [9]. The animals were fasted overnight with water *ad libitum*. The starting dose of 5 mg/kg of both ethanolic and aqueous extracts was administered orally to three animals in each group. If mortality was observed in two or three animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again in three animals to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300, and 2000 mg/kg body weight. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days.

Preliminary phytochemical studies

The different extracts were then subjected to qualitative phytochemical screening for the identification of the phytoconstituents. While petroleum ether, benzene, chloroform does not show any appreciable tests for the presence of different phytoconstituents, ethanolic and aqueous extracts showed positive tests for the presence of glycosides and flavonoids.

In order to follow the reasonable method of isolation of active ingredients from the plants, "activity guided" fractionation was followed. To achieve this, analgesic and anti-pyretic activities of

ethanolic and aqueous extracts were carried out at dose levels of 100 mg/kg, 200mg/kg and 400 mg/kg.

Experimental animals

Healthy Wistar albino rats of both sexes 200–220 g was used for the study. Also albino mice of both sexes weighing between 20 – 25g were used. They were individually housed and were allowed free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee, (IAEC) approved the use of animals for the present study, (Ethical clearance number: 711/02/a/CPCSEA).

Analgesic Activity

Hot Plate Method

The hot plate method described by Turner (1965) was followed for the assessment of analgesic activity. Albino mice were introduced to a hot plate maintained at $55 \pm 0.5^\circ\text{C}$. The reaction time to the thermal stimulus was recorded as the time interval from introduction of the animal to the plate until the first lick of the limbs or the first jump of the animals. The test groups received aqueous and ethanolic extracts of *Ficus benghalensis* at different dose levels (100, 200 & 400 mg/kg) prepared as suspension in 2% Tween 80 orally, the standard group received pentazocine (10mg/kg, i.p.) [10] and control group received only 1 ml of 2% tween 80 solution. The

reaction times were determined before and after 30 minutes, 1 hour, 2 hours and 3 hours period with reference to the control group receiving only vehicle.

Acetic Acid Induced Writhing

Acetic acid solution at a dose of 10ml/kg (0.6%) was injected i.p. and the number of writhes during the following 15 minutes period was observed. The test groups received aqueous and ethanolic extracts of *Ficus benghalensis* at different dose levels (100, 200 & 400 mg/kg) prepared as suspension in 2% tween 80 orally, the standard group received Aspirin (10mg/kg, i.p.) [10] and control group received only 1 ml of 2% tween 80 solution.

Significant reductions in number of writhes by drug treatment as compared to vehicle treatment animals were considered as a positive analgesic response. The percent inhibition of writhing was calculated [10].

$$\% \text{ Inhibition} = \frac{W_c - W_r}{W_c} \times 100$$

Where,

W_c = Mean number of writhes in control group.

W_r = Number of writhes in test group.

Table 1 Phytochemical Screening of different extracts of *Ficus benghalensis*

Tests	Petroleum Ether Extract	Benzene Extract	Chloroform Extract	Ethanol Extract	Aqueous Extract
1. Tests for sterols					
a. Test solution+Conc. H ₂ SO ₄	-ve	-ve	-ve	-ve	-ve
b. Libermann Burchard's Test	-ve	-ve	-ve	-ve	-ve
c. Test solution + sulphur	-ve	-ve	-ve	-ve	-ve
d. Salkowski test	-ve	-ve	-ve	-ve	-ve
2. Tests for Glycosides					
a. Keller Killiani's Test	-ve	-ve	-ve	+ve	+ve
b. Balget's Test	-ve	-ve	-ve	+ve	+ve
c. Bromine water Test	-ve	-ve	-ve	+ve	+ve
d. Legal's Test	-ve	-ve	-ve	+ve	+ve
e. Raymonds Test	-ve	-ve	-ve	+ve	+ve
3. Test for Saponins					
a. Haemolytic Test	-ve	-ve	-ve	-ve	-ve
b. Foam Test	-ve	-ve	-ve	-ve	-ve
4. Test for Tannins					
a. Gelatin Test	-ve	-ve	-ve	-ve	-ve
b. Ferric Test	-ve	-ve	-ve	-ve	-ve
5. Test for Alkaloids					
a. Dragendroff's Test	-ve	-ve	-ve	-ve	-ve
b. Mayer's Test	-ve	-ve	-ve	-ve	-ve
c. Hager's Test	-ve	-ve	-ve	-ve	-ve
d. Wagner's Test	-ve	-ve	-ve	-ve	-ve
6. Test for Carbohydrates					
a. Barfoed's Test	-ve	-ve	-ve	-ve	-ve
b. Benedict's Test	-ve	-ve	-ve	-ve	-ve
c. Molisch's Test	-ve	-ve	-ve	-ve	-ve
7. Test for Flavonoids					
a. Shinoda Test	-ve	-ve	-ve	+ve	+ve
b. Alkaline Reagent Test	-ve	-ve	-ve	+ve	+ve
c. Ferric Chloride Test	-ve	-ve	-ve	+ve	+ve
d. Lead Acetate Test	-ve	-ve	-ve	+ve	+ve
e. Zn-HCl reduction Test	-ve	-ve	-ve	+ve	+ve

+ve indicates positive result –ve indicates negative result

Antipyretic Testing

Initial rectal temperatures of rats were recorded using a six channel tele-thermometer for 1 min. Rats were made hyperthermic by subcutaneous injection of 20% yeast suspension at a dose of 1 ml/100 gm body weight. When the temperature was at peak (18 hours after yeast injection) the rectal temperature were again recorded. Those animals that showed a rise in rectal temperature of more than 1.2°C were used [11]. Different doses of aqueous and

ethanolic extracts of *Ficus benghalensis* were given orally as a suspension prepared in 2% tween 80 solution. Animals were divided into eight groups of six animals each. First group received 1 ml of 2% tween 80 solution orally and served as control. Second, third, fourth, fifth, sixth, seventh and eight groups received standard antipyretic agent i.e. paracetamol suspension (100 mg/kg) [12], ethanolic extract (100 mg/kg), ethanolic extract (200 mg/kg), ethanolic extract (400 mg/kg), aqueous extract (100 mg/kg),

aqueous extract (200 mg/kg), aqueous extract (400 mg/kg) respectively. The rectal temperatures of animals were recorded at 30 minutes intervals for 4 hours following the administration of tween 80, standard drug and plant extracts [12].

Statistical Analysis [13]

All the results obtained from various activities, as described above, were analyzed statistically by using Student's t test and $p < 0.05$ were considered significant.

RESULTS

Phytochemical Screening

Phytochemical screening of the ethanolic and aqueous extracts of *Ficus benghalensis* showed the presence of flavonoids and glycosides as shown in Table 1.

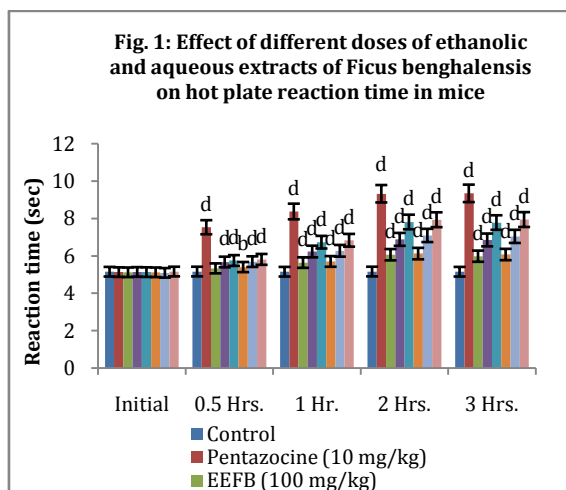
Acute toxicity studies

In the acute toxicity studies no signs of toxicity or mortality were observed at 2000 mg/kg dose level. Therefore we have taken 200 mg/kg as the average therapeutic dose and made variations by taking 100 mg/kg as lower dose and 400 mg/kg as higher dose.

Analgesic Activity

Hot Plate Method

From the result it can be deduced that the extract has shown dose dependant activity. After administration of the ethanolic and aqueous extracts at all the three dose levels, there is statistically significant increase in the hot plate reaction time. But the increase is comparable to the standard drug, pentazocine only at 400 mg/kg dose level as shown in figure 1.

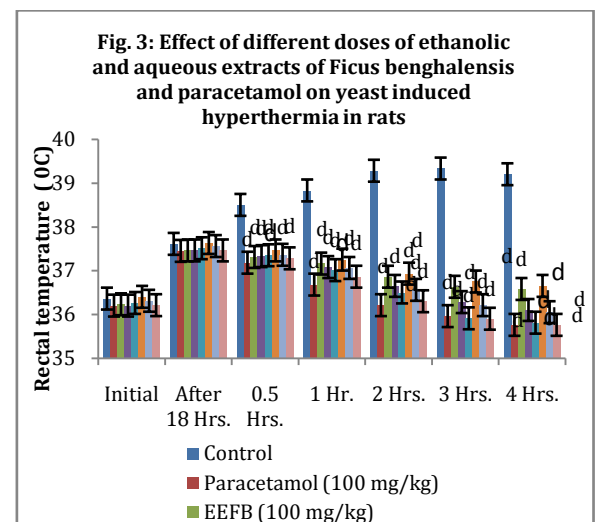
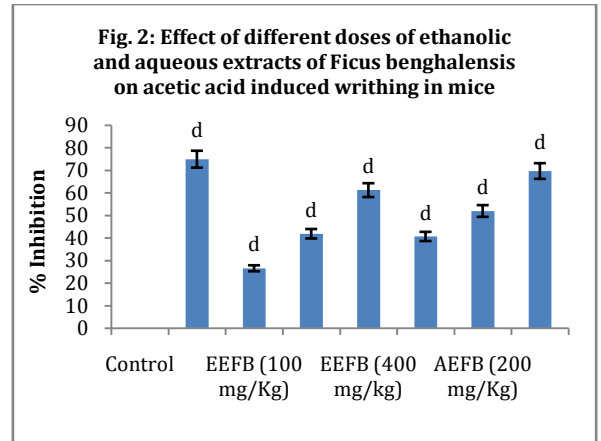


Acetic Acid Induced Writhing

The ethanolic extract at dose levels of 100, 200 and 400 mg/kg exhibited 26.61, 41.93 & 61.28 % inhibition of writhing and the aqueous extract at dose levels of 100, 200 and 400 mg/kg exhibited 40.72, 52.02 & 69.75 % inhibition of writhing as compared to that of 75.00% inhibition shown by Aspirin. It is quite evident from the result that the extract at 400 mg/kg showed comparable activity to that of Aspirin as shown in figure 2.

Anti-pyretic Activity

The anti-pyretic activity of the ethanolic and aqueous extracts of *Ficus benghalensis* has been shown in Fig. 3, which showed significant activity at all the three dose levels. The results were comparable to that of Paracetamol, a prototype of anti-pyretic drug as shown in fig. 3:



DISCUSSION

The present study establishes the analgesic and anti-pyretic activities of the ethanolic and aqueous extracts of *Ficus benghalensis* in the models used. Since antipyretic and analgesic activities are commonly mentioned as characteristic of drugs or compounds which have an inhibitory effect on prostaglandin biosynthesis [14], the yeast induced hyperthermia in rat model was, therefore, employed to investigate the antipyretic activity of this plant. It was found that the aqueous extract at the dose of 400 mg/kg showed a significant decrease in rectal temperature similar to that shown by the standard drug, paracetamol. These results seem to support the view that the extract has some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature [15].

Likewise, the analgesic activity of ethanolic and aqueous extracts of the plant was evaluated using the hot plate method and writhing test in mice. The hot plate method is useful in detecting centrally acting analgesics [16] whereas acetic acid induced writhing method is useful to detect peripheral analgesic effects. Acetic acid, which is used as an inducer for writhing syndrome, causes algia by liberation of endogenous substances, which then excite the pain nerve endings [11]. The fact that the ethanolic and aqueous extracts of *Ficus benghalensis* showed analgesic activity in both the models studied, indicate that this effect could be due to the presence of two components; one acting centrally and the other via peripheral route [16].

CONCLUSION

The ethanolic and aqueous extracts of *Ficus benghalensis* have shown significant analgesic and antipyretic activities in all the animal models used as seen from the above results. Therefore the

present study had verified the traditional use of *Ficus benghalensis* in pain and fever. As the phytochemical screening has shown the presence of flavonoids and glycosides in both ethanolic and aqueous extracts, the potent activity may be attributed to the presence of these phytoconstituents.

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REFERENCES

- Patel MA, Patel PK, Patel MB. Effects of ethanol extract of *Ficus benghalensis* (bark) on inflammatory bowel disease. Indian J Pharmacol [serial online] 2010 [cited 2011 Jan 6]; 42: 214-8. Available from: <http://www.ijp-online.com/text.asp?2010/42/4/214/68420>
- <http://www.hort.purdue.edu/newcrop/default.html>. West Lafayette: Purdue University; 2003 [updated 2003 January 01; cited 2004]. Available from: <http://www.hort.purdue.edu/newcrop/cropfactsheets/ficus.html>.
- Gabhe SY, Tatke PA, Khan TA. Evaluation of the immunomodulatory activity of the methanol extract of *Ficus benghalensis* roots in rats. Indian J Pharmacol. 2006; 38:271-5.
- Deshmukh VK, Shrotri DS, Aiman R. Isolation of a hypoglycemic principle from the bark of *Ficus bengalensis*. Indian J Physiol Pharmacol. 1960; 4:182-5.
- Shukla R, Gupta S, Gambhir JK, Prabhu KM, Murthy PS. Antioxidant effect of aqueous extract of the bark of *Ficus benghalensis* in hypercholesterolaemic rabbits. J Ethnopharmacol. 2004; 92:47-51.
- Taur DJ, Nirmal SA, Patil RY, Kharya MD. Antistress and anti-allergic effects of *Ficus benghalensis* bark in asthma. Nat Prod Res. 2007; 21:1266-70.
- Aswar M, Aswar U, Watkar B, Vyas M, Wagh A, Gujar KN. Anthelmintic activity of *Ficus benghalensis*. International Journal of Green Pharmacy 2008; 2(3): 170-2.
- Augusti KT. Hypoglycemic action of bengalensin: A glucoside isolated from *Ficus benghalensis* Linn., in normal and alloxan diabetic rabbits. Indian J Physiol Pharmacol. 1975; 19: 218-20.
- <http://www.oecd-ilibrary.org/OECD>, Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects, OECD Publishing; 2002 [updated 2002 Feb 08]. Available from: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788;jsessionid=473a34p6eq17.delta.
- Puchchakayala G, Laxmi P, Diwakar B. Antinociceptive and anti-inflammatory effects of *Cleome chelidoni* linn. roots in experimental animals. Phcog. Mag. 2008, 4(13): 32-36.
- Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJC. Anti-inflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. J. Ethnopharmacol 2003, 84: 31-35.
- Narayanan N, Thirugnanasambantham P, Viswanathan S, Vijayasekaran V, Sukumar E. Antinociceptive, anti-inflammatory and antipyretic effects of ethanol extract of *Clerodendron serratum* roots in experimental animals. J. Ethnopharmacol 1999, 65: 237-241.
- Kulkarni SK. Handbook of Experimental Pharmacology. 2nd ed. New Delhi: Vallabh Prakashan; 1993.
- Vane JR. The evaluation of non-steroidal anti-inflammatory drug and their mechanism of action. Drug 1987; 33:8-27.
- Milton AS. Prostaglandins and fever. Trends in Pharmacological Sciences 1982; 40: 490-492.
- Radhakrishnan R, Zakaria MNM, Islam MW., Kamil M., Ismail A., Chan K. et al. Analgesic and Anti-Inflammatory Activities of *Teucrium stocksianum*. Pharm. Bio. 2001; 39 (6): 455-459.