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Evaluation of analgesic activity and phytochemical screening of *Ficus bengalensis* Linn Bark

Shital S. Chavan*[1], Ravindra S. Jadhav[2], Shital S. Kolhe[2], Rajendra S. Bhambar[3] and Vijay D. Tambe[2]

[1]*Department of Pharmacognosy, Government College of Pharmacy, Ratnagiri, Maharashtra, India*
[2]*Department of Pharmacognosy, Pravara Rural College of Pharmacy, Loni, Maharashtra, India*
[3]*Department of Pharmacognosy, Panchavati College of Pharmacy, Nasik, Maharashtra, India*

ABSTRACT

Ficus bengalensis Linn. (Family:Moraceae) is a large branching tree with numerous aerial roots occurring all over India. Currently available drugs for the management of pains, fever and inflammation conditions are presents with many known adverse effects, hence the search for new drugs from plants which hitherto may be harmless to humans. For this purpose, Ficus bengalensis Linn (Family:Moraceae) was screened for its phyto contents and analgesic properties using hotplate and tail immersion method with mice. The result of the preliminary Phytochemical studies revealed the presence of tannins, flavonoids, saponins, alkaloids, carbohydrates and phenolics in the plant as a whole. The analgesic study showed that the methanolic extract of the bark showed significant activity as compared to pentazocine used as a standard drug. Tannins, flavonoids, alkaloids and saponins have been reported to be responsible for the analgesic and anti-inflammatory activities in many medicinal plants of this family. These results may explain the use of the plant for the management of pains and its related ailments in the locality where it is very common.

Key words: Analgesic, *Ficus bengalensis*, Hot plate method, Tail immersion test

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INTRODUCTION

An analgesic, or painkiller, is any member of the group of drugs used to achieve analgesia-relief from pain. According to International Association for the study of Pain (IASP), pain as a sensation is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage [1, 2]. Analgesic drugs act in various ways on the peripheral and central nervous system. Analgesics are drugs used to treat or reduce pain and the classical analgesic drugs notably opiates and non steroidal anti-inflammatory drugs have their origin in natural products but many synthetic compounds that act by the same mechanism have been developed and are associated with serious adverse effects such as ulceration, gastrointestinal bleeding, additive potential, respiratory distress, drowsiness, nausea etc [3,4]. On the other hand drugs of plant origin have been used for management of diseases for many centuries and have not been reported of any deleterious effects to their hosts. Hence *Ficus bengalensis* Linn (Moraceae) was selected for this study. According to Ayurveda and trado-medicinal applications the Indian Banyan tree is astringent to bowels and useful in treatment of biliousness, ulcers, vomiting, vaginal complains, fever, inflammations and leprosy. According to Unani system of medicine, the latex is aphrodisiac, tonic and useful in piles, nose-diseases and gonorrhea. The aerial root is use in syphilis, biliousness, dysentery and inflammation of liver; it is also used in treatment of tooth ache, tooth picks, diabetes.

A decoction of bark is to be prepared and consumed twice daily shows hypoglycemic activity. Ayurvedic practitioners in India are using the milky juice (latex) of stem bark of *F.bengalensis* for the treatment of rheumatism

and other inflammatory diseases. The water extract of *F. bengalensis* bark has been reported to possess hypocholesterolaemic and hypolipidaemic effects. Aqueous and ethanolic extracts of *F.bengalensis* were investigated for antibacterial activity against *Pseudomonas aeruginosa*, *Proteus mirabilis, Stapylococcus aureus, Bacillus cereus, Alcaligenes faecalis* and *Salmonella typhimorium*. Bark extracts of *F.bengalensis* for its antiallergic and antistress potential in asthma by milk-induced leukocytosis (antistress effect) and milk-induced eosinophilia (antiallergic effect). *F. bengalensis* bark aqueous extract (500 mg/kg body weight/day) reduced the inflammation and swelling in pancreatic tissue and restored the levels of serum electrolytes [5, 6, 7].

Phytosterolin, ketones, Flavonoids, sterols, oentacyclic, triterpenes, triterpenoids, Furocoumarin, tiglic acid ester. Three ketones: 20-tetratriacontene-2-one (1), 6-heptatriacontene-10-one (7), pentatriacontan-5-one (13), and two other compounds, beta-sitosterol-alpha-D-glucose and meso-inositol has been reported from plant *F. bengalensis* Linn. [5, 6, 8]. The present study was undertaken to evaluate the analgesic activity of bark of *F. bengalensis* Linn and to find phyto constituents responsible for analgesic activity.

MATERIALS AND METHODS

Plant material

Bark of *Ficus Bengalensis* was collected from Ahmednagar district, Vadgaonpan, authenticated, having voucher no. FICUBSHIS2. Cleaned and dried at room temperature in shade, away from direct sunlight and ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of extract

Large difference in particle size of crude drug results in long extraction time as the coarse particles increases the extraction time and fine may form bed, so the powdered material was sieved through 60-120 mesh to remove fine and the powder was subjected for further study. Hot continuous extraction Technique; Soxhlet Extraction was used for sequential extraction with a series of solvents of increasing polarity i.e. Pet Ether and ethanol. The marc after exhaustive pet ether extraction was air-dried and subjected to extraction with methanol (high polarity solvent). The percentage yield was calculated using the formula below and the extract stored in a refrigerator at 15°C until time of use.

$$\%\text{yield} = \frac{\text{weight of extracted material}}{\text{weight of original plant material used}} X \frac{100}{1}$$

Animals

Young Swiss-albino mice of either sex, aged 4-5 weeks, average weight 20-25 gm were used for the experiment. They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase with access to food and water ad libitum. The set of rules followed for animal experiment were approved by the institutional animal ethical committee [9].

Acute Toxicity Test

Toxicity studies conducted as per internationally accepted protocol drawn under OECD guidelines in Swiss albino mice at a dose level up to 2000 mg/kg. Mice were fasted for overnight and maintained with water *ad libitum* and separated into different groups (n= 6). In fixed dose method, dose started from 2000 mg/kg body weight. The next day the product (suspended in 5% tween 80 solutions) was administered orally at a dose of 2000 mg/kg. After administration of the test compounds, animals were observed individually and continuously for 30 min, 2 hr, and 24 hr to detect changes in the autonomic or behavioral responses and also for tremors, convulsion, salivation, diarrhea, lethargy, sleep, and coma and then monitored for any mortality for the following 14 days [10,11,12].

Phytochemical screening

Phytochemical screening of the prepared extracts was conducted with various qualitative tests to identify the presence of chemical constituents. To perform the tests the following chemicals and reagents were used: Carbohydrates with Molisch's test, glycoside with water and sodium hydroxide solution, saponins with the capability of producing suds, steroids with chloroform and sulphuric acid, flavonoids with Mg and HCl, tannins with ferric chloride solution, gum with Molish reagents and concentrated sulfuric acid. Alkaloids were tested with Mayer's reagent, Hager's reagent and Dagendorff's reagent. These were identified by characteristic color changes using standard procedures [9, 13, 14].

Analgesic activity

Hot plate method

Central analgesic activity of petroleum ether, and methanol extract was evaluated using hot plate method. The mice of either sex were divided into four groups of six animals each. The first group served as control and received only vehicle (2% DMF), second group was administered standard drug pentazocine (10mg/kg, IP) dissolved in vehicle. The animals of third and fourth group were treated with petroleum ether and methanol extracts (50 mg/kg, i.p., each). The animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5 °C. A cut off period of 15s was observed to avoid damage to the paw [15]. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90, 120,150,180 min after oral administration of the samples [16, 17, 18]. 0 min readings are the predrug reaction time.

Tail immersion test

The procedure is based on the observation that morphine-like drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55 °C. Mice of either sex were divided into four different groups each containing six animals. The animals were marked individually, weighed and numbered appropriately. The lower 5 cm portion of each tail was immersed in beaker of water maintained at 55 ± 0.5 °C. The time, in seconds, for tail withdrawal from the water was recorded as the reaction time at the time interval of 15, 30, 45 and 60 minutes, with a cut-off time for immersion set at 10 s. The reaction time was measured 1 h before and after oral administration of extract (300 mg/ kg) or PSS (10 mL/kg). Second group was administered standard drug pentazocine (PTZ, 5 mg/kg p.o.) [19, 20].

Statistical analysis

The results of statistical analysis for animal experiment were expressed as mean ± SEM and were evaluated by ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. The $p<0.05$, 0.001 were considered to be statistically significant.

RESULTS

Plant Extraction

The yield of the stem bark extract was 5.25% w/w dry matter and was dark in colour.

Acute Toxicity Test

Acute toxicity test of the extract produced no death or signs of toxicity after 24 hours even at the dose of 3000 mg/kg which shows that the extract was well tolerated.

Phytochemical Screening

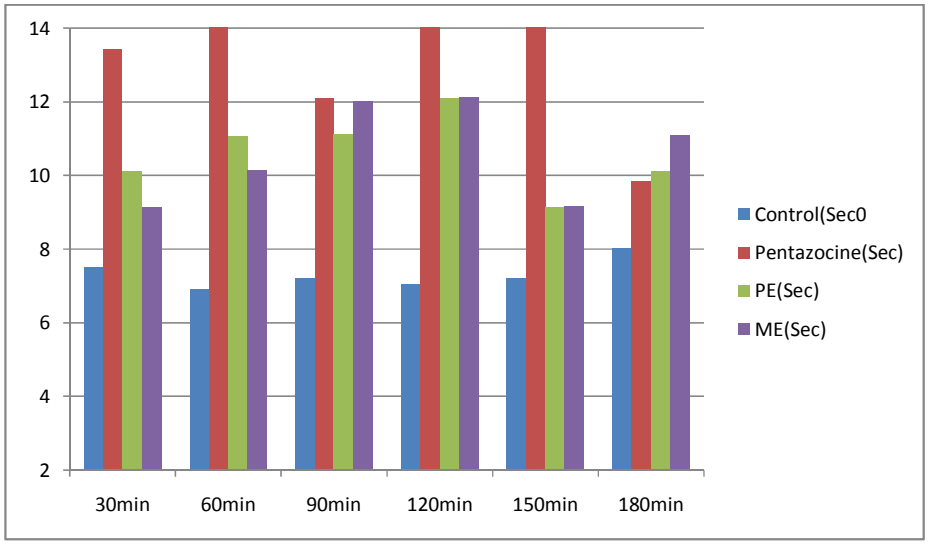
Phytochemical analysis of the pet ether and methanolic extract of *Ficus bengalensis* Bark revealed the presence of carotenoids, lipids, free sterol, triterpens and alkaloids, carbohydrates glycosides, tannins, resinous substances respectively.

Hot Plate Method

All the extracts of Ficus bengalensis bark showed significant analgesic activity at 50 mg/kg, i.p. dose as shown in table 1. Analgesic activity was comparable with standard drug pentazocine. Among all the extracts, methanol extract of bark of *Ficus bengalensis* showed highest increase in reaction time. Potency increases from petroleum ether, methanol. From the result it may be concluded that tannins compounds plays important role in analgesia. Methanolic extracts contain tannins compounds so it shows significant analgesic activity compare to petroleum ether.

Table 1: Effect of pet ether and methanolic extracts of *Ficus bengalensis* bark on latency to hot plate test in mice
PEFB: Pet ether extract of bark of *Ficus bengalensis* ; MEFB methanol extract of bark of *Ficus bengalensis*

Treatment	Latency to lick the paws (sec±SEM)					
	30 min	60 min	90 min	120 min	150 min	180min
Vehicle	7.5±0.219	6.9 ±0.223	7.2±0.286	7.05±0.232	7.2±0.143	8.04±0.223
Pentazocine	13.45±0.286	14.84±0.391	12.09±0.243	15.06±0.321	14.96±0.286	9.84±0.243
PEFB	10.11±0.186	11.07±0.165	11.20±0.362	12.1±0.221	9.13±0.154	10.12±0.365
MEFB	9.12±0.143	10.13±0.186	12.02±0.143	12.13±0.189	9.15±0.176	11.10±0.196



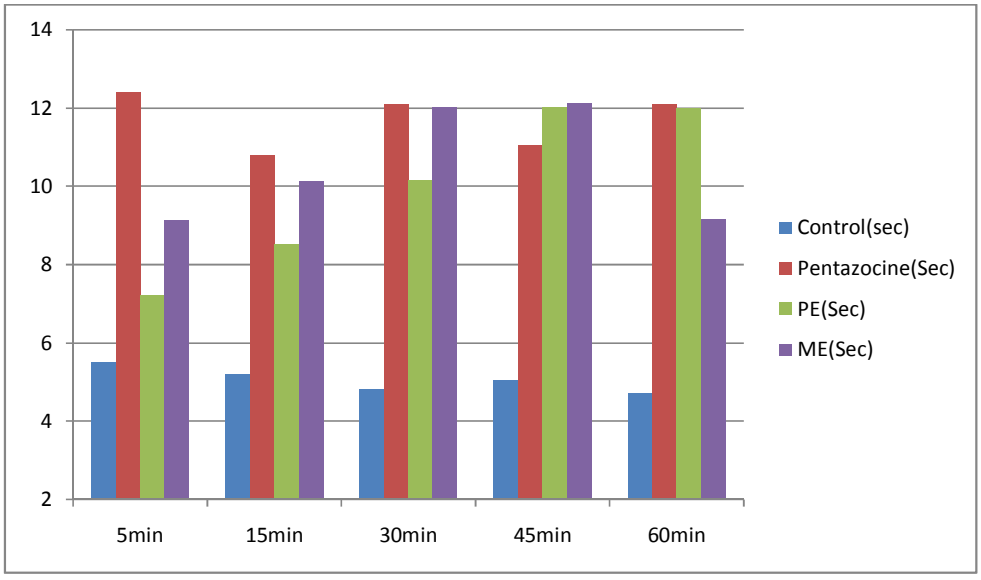
Graph 1: Effect of pet ether and methanolic extracts of *Ficus bengalensis* bark on latency to hot plate test in mice

Tail Immersion Method:

All the extracts of Ficus bengalensis bark showed significant analgesic activity at 50 mg/kg, i.p. dose as shown in table. Analgesic activity was comparable with standard drug pentazocine. Among all the extracts, petrolium ether extract of *Ficus bengalensis* bark showed highest increase in reaction time. Methanolic extracts contain tannins compounds so it shows significant analgesic activity compare to petroleum ether.

Table 2: Effect of pet ether and methanolic extracts of *Ficus bengalensis* bark on Tail immersion test in mice
PEFB: Pet ether extract of bark of *Ficus bengalensis* ; MEFB methanol extract of bark of *Ficus bengalensis*

Treatment	Tail Flick Time SEM (sec)				
	5 min	15 min	30min	45 min	60 min
Vehicle	5.5±0.217	5.2±0.187	4.8±0.201	5.05±0.274	4.7±0.303
Pentazocine	12.4±0.262	10.8±0.287	12.09±0.264	11.06±0.117	12.1±0.265
PEFB	7.2±0.177	8.5±0.197	10.15±0.316	12.01±0.132	12±0.287
MEFB	9.12±0.287	10.13±0.324	12.02±0.245	12.13±0.156	9.15±0.217



Graph 2: Effect of pet ether and methanolic extracts of *Ficus bengalensis* bark on Tail immersion test in mice

DISCUSSION

Anti-nociceptive models; hot plate and tail immersion tests were used to evaluate the analgesic activity of *Ficus bengalensis*. Since tests of analgesic drugs commonly measure nociception and involves the reaction of animals to painful stimuli [21]. The stimulus may be thermal (tail immersion or hot plate tests), chemical (acetic acid-induced writhing or formalin tests) or mechanical (tail or paw pressure tests) [22]. The methanolic and pet ether bark extract of *Ficus bengalensis* produced no death or signs of toxicity even at the dose of 3000 mg/kg which suggests that the extract was well tolerated by the mice and that the doses used were safe.

The methanolic extract of *Ficus bengalensis* bark showed a dose-dependent and significant ($P < 0.001$) increase in the pain threshold at 60 min post-treatment with dose of extract in the tail immersion, and Hot plate tests. The effects of the extract were significantly ($P < 0.001$) lower than those produced by pentazocin in the same tests.

The tail immersion and hot plate models have been used to study centrally acting analgesics [23, 24]. In these tests, the nociceptors are sensitise by sensory nerves and the involvement of endogenous substances such as prostaglandins are minimized. Thus from the results, we can conclude that the analgesic activity of *Ficus bengalensis* may be fully mediated through central mechanism.

CONCLUSION

In conclusion, we can confirm that the methanolic extract of *Ficus bengalensis* bark shows central analgesic properties. However, further study is needed in order to understand the precise mechanism. In future experiments, studies with purified fractions of the extract can be conducted for further pharmacological and toxicological characterization, such as the research of the mechanisms involved in the central analgesic effect.

REFERENCES

[1] Merskey HM., *Pain, Suppl*. **1986**; 3(5): 215-221.
[2] Michael YD, Dubois CG, Allen HL. Chronic Pain Management in: Healy TEJ, Knight. PR eds. Wylie and Church-Davidson's: A Practice of Anaesthesis, 7th ed. London. Hodder Arnold, **2003**; 1235-1239.
[3] Laurence DR, Benneth PN, Brown MJ. Clinical Pharmacology. 8th edn. Edinburgh: ChurchHill Livingstone; **1997**.
[4] Mate GS, Naikwade NS, Chowki CSA, Patil SB. Evaluation of Anti-nociceptive Activity of *Cissus quadrangularis* on Albino Mice. Int J Green Pharm. **2008**; 2:118–121.
[5] Daniel RS, Devi KS, Augusti KT, Sudhakaran NCR. *Ind. J. Exp. Biol*. **2003**; 41(4): 296-303.
[6] Shukla R, Anand K, Prabhu KM, Murthy PS. *Ind. J. Clin. Biochem*. **1995**; 10: 14–18.
[7] Taur DJ, Nirmal SA, Patil RY, Kharya MD. *Nat. Prod. Res*. **2007**; 21(14): 1266-1270.
[8] Sharad S, Mamta C, Edwin E, Shruti S, Hemant S. *Int. J. Diabetes. Dev. Ctries*. **2007**; 27(2): 56-59.
[9] Zulfiker AHM, Rahman MM, Hossain MK, Hamid K, Mazumder MEH, Rana MS. *Biology and Medicine*. **2010**; 2(2):42-48.
[10] Stitzel K, Carr G. Statistical basis for estimating acute oral toxicity comparison of OECD Guidelines 401, 420, 423 and 425. Appendix O-1; **1999**.
[11] Banger OP, Jarald EE, Asghar S, Ahmad S. *International Journal of Green Pharmacy*. **2009**; 211-14.
[12] Chavan SS, Jadhav RS, Kharat D, Mankar SD, Godge RK. BJPR. **2015**; 6(4): 255-260.
[13] Ghani A. Medicinal plants of Bangladesh with chemical constituents and uses. 2nd edn. Asiatic Society of Bangladesh, Dhaka, Ramna. **2003**; 184.
[14] Quibria T, Das BK, Hasan T, Uddin MA, Alam MM. *American Journal of Pharmaceutical Sciences and Nanotechnology*. **2014**; 1(1):1-10.
[15] Franzotti EM, Santos CVF, Rodrigues HMSL, Mourao RHV, Andrade MR, Antoniolli AR. *Journal of Ethnopharmacology*. **2000**; 72: 273-8.
[16] Eddy NB, Leimback D. *Journal of Pharmacology and Experimental Therapeutics*. **1953**; 107:385–393.
[17] Kulkarni, SK. Hand Book of Experimental Pharmacology. Vallabh Prakashan, Delhi, India, **1999**; 117.
[18] Toma W, Graciosa JS, Hiruma-Lima CA, Andrade FDP, Vilegas W, Souza Brita ARM. *Journal of Ethnopharmacology*. **2003**; 85: 19–23.
[19] Janssen PA, Niemegeers CJ, Dony JG. *Arzneimittel Forschung/Drug Research*. **1963**; 6: 502-507.
[20] Vyas S, Agrawal RP, Solanki P, Trivedi P. *Acta Pol Pharm*. **2008**; 65(4):473-6.
[21] Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th edn. New Delhi India: Elsevier Science ltd; **2003**.
[22] George KA, Eric W, David DO, George AK. *Pharmacog Mag*. **2009**; 17:49–54.
[23] Woolfe G, MacDonald AD. *J Pharmacol Exp Ther*. **1994**; 80:300.

[24] Bachlav RS, Gulecha VS, Upasani CD. *Indian J Pharmacol*. **2009**; 41(4):158–161.