

EVALUATION OF COMPARATIVE ANALGESIC ACTIVITY OF TWO *FICUS* SPECIES

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ABSTRACT

The main objective of study involves ethanolic extract of the leaves of *Ficus bengalensis* and *Ficus glomerata* was investigated for its acute analgesic potential in animals. Swiss mice were used for the experiment respectively. Analgesic activity determined by acetic acid induced writhing and formalin induced paw biting and licking method. Various concentrations (100, 200 and 400 mg/kg) were tested in the bioassay. In acetic acid induced writhings, both the extracts shown significant activity ($p < 0.01$). *Ficus glomerata* shows more reduction in pain than *Ficus bengalensis* as compared to reference standard Diclofenac. In formalin induced paw biting and licking test, the ethanolic extract of *F. bengalensis* and *F. glomerata* shown significant ($p < 0.01$) results. *Ficus glomerata* shows more reduction in pain than *Ficus bengalensis* as compared to reference standard Indomethacin. The leaves extract of *Ficus bengalensis* and *Ficus glomerata* contains alkaloids, carbohydrates, proteins, cardiac glycosides, steroids, flavonoids and tannins alkaloids, and phenolic compounds. It is revealed from the screening models used that the ethanolic extract of this plant shows the analgesic activity. The activity was attributed to the presence of phytoconstituents in the tested extract.

Keywords: *Ficus bengalensis*, *Ficus glomerata*, Moraceae, Analgesic, Diclofenac, Indomethacin.

INTRODUCTION

There is evidence of herbs having been used in the treatment of diseases and for revitalizing body system in almost all ancient civilization. Ayurveda, the Science of Life, has provided a rationale basis for treatment of various ailments such as pain which was very common complications in human beings. Several plants are claimed and proved to possess analgesic activity.¹

The drugs used in the management of the severe pain have been designated as analgesics. Pain is clinically classified into two types viz. acute and chronic. Acute pain is temporary and instantaneous. In contrast chronic pain is continuous, gradual in onset and refractory to treatment. Psychogenic pain is said

to exist when no anatomic or physiologic reason is available, and relief may be obtained by the use of placebos or sedatives.²

Ficus bengalensis, commonly known as Vad is a very large tree, sending down many aerial roots from the branches. Leaves are coriaceous, ovate to elliptic, obtuse, entire, glabrous or minutely pubescent beneath the base. All parts of the plant are acrid, sweetish, astringent to the bowels, useful in the kapha, ulcers, vomiting, vaginal complaints, fever and inflammations. The leaves are good for leprosy. The milky juice is aphrodisiac, tonic, maturant, lessens inflammation, useful in piles, diseases of nose, gonorrhoea. Externally the juice is applied for pains in rheumatism and lumbago.

Ficus glomerata commonly known as Umbara is an evergreen tree 15-18 m. in height. Young shoots are glabrous, pubescent. Leaves are ovate oblong or elliptic- lanceolate, tapering to a bluntish point at the apex with entire margins. All parts are cooling, sweet, acrid, anti dysenteric. The root is useful in hydrophobia. The bark is cooling, galactagogue and good for gravid uterus. The unripe fruit is acrid, astringent to bowels, tonic, useful in kapha, leucorrhoea and blood diseases. The ripe fruit is acrid, sweet, cooling, useful in blood diseases, burning sensations, fatigue, urinary discharges, thirst, leprosy, menorrhagia, nose bleeding.

MATERIALS AND METHODS

Plant material

The leaves of *Ficus bengalensis* and *Ficus glomerata*, were collected from Mahatma Phule Krushi Vidyapith, Rahuri, Ahmednagar and was authenticated.

Preparation of the extract

The collected leaves of both the plants were separated from impurities, dried properly and coarsely powdered in a mechanical grinder. The powder (50 g) was extracted in a soxhlet extractor with ethanol and total of 50 cycles were run to obtain thick slurry. This slurry was then vacuum evaporated to yield a solid extract. The dried ethanolic extract of both the plants were stored in a well closed, air tight and light resistant borosil glass container.

Preliminary phytochemical screening

In order to determine presence of alkaloids, glycosides, flavonoids, tannins, steroids, saponins a preliminary phytochemical study with plant extracts was performed³.

Pharmacological evaluation

Animals Used

Male Swiss mice (5-6 weeks old) weighing 20-25 g (Wistar strain) were housed in groups of six in Standard laboratory conditions of temperature ($23 \pm 1^{\circ}\text{C}$), relative humidity (55.5 %), lighting (08 to 20 hrs.) with food (Lipton India Ltd. Pellets) and water freely accessible⁴. They were transferred to laboratory at 1 hr. before the begin of the experiment. The experiments were performed in the light portion.

Drugs and chemicals

Diclofenac, Indomethacin, Ethanol (Qualigen Fine Chemicals, Mumbai)

Acute oral toxicity studies^{5,6}

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development-guidelines 425. As per the test female albino mice (Wistar Strain) was fasted overnight and was given increased doses upto 5000 mg/kg of ethanolic extract of both the plants orally. The next day, the animal was observed for 24 hr, with special attention during first 4 hr, and daily thereafter for a period of 14 days, for any sign of toxicity or mortality. Likewise five animals were dosed and observed one followed by other until the 50 % of the animals will die. All the data were placed in the computer guided statistical program-AOT425 statPgm and from this software the LD₅₀ of the ethanolic extract of *Ficus bengalensis* and *Ficus glomerata* was calculated.

Evaluation of analgesic activity

1. Acetic acid induced writhing⁷

A variety of chemical agents have been used for producing pain. The I. P. administration of noxious chemical substances to mice and cats produces peritoneal irritation, which elicits a writhing response. Many chemical agents have been reported to produce writhing but acetic acid and phenylbenzoquinone are the two most commonly used irritants⁸. In the evaluation of peripherally acting analgesic activity, the abdominal constrictions were induced by intraperitoneal injection of acetic acid (0.6% v/v) according to the procedure described by Costa et al. The animals were divided into eight groups containing 10 mice (n=10) each. Various groups were treated with FBE (100, 200 and 400 mg/kg, i.p.) and FGE (100, 200 and 400 mg/kg, i.p.), Diclofenac (100 mg/kg, i.p.) as standard⁹. And saline (10 ml/kg, i.p.) as a control. Acetic acid was injected (0.2 ml/10 g, 0.6 % , i.p.) after 30 minutes following the dose administration, and after this each mice was placed in a jar for observation. Number of writhes were counted for 30 minutes and registered. Observations were compared with control and percentage inhibition in writhes calculated. Analgesia was calculated as the % inhibition of abdominal constrictions.

$$\% \text{ Inhibition} = \frac{\text{Control mean} - \text{Test mean}}{\text{Saline (control) group mean}} \times 100$$

2. Formalin test

The formalin test was carried out by the method of¹⁰. The animals were divided into eight groups containing 10 mice (n= 10) each. Various groups were treated with FBE (100, 200 and 400 mg/kg, i.p.) and FGE (100, 200 and 400 mg/kg, i.p.), Indomethacin (10 mg/kg, i.p.) as a standard and saline (10 ml/kg, i.p.) as a control. 30 min after treatment all animals were injected with 20µl of formalin in saline solution into the subplanter region of the left hind paw. Each animal was placed in the glass jar and observed for 30 min. the first period (early phase)was recorded 0-5 min(Biting response) after the injection of formalin and the second period (late phase) was recorded 10-30 min (Licking response) after the injection. The time (sec) spent in licking responses of the injected paw were measured as an indicator of pain response.

$$\% \text{ Inhibition} = \frac{\text{Control mean} - \text{Test mean}}{\text{Saline (control) group mean}} \times 100$$

Statistical analysis

The data on biological studies were reported as Mean \pm Standard Error Mean (SEM, n = 10). p values < 0.01 when compared to standard drugs. The statistical analysis was carried out by one way ANOVA using the Dunnet's t test.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the ethanolic extract of the leaves of *Ficus bengalensis* shows presence of alkaloids, carbohydrates, proteins, cardiac glycosides, steroids, flavonoids and tannins while ethanolic extract of leaves of *Ficus glomerata* shows presence of alkaloids, carbohydrates, proteins, cardiac glycosides, steroids, and phenolic compounds.

In acetic acid induced writhings, both the extracts shown significant activity (p<0.01). *Ficus glomerata* shows more reduction in pain than *Ficus bengalensis* as compared to reference standard Diclofenac.

In formalin induced paw biting and licking test , the ethanolic extract of *F. bengalensis* and *F. glomerata* shown significant (p<0.01) results. *Ficus glomerata* shows more reduction in pain

than *Ficus bengalensis* as compared to reference standard Indomethacin.

CONCLUSION

The abdominal writhing response induced by acetic acid is sensitive process to establish peripherally acting analgesics. Local peritoneal receptors are responsible for abdominal writhing action. Intraperitoneal administration of acetic acid causes an increase in of PGE2 and PGF2a and produce analgesia by inducing capillary permeability and liberating endogenous substances like serotonin, histamine, prostaglandins, bradykinin, and substance P that sensitize pain nerve endings. It has been suggested that acetic acid stimulates the valinoid receptors and bradykinin B2 receptors in the pathway comprising sensory afferent C-fibers¹¹. Therefore, the observed activity may be due to interfering the synthesis or release of endogenous substances or desensitization of nerve fiber which carry pain sensation.

In acetic acid induced writhings, both the extracts shown significant activity (p<0.01) but *Ficus glomerata* shows more reduction in pain than *Ficus bengalensis* as compared to reference standard Diclofenac. Similarly in formalin induced paw biting and licking test , the ethanolic extract of leaves of *F. bengalensis* and *F. glomerata* shown significant (p<0.01) results. *Ficus glomerata* shows more reduction in pain than *Ficus bengalensis* as compared to reference standard Indomethacin. So it can be concluded that *Ficus glomerata* is more potent than *Ficus bengalensis* in peripheral analgesia. However further study is necessary in connection to phytochemistry and the characterization of phytoconstituents responsible for peripheral analgesic activity from both the plants.

Flavonoids are known to target prostaglandins which are involved in the acute inflammation and pain perception¹². It can be concluded that ethanolic extracts of *Ficus bengalensis* and *Ficus glomerata*. Linn possess analgesic activities may be due to the presence of flavonoids and other polyphenolic moieties present in it, which seems to support the use of these plants in traditional medicine.

Table 1: Effect of ethanolic extract of *F. bengalensis* (FBE) and *F. glomerata* (FGE) on acetic acid induced writhing

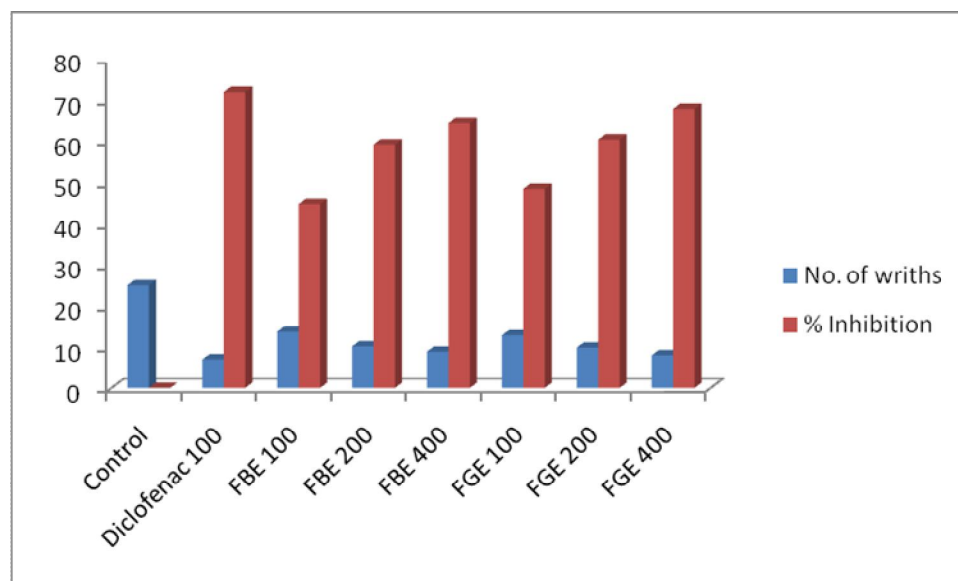
S. No.	Group	Dose (mg/kg) and route	No. of writhings	% Inhibition
1	Control	10 ml/kg, i.p.	25.00 ± 0.9545	-----
2	Diclofenac	100, i.p.	7.00 ± 1.033 ^{**}	72.00
3	FBE	100, i.p.	13.80 ± 1.263 ^{**}	44.8
4	FBE	200, i.p.	10.20 ± 1.104 ^{**}	59.2
5	FBE	400, i.p.	8.900 ± 1.320 ^{**}	64.4
6	FGE	100, i.p.	12.90 ± 0.8360 ^{**}	48.40
7	FGE	200, i.p.	9.90 ± 1.312 ^{**}	60.40
8	FGE	400, i.p.	8.00 ± 1.317 ^{**}	68.00

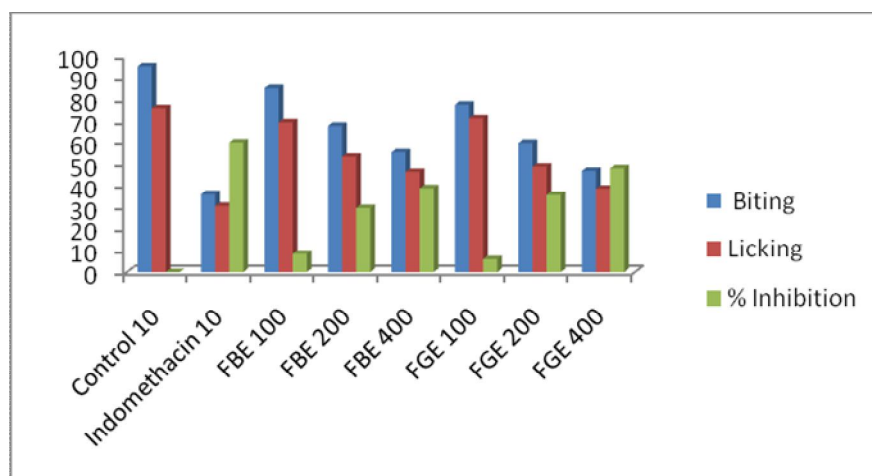
Values are mean ± SEM, (n=10), ^{**}P<0.01 v/s control.

Table 2: Effect of ethanolic extract of *F. bengalensis* (FBE) and *F. glomerata* (FGE) on Formalin induced biting and licking

S. No.	Group	Dose (mg/kg) and route	First phase (sec.) (Biting)	Second Phase (Sec.) (Licking)	% Inhibition
1	Control	10 ml/kg, i.p.	95.40 ± 6.817	75.80 ± 7.879	-----
2	Indomethacin	10, i.p.	36.00 ± 2.530 ^{**}	30.50 ± 1.600 ^{**}	59.76
3	FBE	100, i.p.	85.10 ± 2.873 ^{**}	69.40 ± 2.504 ^{**}	08.44
4	FBE	200, i.p.	67.80 ± 2.901 ^{**}	53.40 ± 4.719 ^{**}	29.55
5	FBE	400, i.p.	55.40 ± 2.880 ^{**}	46.40 ± 2.067 ^{**}	38.78
6	FGE	100, i.p.	77.50 ± 3.246 ^{**}	71.30 ± 2.833 ^{**}	05.93
7	FGE	200, i.p.	59.50 ± 2.034 ^{**}	48.80 ± 3.147 ^{**}	35.62
8	FGE	400, i.p.	46.90 ± 1.696 ^{**}	38.60 ± 2.596 ^{**}	48.02

Values are mean ± SEM, (n=10), P<0.05, ^{**}P<0.01 v/s control.

**Graph 1: Effect of ethanolic extract of *F. bengalensis* (FBE) and *F. glomerata* (FGE) on acetic acid induced writhing**



Graph 2: Effect of ethanolic extract of *F. bengalensis* (FBE) and *F. glomerata* (FGE) on Formalin induced biting and licking

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