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Analgesic and Antipyretic Activity of the Bark Extracts of *Ficus bengalensis* Linn.

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ABSTRACT: Background: The traditional information suggesting that the *Ficus bengalensis* Linn, belonging to family of *Moraceae,* possesses therapeutic activities like antipyretic, analgesic, and wound healing. **Aim:** In the present work attempts were made to study detail phytochemical investigation and pharmacological action, particularly anti-pyretic and analgesic activities of bark of *F. bengalensis*. **Method:** The acute toxicity study of extracts of the *F. bengalensis* Linn were showed 50 % of mortality at dose of 1000 mg/kg. Hence 1/10th of the same dose for all these extracts was taken as therapeutic dose i.e. 100 mg/kg. The animals were fevered by injection of Brewer's yeast suspension (10 mg/kg) subcutaneously in back below the nape of neck. The petroleum ether, chloroform, ethanol, and aqueous extract were fed to fevered rats. Similarly, in case of analgesic activity the rats were kept on fasting for 24 h. Then all these extracts were administered orally (100 mg/kg) 60 min prior to the commencement of the estimation of reaction time. And finally the animal models were subjected for hot plate and tail immersion analgesic activity. **Results:** Ethanolic extract showed significantly decrease in elevated body temperature, while petroleum ether, chloroform, and aqueous extracts did not show a significantly decreased in elevated body temperature. However, the ethanolic extract showed more significant analgesic activity as compared to any other extracts. **Conclusion:** It could be concluded that the ethanoic extract of *F. bengalensis* showed more significant antipyretic and analgesic activities.

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INTRODUCTION:

The use of plant for treating various diseases predates human history and forms the origin of much of the modern medicine. Long before the advent of modern medicine, herbs were the main stream remedies for nearly all ailments [1-2]. People commonly diagnosed their own illness, prepared and prescribed their own herbal medicines or brought them from the local apothecaries [4-5]. Herbal medicines are being used increasingly as dietary supplements to fight or prevent

common maladies like cancer, heart attacks, and depression. When added to food as supplements, herbs have also been termed as "nutraceuticals". Herbal remedies are unpurified plant extracts containing several constituents, which often work together synergistically. According to World Health Organization, herbal medicine is defined as plant derived material or preparation, which contains raw or processed ingredients from one or more plants with therapeutic values.

F. bengalensis Linn. is also commonly known as Banyan tree. Bark of *F. bengalensis* Linn is reported to posses' anti-diabetic, hepatoprotective, anthelmintic, antioxidant, immunomodulatory and anti-diarrhoeal activity, while anti-pyretic and analgesic activity of bark is still not scientifically investigated. Antipyretic and Analgesic compounds in the market still present a wide range of undesired effects leaving an open door for new and better compounds. Natural products are believed to be an important source of new chemical substance with potential therapeutic applicability. Several plant species traditionally used as antipyretic and analgesic. The use of plant preparation and medicaments for various diseases started from ancient period of time. Folk medicine provides a valuable approach in search for development of new and useful therapeutic agents like antipyretic, analgesics, hepatoprotective, anticancer and nutraceuticals [6-7].

Literature survey for the plant of *F. bengalensis* Linn revealed that this plant is having anti-diabetic, hepatoprotective, anthelmintic, antioxidant, immunomodulatory and anti-diarrhoeal activity. However, the tribal people in satpuda range use the bark of *F. bengalensis* for its anti-pyretic and analgesic activity, but the anti-pyretic and analgesic activity of *F. bengalensis* Linn is still not reported. Hence it was thought worthwhile to study antipyretic and analgesic activity of *F. bengalensis* Linn [8-10].

MATERIALS AND METHODS:

The ethanol, petroleum ether, and chloroform were procured from HiMedia, New Delhi. The Brewer yeast and Aspirin were purchased from S.D. Fine Chem, Mumbai. All other chemicals and reagents used in this research study were of analytical grades and procured from the authorized dealer.

Procurement and Authentication of Plant Material:

For the present study, barks of F. bengalensis Linn were procured from the local market of Faizpur. Bark and different plant parts were authenticated at the Botanical Survey of India, Pune. The authentication number was BSI/WC/TECH./2008/356.

Drying and Size Reduction:

Bark of *F. bengalensis* Linn were dried in shade under normal environmental conditions and then subjected to size reduction to coarse powder. The coarse powdered material was stored in air tight polythene bags [11].

Extraction:

The coarse powder material was charged into the Soxhlet extractor (Borosil, India) and hot continuous successive extraction was carried out using different solvents (Water, ethanol, chloroform, and petroleum ether), on basis of their increasing order of polarity. Each time before extracting with the next solvent, the powdered material was air-dried and each extract was concentrated by distilling off the solvent to obtain the crude extractive. The drug was extracted with each solvent till completion of extraction (40 cycles) [12-13].

Preliminary Phytochemical Screening:

The extracts were subjected to preliminary phytochemical screening for the detection of various plant constituents [12,13].

Salkowaski Test:

Residue of each extract (few mg) was taken in chloroform (2 ml) and conc. sulphuric acid (2 ml) was added from the side of the test tube. The test tube was shaken for few minutes. The development of red color in the chloroform layer indicated the presence of sterols [14].

Liebermann's Test:

To the residue (few mg) acetic anhydride (few ml) was added and gently heated. The content of the test tube was cooled. Few drops of concentrated sulphuric acid were added from the side of the test tube. A blue colour gave the evidence of presence of sterols [15].

Liebermann-Burchard Test:

The residue (few mg) was dissolved in chloroform and few drops of acetic anhydride were added to it, followed by concentrated sulphuric acid from the side of the tube. A transient colour development from red to blue and finally green indicated the presence of sterols.

Test for alkaloids:

The residue (5 ml, 1.5 % v/v) of each extract was taken separately in hydrochloric acid and filtered. The filtrate was then used for testing alkaloids.

***Dragendorff's Reagent*:**
The Dragendorff's reagent was sprayed on Whatmann No. 11 filter paper and the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with Dragendorff's reagent, with the help of a capillary tube. Development of an orange red colour on the paper indicated the presence of alkaloids [16].

***Hager's Reagent*:**
A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was obtained indicating the presence of alkaloids.

***Mayer's Reagent (Potassium Mercuric Iodide Reagent)*:**
The Mayer's reagent was prepared as follows: mercuric chloride (1.36 g) was dissolved in distilled water (60 ml). Both the solutions were mixed and diluted to 100 ml with distilled water. To a little of the test filtrate, taken in a watch glass; a few drops of the above reagent were added. Formation of cream colored precipitate showed presence of alkaloids.

***Wagner's Reagent (Iodine-Potassium Iodide)*:**
Iodine (1.27 g) and potassium iodide (2 g) were dissolved in water (5 ml) and the solution was diluted to 100 ml with water. When few drops of this reagent were added to the test filtrate, a brown precipitate was formed indicating the presence of alkaloids.

***Test for saponins*:**
***Foam Test*:**
A few mg of the test residue was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. If a stable, characteristic honeycomb like forth is obtained saponins are present.

***Haemolysis Test*:**
A little of test residue was dissolved in normal saline in such a way that the solution (5 ml) represented the crude drug (1 g.). In a series of 5 test tubes, doses (0.2, 0.4, 0.6, 0.8, and 1 ml) were added and volume was made up to 1 ml in each case with normal saline. 1 ml of diluted blood (0.5 ml of rabbit's blood diluted to 25 ml with normal saline) was added to each tube and changes observed. If hemolysis of blood occurs the saponins are present [17].

***Test for flavonoids (shinoda test)*:**
A small quantity of test residue was dissolved in ethanol (5 ml, 95 % w/v) and treated with few drops of concentrated hydrochloric acid and magnesium metal (0.5 g). The pink, crimson or magenta color is developed within a minute or two, if flavonoids are present.

Evaluation of Antipyretic and Analgesic Activity:
***Animal Selection*:**
Wistar strain albino rats (150 to 200 g) were used for anti-pyretic model. Rats were kept in polypropylene cages and led on standard laboratory diet i.e. oil extracted groundnut feed was given. The animals were exposed to 12 h. cycle of darkness and light. The bedding materials of cages were changed every day. Rats were divided into six groups. Each group contained six animals [18].

***Antipyretic Activity by Yeast Induced Pyrexia Method*:**
A suspension of Brewer's yeast (15 %) in normal saline water (0.9 % w/v) was prepared. Five groups each containing 6 rats of either sex were taken. The thermocouple was inserted 2 cm deep into the rectum and the rectal temperatures were recorded. The animals were fevered by injection of brewer's yeast suspension (10 mg/kg) subcutaneously in the back below the nape of the neck. The sight of injection was massaged in order to spread the suspension beneath the skin. The room temperature was kept at 22 to 24 ºC. Immediately after yeast administration, food was withdrawn, and then the rise in rectal temperature was recorded. The measurement was repeated after 30 minutes. The dose of the test compound and standard drug was given orally. The rectal temperature was recorded again after 30, 60, 120 and 180 min [19]. Aspirin (100 mg/kg) was selected as a standard drug. The various extracts were dissolved in normal saline water with the help of Gum acacia (2 % w/v).

***Analgesic activity by Hot Plate Method*:**
In this Hot Plate Method, animals from each group were placed on the hot plate, which is commercially available, consists of an electrically heated surface. Temperature of this hot plate is maintained at 55 to 56 ºC and observation is done up to the time until either paw licking or jumping was noted. Then the average basal reaction time was noted before and after 30, 60, 90, and 120 min following oral administration of the drugs and test compounds [20].

Table 1. Phytochemical Investigation of extracts of the *F. bengalensis* Linn.

Test	Pet. Ether extract	Chloroform extract	Ethanol extract	Water extract
Carbohydrates	-	-	+	+
Gums	-	-	-	+
Mucilage	-	-	+	+
Proteins	-	-	+	-
Amino Acids	-	-	+	-
Fats and Oils	+	-	-	-
Sterols and triterpenoids	+	+	+	+
Cardiac glycosides	-	-	+	+
Anthraquinone glycosides	-	-	+	+
Cyanogenetic glycosides	-	-	-	-
Coumarin glycosides	-	-	-	-
Alkaloids	-	-	-	-
Saponins	-	-	+	-
Flavonoids	+	+	+	+
Tannins phenolic compounds	-	-	+	+

(+) = Present, (-) = Absent.

***Analgesic activity by Tail Immersion Method*:**

Tail immersion method was used to determine the analgesic activity. Rats of wistar strain were randomly divided into a six groups having six animals in each and they were fasted overnight but during the experiment had free access to water. All the extracts were administered orally (100 mg/kg) 60 min prior to the commencement of the estimation of reaction time. The temperature of the water in the organ bath was set at 55 ± 0.5 ºC with the help of thermostat. The reaction time was determined by immersing the tail in hot water and the time taken by the rat to withdraw its tail clearly out of water was noted. Observations were repeated at an interval of 30 min up to 120 min [20].

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening of Extracts:

The results of preliminary phytochemical screening of different extracts of bark of *F. bengalensis* Linn are given in Table 1.

Antipyretic Activity:

Extracts obtained were subjected to evaluate antipyretic activity by yeast induced fevered rats. Aspirin (100 mg/kg) was taken as standard drug. Ethanolic extract showed significant decrease in elevated body temperature, while petroleum ether extract, chloroform extract and water extract did not show a significant decrease in elevated body temperature as compared to standard drug.

The animals were fevered by injection of Brewer's yeast suspension (10 mg/kg) subcutaneously in back below the nape of neck. The petroleum ether, chloroform, ethanol, and aqueous extract were fed to fevered rats. Ethanolic extract showed significantly decrease in elevated body temperature, while petroleum ether extract, chloroform extract, and aqueous extract did not show a significantly decreased in elevated body temperature.

Analgesic activity:

Hot Plate Method:

Extracts obtained were subjected to evaluate for analgesic activity by hot plate method using rats as animal model. Aspirin (100 mg/kg) was taken as standard drug. Ethanolic extract of bark of *F. bengalensis* shows more significant activity, while pet-ether extract, chloroform extract and water extract does not show significant analgesic activity as compared to standard drug.

Tail Immersion Method:

Extracts obtained were subjected to evaluate for analgesic activity by tail immersion method using rats as animal model. Aspirin (100 mg/kg) was taken as standard drug. Ethanolic extract of bark of the *F. bengalensis* shows more significant activity, while pet-ether extract, chloroform extract and water extract does not show significant analgesic activity as compared to standard drug.

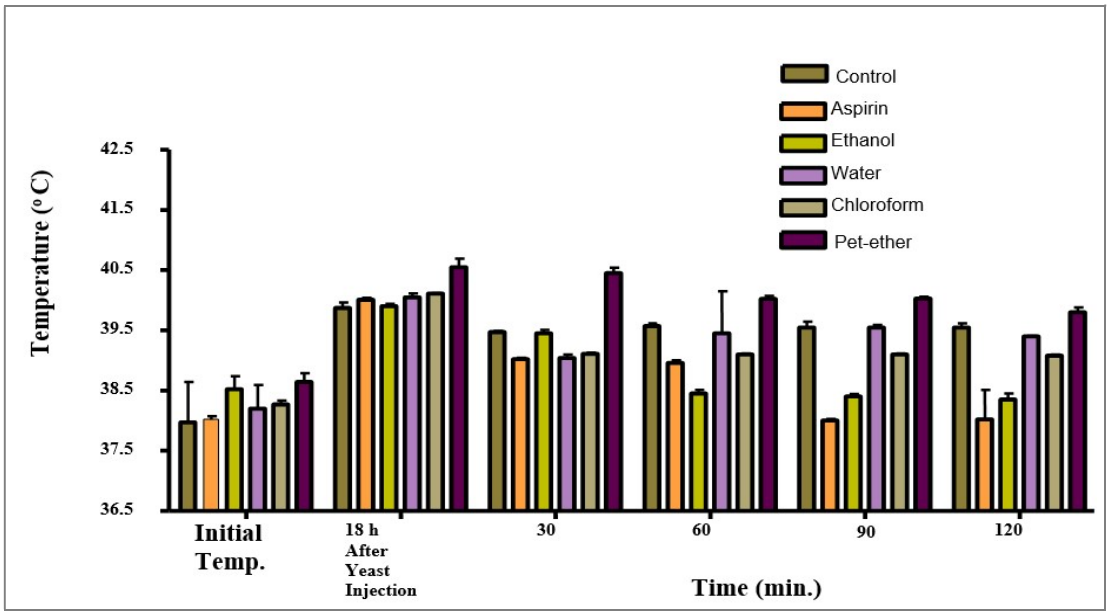

Fig 1. Result of Yeast Induced Pyrexia Method.

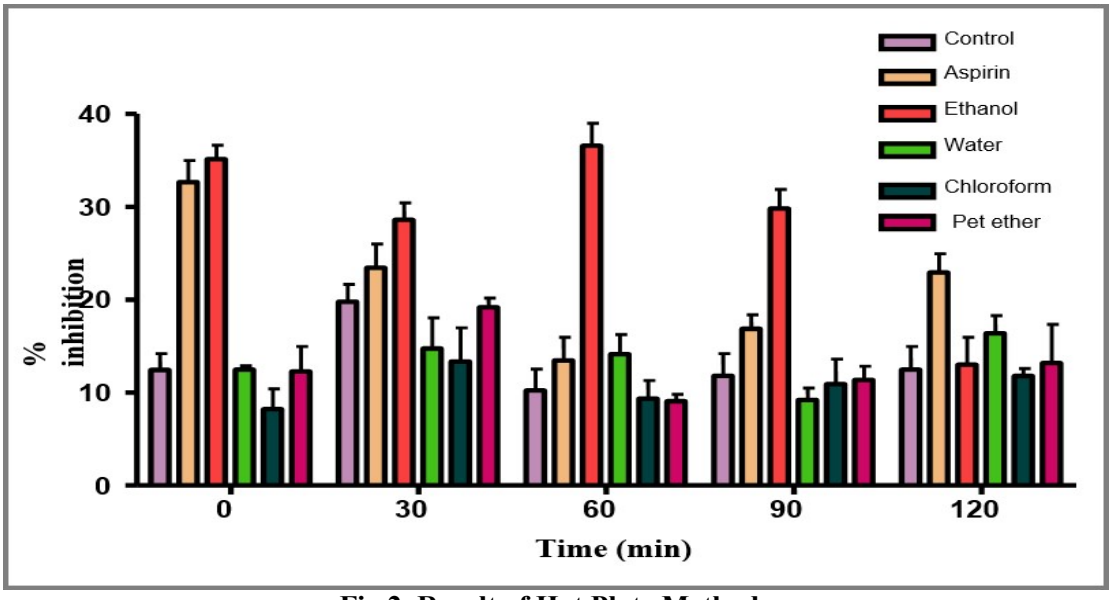

Fig 2. Result of Hot Plate Method.

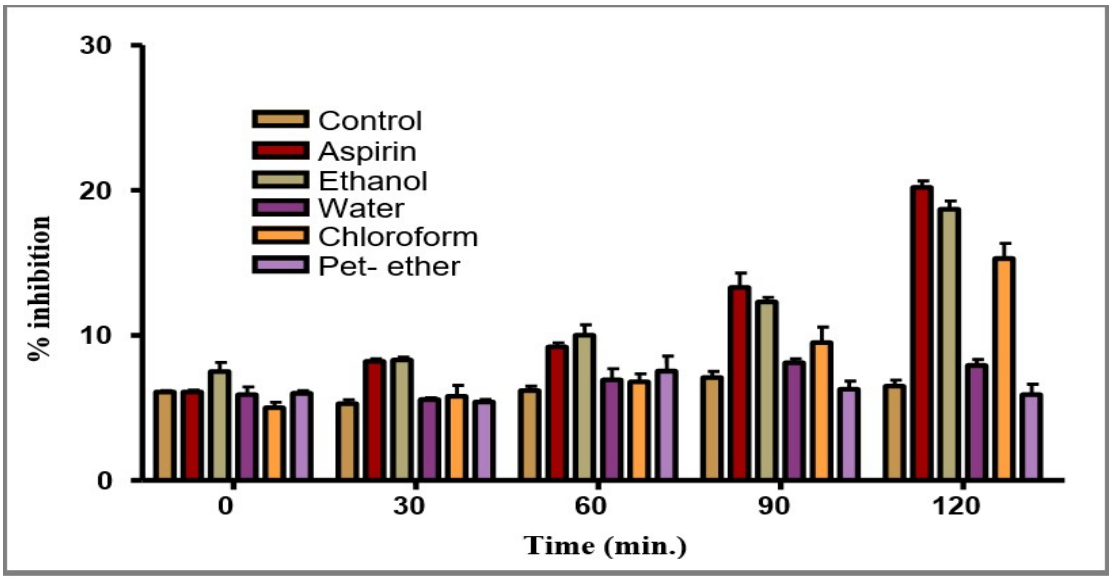

Fig 3. Result of Tail Immersion Method.

Analgesic activity the rats were kept on fasting for 24 h. Then all these extracts were administered orally (100 mg/kg) 60 min prior to the commencement of the estimation of reaction time. And finally the animal models were subjected for hot plate and tail immersion analgesic activity. However, the ethanolic extract showed more significant analgesic activity as compared to any other extracts.

CONCLUSION:

The acute toxicity study of extracts of the F. bengalensis Linn were showed 50 % of mortality at dose of 1000 mg/kg. Hence 1/10th of the same dose for all these extracts was taken as therapeutic dose i.e. 100 mg/kg. The animals were fevered by injection of Brewer's yeast suspension (10 mg/kg) subcutaneously in back below the nape of neck. The petroleum ether, chloroform, ethanol, and aqueous extract were fed to fevered rats. Ethanolic extract showed significantly decrease in elevated body temperature, while petroleum ether extract, chloroform extract, and aqueous extract did not show a significantly decreased in elevated body temperature.

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