Anti-Rheumatic and Antioxidant activity of extract of Stem bark of *Ficus bengalensis*

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Abstract

Ficus bengalensis Linn (Moraceae) is commonly known as Banyan tree or Bargad. It possesses medicinal properties and in ayurveda is used in diuretic, hypoglycemic, anti-inflammatory agent and in diarechea.

The analgesic, anti rheumatic and ant-oxidant activity of the methanolic extract of the bark of Ficus bengalensis (MFB) were studied at doses of 100, 200 and 300 mg/kg (i.p) using the Freund's Complete Adjuvant induced arthritis model, the Formalin induced arthritis model and the Agar induced arthritis model. The extract produced marked inhibitory effect on edema especially on secondary immunological arthritis and caused graded inhibition of both phases of Formalin- induced pain .The present study validates the traditional use, demonstrating that the methanolic extract of bark of Ficus bengalensis possesses dose –dependent anti-rheumatic activity in all the models with a possibility of acting through the central and peripherally mediated activities. The DPPH and hydrogen peroxide model demonstrated positive antioxidant activity in a concentration dependent manner(100µg/ml).

Keywords: *Ficus bengalensis; Formalin test; Agar induced arthritis; Freunds Complete Adjuvant induced arthritis. DPPH.*

Introduction

Traditional and folklore medicines play an important role in healthcare. There exists a plethora of knowledge and benefits of herbal drugs in our ancient literature of Ayurvedic and Unani medicine. According to the WHO, 80% of the population in developed countries relies on traditional medicine for their primary healthcare. Exploration of the chemical constituents of plants and pharmacological screening would provide the basis for developing new lead molecules in strategic favor of Natural product Drug Discovery. This shows the need for planned activity guided phyto-pharmacological evaluation of herbal drugs.

Ficus bengalensis (Moraceae, Mulberry family) commonly known as Banyan tree, has more than 800 species and 2000 varieties of *Ficus* species.[1]

Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints and can affect multiple other organs of the body. It can cause joint destruction and functional disability and is a common ailment amongst the geriatric patients, who are prescribed pain relieving anti- inflammatory drugs and DMARD causing adverse effects on the liver, kidney and GIT.
Previous studies have attributed a variety of salutary physiological effects to this species. *Ficus bengalensis* is used in ayurveda for diarrhea, dysentery, piles, as a hypoglycemic, diuretic, tonic, astringent, in rheumatism, applied to gums to lessen inflammation.[2]

Previous, phytochemical studies carried out reported on the presence of three isolated ketones[3] haracterized as, 20-tetratriaconthene -2-one; 6-heptatriacontene-10-ione, and pentatriacontan-5-one. A dimethoxy derivative of leucocyanidin[4] characterized as 3-O-beta–D-galactosyl cellobioside and bengalenoside [5] were reported on exhibiting hypoglycaemic activity. A glycoside,

perlargonidin characterized as 3-O-alpha-L-rhamnoside was isolated from the bark[6]. Two flavonoids[7] characterized as 5,7-dimethyl ether of leucopelargonidin, a 3-O-alpha –L rhamnoside and 5'3' dimethyl ether of lecocyanidin, a 3-O-alpha D galactosyl cellobioside exhibited antioxidant action in hyperlipidemic rats. Leucodelphinidin[8] exhibited hypoglycaemic activity. Further study reveals the antioxidant activity of the aqueous bark extract[9], immunomodulatory activity of methanolic extract of aerial roots[10] and the anti-inflammatory activity of the ethanolic bark extract using the carrageenan induced anti-inflammatory model.[11]

Although a lot of work pharmacological investigation has already been carried out but this sacred tree possess exhorbant properties and a lot more is yet to be explored. It was hence thought fruitful to carry out an in depth study on the anti rheumatic activity of the methanolic extract of the bark using different models.

The total ash, water soluble / acid insoluble ash values were found to be 10.55%, 7.7 % and 3.62 % respectively. Moisture content of drug was found to be 0.124 gm. It yielded 1.9 % in petroleum ether, 1.3 % in acetone extract, 4.3 % in methanol, 4.6 % in ethanol and 6.5 % in water. Phytochemical investigation showed the presence of sterols, flavanoids, phenols, tannins, alkaloids, glycosides, carbohydrates and saponins in the ethanol, methanol, acetone and water extracts. Tannins and flavonoids were absent in petroleum ether (table-1).

Material and Methods

Plant material and extraction: The crude drug (bark) of the plant *Ficus benghalensis* were collected from "Dashoda Garden" Near Toll naka, Khandwa road, Indore (M.P.) and authenticated by Prof. Diwanji.

The roots were shade-dried, powdered and stored in airtight containers. The powder was subjected to successive Soxhlet extraction using solvents of varying polarity; petroleum ether (60^0-80^oC), benzene, ethyl acetate, acetone, ethanol, methanol and water. The solvent was removed under reduced pressure to obtain a total of six extracts. After about forty siphons of each solvent extraction step, the material was concentrated by evaporation. The extracts were standardised with respect to their physico -chemical parameters such as consistency, pH and extractive value. All the extracts were subjected to qualitative chemical tests to determine the nature of the phytoconstituents[12,13] viz. carbohydrates, glycoside, alkaloid, amino acid, flavanoids, fixed oil, tannins, gum and phytosterols. This showed the presence of various constituents in different extracts.

Solvents for Extraction: Methanol, Ethanol, Acetone, Petroleum Ether AR (60-80^0C)-, Benzene, Ethyl Acetate from SD Fine Chemicals Ltd. Mumbai.
Reagents: for phytochemical screening.
Chemicals: Formaldeyde, agar, from SD Fine Chemicals Ltd. Mumbai, Acetyl salicylic Acid and Freunds Adjuvant from Sigma Aldrich and Indomethacin from Microlab, Banglore.

Animals: Healthy Adult Swiss albino rats (150-200g) of both sexes were obtained from Instinimal Health and Veterenary Biological, Mhow, Indore, M.P and used throughout the study. They were housed in microlon boxes in a controlled environment conditions (22-28 ^{0} C, 60-70% relative humidity, 12-h dark/light cycle) and were fed with standard rat feed (m/s Hindustan lever Ltd, Bangalore, India) and water *ad libitum.* Prior to their use they were allowed two weeks for acclimatization within work area environment. The experiments were conducted as per guidelines of CPCSEA, Chennai, India after obtaining animal ethical committee clearance.

Acute toxicity study: Acute toxicity study of MFB was carried out in rats according to OECD 423 guidelines[14]. Different doses of MFB were administered up to 5000mg/Kg (p.o) and the animals observed for a period of 72 hr for behavioral changes, toxic reactions and mortality.

Pharmacological Tests Models: Just prior before use the Methanolic dried extract of *Ficus bengalensis* MFB was dissolved in a mixture of propylene glycol and water (1:4).[15]
Adjuvant –induced arthritis in rats: Experimental arthritis was induced in rats according to method of Newbould[16] . The right footpad of each rat was injected subcutaneously

with 0.1 ml of Freund's Complete Adjuvant agent (FCA, sigma). The extract at three dose levels (100, 200, 300 mg/Kg.i.p), distilled water and Acetyl salicylic acid (ASA) at 10mg/Kg were given daily for 16 consecutive days to the treatment groups, control group and reference group respectively. Treatment started from day 8th after FCA injection. The assessment of the antiarthritic activity was done by measuring the mean paw edema on the 8th, 12, 16 and 24 days post injection of FCA.

Formalin –induced Arthritis: The test was performed according to Dubuissson and Dennis [17] .The extract was dissolved in a mixture of propylene glycol and water (1:4).The extracts (100, 200, 300 mg/Kg) and ASA (10mg/Kg.) were administered i.p in a volume of 1.5-2ml.Control group received only vehicle (1.5-2ml) Briefly 30 min after treatment, 50 µl (2.5% v/v in distilled water) of formaldehyde was injected subcutaneously into the plantar surface of the hind paw of rats. The behavioral responses to nociception were noted and the time spent licking, biting or scratching the injected hind paw was recorded up to 30min. The first 5 min was considered as early phase (neurogenic phase) and the period of 15-30 min as late phase (inflammatory phase).

Agar- induced edema of the rat paw: The rat paw edema method was used, whereby agar is the edematogenic agent. Acute inflammation was measured in terms of change in volume of the rat hind paw induced by subplantar injection of agar [18,19] . Animals (n =5 group) received 100, 200, 300 mg/Kg of MFB administrated orally. Edema was induced one hour later with agar (0.1ml) injected into the sub plantar region of the right hind paw of the rats. The volume of distilled water displaced by the treated paw was measured before and 1,2,3 and 4 hr after induction of edema using a plethysmometer (model 7159, Ugo Basile, Varese ,Italy). Control groups received either equivalent volume of the vehicle (distilled water) or indomethacin (100mg/Kg). Inflammation was assessed as the difference between the zero time volume of the treated paw (Vo) and the volume at the various times (Vt) after the administration of the phlogistic agent. Percent inhibition of edema[20] was calculated using the relation:

Inhibition of edema (%) = 100 [1-{(a-x)/ (b-y)}].
Where a= mean paw volume of treated rats at various time after agar injection, x = mean paw volume of treated rats before agar injection, b= mean paw volume of control rats at various time after agar, y = mean paw volume of control rats before agar injection.

Anti-oxidant activity

Preparation of DPPH Solution: Solution of DPPH (0.1mM) in methanol was prepared by dissolving 1.9 mg of DPPH in methanol and volume was made up to 100ml with methanol. The solution was kept in darkness for 30 minutes to complete the reaction.

Preparation of Hydrogen Peroxide Solution: 0.2M Potassium dihydrogen phosphate and 0.2M sodium hydroxide solutions were prepared as per the Indian Pharmacopoeia 1996 standards. 50 ml of Potassium dihydrogen phosphate solution was placed in a 200 ml volumetric flask and 39.1 ml of 0.2M sodium hydroxide solution was added in this and finally volume was made up to 200 ml with distilled water to prepare phosphate buffer (pH-7.4). 50 ml of phosphate buffer solution was taken and an equal amount of hydrogen peroxide was added in this to generate the free radicals and solution was kept a side to complete the reaction.

Determination of Anti-Oxidant Activity: 1ml of DPPH solution was added to 1ml of different extracts and allowed to stand at room temperature for 30 min, and then absorbance was measured at 517 nm in a spectrophotometer. Similarly 1ml Extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution and the absorbance was measured at 230 nm in a spectrophotometer.
The percentage inhibition was measured by following formula[27].

$$\% \text{ inhibition} = (Ac-At) \times 100/Ac$$

Ac is the absorbance of control
At is the absorbance of test sample

Statistical analysis: The experimental data were expressed as Mean ±, standard deviation and statistically assessed by one- way analysis of variance (ANOVA). Difference between drug treated groups and control group was evaluated by student's *t* test. $P< 0.05$ was considered significant.

Results and Discussion

Acute Toxicity Study: LD-50 value by oral route could not be determined as no mortality was observed up to 4g / Kg dose level. No toxic reactions were observed at none of the doses employed.

Effect on rat paw edema: Table 2 shows the mean change in paw edema and inhibition rate after administration of **Freunds Complete Adjuvant** and MFB extract. The left hind paw also developed edema in addition to the right footpad. The different doses of MFB exhibited anti-inflammatory activity which was maintained until the experiment was terminated on day 24.

The results of FCA-induced edema arthritis in rats show that the extracts at doses of 200 and 300 mg/kg exhibited significant anti-inflammatory effects, especially on the secondary immunological arthritis.

The **Formalin test** is sensitive and valid model for various classes of analgesic drugs [21]. It produced a biphasic response and different analgesics may act differently in early and late phases of the test[22]. Administration of the extract demonstrated dose dependent inhibition, as shown in table 3 in both early (53.8 %, 69.9%) and late phase (70.41%, 78.42%) licking response, at the tested doses of 200 and 300 mg/kg respectively (table 3). While ASA caused significant inhibition (67.72%) of late phase but not early phase (13.57%). This test can be used to suggest the possible mechanism of antinociceptive effect of a proposed analgesic[23]. Centrally acting drugs such as opioids inhibit both phases equally[24] while peripherally acting drugs inhibit only the late phase. The late phase seems to reflect an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs [21,25]. The inhibition of both phases of pain as observed with the extract showed that they contain analgesic principles acting both centrally and peripherally. Formalin induced pain is caused primarily by peripheral tissue inflammation[26].

Administration of the extract significantly ($P<0.05$) suppressed the development of acute edema of rat paw induced by **agar** at 3 doses tested, as shown in table 4. The extract exhibited highest inhibition at a dose of 300mg/Kg which was as good as indomethacin. A combination of Indomethacin (100mg) and the extract (100mg) showed the maximum inhibitory effect.

DPPH and Hydrogen peroxide methods exhibited inhibition of free radicals by Methanolic extract of bark of *Ficus bengalensis.* A 100 µg/ml of methanol extract exhibited 85.46 and 88.62 percent inhibition of DPPH and Hydrogen peroxide free radicals respectively. It exhibited low IC50values, 51.83 µg/ml and 44.73 µg/ml for DPPH and Hydrogen peroxide models respectively.

RA is an autoimmune disease characterized by chronic inflammation, hyperproliferation of the synovial lining and cartilage destruction. It is reported that the methanolic extract contains several phytochemicals like terpenoids, alkaloids, glycosides, flavonoids, steroids as shown in table. The presence of flavonoids, tannins, saponin, steroids may attribute to its anti-rheumatic activity through the anti-inflammatory activity as well as modifying the autoimmune system. In addition, the preliminary acute toxicity test obtained by this plant indicates it has reasonable safety margins with regards to acute toxicity.

Conclusion

The methanolic extract of the plant exhibited significant anti- rheumatic activity and anti-oxidant activity, based on the various models. *Ficus bengalensis* could be a potential source of natural antioxidants. Further bioactive isolation could be carried out to isolate the bioactive molecule responsible for the anti-rheumatic activity, which could be considered as a drug candidate.

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References

1. Kirtikar K. R. and Basu B. D., Indian Medicinal Plants, International Book Distributor, Dehradun, India, 2312 **(2004)**

2. Nadkarni K. M., Indian Materia Medica, Edn, **Vol. I**, Popular Book Depot, 544 **(1955)**

3. Subramanian P. M. and Mishra G. S., Chemical constituents of *Ficus bengalensis, Pol. J. Pharm.,* **30(4),** 559-62 (**1978)**

4. Augusti K. T. and Daniel R. S., Antidiabetic effect of Leucocyanidine derivative isolated from bark of *F.bengalensis, Indian J. Biochem Biophy,* **29**, 380-82 **(1992)**

5. Augusti K. T., Hypoglycaemic action of bengalenoside, a glycoside, isolated from *F. Bengalensis* in normal and alloxan diabetic rabbits, *Indian J. Physiol. Pharmacol.,* **19**, 218-220 **(1975)**

6. Augusti K. T. and Cherian B., Effect of Leucopelargonin derivative from *Ficus bengalensis* Linn, On diabetic dogs, *Ind J. Med .Res.,* **99**, 82-86 **(1994)**

7. Cherian S. and Augusti K. T., Antidiabetic effect of Leucopelargonidin, *Indian Exp*. Bio, **31 (1)**, 26 -29 **(1993)**

8. Geetha A. and August K. T., Hypoglycaemic effects of Leucodelphinidin derivative isolated from *F. bengalensis, Ind. J. Physiol Pharmacol,* **38,** 220 **(1994)**

9. Rimi Shukla and Shweta Gupta, Antioxidant effect of aqueous extract of bark of F. Bengalensis, *J. of Ethnopharmacology*, **92 (1)**, 47-51 **(2004)**

10. Gabhe S. Y., Tatke P. A. and Khan T. A., Evaluation of immunomodulatory activity of methanol extract of *F.bengalensis, Ind. J. Pharmacology,* **38(4)**, 271-275 **(2006)**

11. Patil V. V. and Pimpirikar R. B., Pharmacognistical studies and evaluation of anti-inflammatory activity of *Ficus bengalensis, Journal Young Pharmacists,* **1**, 49-53 **(2009)**

12. Mukherjee K., Pulok 2002, Kokate C. K., 1996, Khandelwal K. R., Practical Pharmacognosy, 67-70 **(2006)**

13. Khandelwal, K. R., Kokate C. K., Gokhale S. R., Practical Pharmacognosy Techniques And Experiments, Nirali Prakashan Publications, 104-106 **(1996)**

14. Zimmermann M., *Ethical guidelines for investigation of experimental pain in conscious animals,* Pain, **16,** 109-10 **(1983)**

15. Organization of Economical and Cooperation Development Guidelines for the Testing of chemicals-Acute Oral Toxicity up and Down Procedure, 425 **(2006)**

16. Newbould B. B., *Brit J. Pharmacol,* **21,** 127 **(1963)**

17. Dubuisson D., and Dennis S. G., *Pain,* **4,** 161 **(1977)**

18. Okoli C. O., Akah P. A., Nwafor S. V. and Adams C. D., Anti-inflammatory activity of hexane leaf extracts *Aspilia Africana*., *J. Ethnopharmacol*. **109,** 219-225 **(2007)**

19. Iwueke A. V., Nwodo O. F. C., Okoli C. O., Evaluation of anti-inflammatory and analgesic activities of *Vitex doniana* leaves, *Af.r J. Biotech,* **5 (20)**, 1929-1935 **(2006)**

20. Perez G. R. M., Anti-inflammatory activity of *Ambrosia artemisiaefolia and Rheo spathaceae, Phytomed,* **3,** 163-167 **(1993)**

21. Hunskaar S., Hole K., *Pain,* **30**, 103, **(1987)**

22. Lee O. K. and Jeong Y. S., Effects of different concentrations of Formalin on Paw edema and Pain Behaviors in Rats, *Journal Korean Med Sci.,* **17**, 81-85 **(2002)**

23. Tjolsen A., Berge O. G., Hunskaar S., Rosland J. H., Hole K., *Pain,* **51,** 5 **(1992)**

24. Shibata M., Ohkubo T., Takahashi H., Inoki R., *Pain,* **38**, 347 **(1989)**

25. Rosland J. H., Tjoisen A. and Hole K., *Pain* **42**, 235 **(1990)**

26. Paras S. Kirankumar H. and Sakshy S., Evaluation of Anti-inflammatory activity of

27. *Cymopsis tetragonoloba* seeds in rodents, *Journal of Pharmacy Research,* **3(1)**, 163-165, **(2010)**

28. Sharma A., Bhardwaj S., Mann A. S., Jain A. and Kharya M. D., Screening Methods of Antioxidant Activity: An Overview, *Phcog Rev,* **(2)**, 232-238 **(2007)**

Table-1: Phytochemical screening of different extracts of the bark of *Ficus bengalensis*

		Petroleum ether	Benzene	Acetone	Ethyl acetate	Methanol	Ethanol	Water
1	Alkaloids	-ve	--	+ve	+ve	+ve	+ve	+ve
2	Carbohydrate	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3	Glycoside							
	Cardiac	-ve	-ve	-ve	-ve	-ve	-ve	+ve
	Saponins	+ve	+ve	+ve	-ve	+ve	+ve	+ve
	Flavonoid	-ve	+ve	+ve	+ve	+ve	+ve	+ve
	Antraquinones	-ve	-ve	-ve	-ve	-ve	-ve	-ve
4	Proteins	-ve	-ve	+ve	-ve	+ve	+ve	+ve
5	Amino acids	-ve	-ve	-ve	-ve	-ve	-ve	-ve
6	Tannins	-ve	-ve	+ve	+ve	+ve	+ve	+ve
7	Fats and oils	-ve	-ve	-ve	-ve	-ve	-ve	-ve
8	Steroids	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Table-2: Effect of methanolic extract on FCA- induced paw edema in rats
Mean change in paw edema (Mean SEM)

Days	Control	ASA	Extract 300mg/kg	Extract 200mg/kg	Extract 100mg/kg
8	1.05 ± 0.14	1.01± 0.16 (3.8)	1.02± 0.13 (3.6)	1.03± 0.09(1.9)	1.05± 0.12 (0)
12	1.13± 0.12	1.03 ± 0.15* (8.8)	0.96± 0.19* (15.0)	1.00±0.14* (11.5)	1.0 1± 0.19* (10.6)
16	1.24± 0.16	1.00 ± 0.17*(19.5)	0.81±0.20** (34.68)	1.03±0.15*(16.92)	1.07 ±0.18* (13.7)
20	1.28 ± 0.18	1.00± .18*(21.88)	0.86± 0.17* (32.81)	0.97± 0.18*(24.22)	1.00 ±0.13(21.89)
24	1.26 ± 0.17	0.92± .20*(26.98)	0.75± .18*(40.48)	0.86±0.13*(31.7)	0.97± 0.19(23.0.)

*Statistically significant from control P< 0.05. .** Statistically significant from control P< 0.01 .Values in parenthesis represent percentage inhibition of edema calculated relative to control. Edema value was measured 2 h after drug administration, which was performed on 8th day after FCA injection

Table 3: Effect of Methanolic extract in Formalin-induced Paw edema in Rats

Treatment	Dose (mg/kg)	Mean S.E.M.of total amount of time (sec),the animals spent Licking or biting paw			
		0 – 5 min	Inhibition (%)	15 – 30 min	Inhibition (%)
Control		68.50 ± 3.09	-	146.65 ± 4.60	-
Extract	100	37.12 ± 2.1*	45.8	76.18 ± 3.7*	48.05
	200	28.2 ± 3.4*	53.8	43.4 ± 4.5*	70.41
	300	21.1 ± 2.6*	69.19	31.65 ± 4.2*	78.42
ASA	100	59.2 ± 7.2	13.51	37.6 ± 8.5**	67.54

Results are expressed as mean ± S.E.Min seconds (N=10). * P< 0.05: ** P< 0.01 compared to control group. (Dunnets test)

Table-4: **Effect of Methanolic Extract on Agar-Induced Paw Edema in Rats**

Treatment (mg/kg)	Mean increase in paw volume (ml)					% Decrease in paw volume at 4 h
	0 h	1 h	2 h	3 h	4 h	
Control	0.77±0.04	1.16±0.01	1.91±0.01	2.12±0.03	2.18±0.01	-
Indomethacin100mg	0.74±0.06	1.0±0.03*	1.08±0.03*	1.1±0.03*	1.25±0.03*	42.66
300mg	0.72±0.07	0.97±0.06*	1.34±0.07*	1.4±0.01*	1.3±0.08*	40.37
200mg	0.75±0.05	1.0 ±0.01*	1.44±0.08*	1.5±0.05*	1.42±0.06*	34.86
100mg	0.81±0.08	0.99±0.01*	1.51±0.06*	1.6±0.07*	1.44±0.04*	33.95
Indomethacin100)+ MFB(100)	0.71±0.06	0.96±0.04*	1.16±0.06*	1.3±0.05*	1.21±0.05*	44.50

n=5.The observations are mean ± SEM. *p<0.05, as compared to control (ANOVA followed by Dunnett's test).

Table-5: Anti-oxidant activity of MFB showing % inhibition and IC50 value (µg/ml)

Conc. of Extract	% inhibition	
	DPPH Method	H2O2 Method
20 µg/ml	23.76	29.44
40 µg/ml	38.42	44.65
60 µg/ml	59.68	67.86
80 µg/ml	75.42	77.22
100 µg/ml	85.46	88.62
IC50 Value µg/ml	51.83	44.73