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Assessment of Anti-inflammatory Activity of Methanolic Extract of Ficus Bengalensis and Its Fractions in Rats

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ABSTRACT

The present study was aimed to evaluate anti-inflammatory activity of methanolic extract of Ficus bengalensis and its fractions against carrageenan and formalin induced inflammation and oedema models in rats. Methanolic extract was prepared and it was fractionated using different solvent in increasing order of polarity such as toluene, ethyl acetate, chloroform and butanol and subjected to anti-inflammatory study. Degree of inflammation was measured plethysmometrically and compared with control and standard drug, diclofenac10mg/kg. All the drugs were administered orally. Treatment of rats with carrageenan and/ or formalin produced a significant inflammation and oedema. Rats pretreated with methanolic extract of bark of Ficus bengalensis 100-200 mg/kg body weight its ethyl acetate fraction exhibited significant reduction in inflammation in both models studied. The results obtained with methanolic extract of bark of F. bengalensis and its ethyl acetate fraction [FB1] was comparable with the standard anti-inflammatory agent diclofenac sodium. Maximum anti-inflammatory effect was found to be at the dose of 150mg/kg in case of methanolic extract and 50mg/kg in case of FB1 in both the models studied in rats. Our data suggest that F. bengalensis bark possesses a potential anti-inflammatory activity.

Keywords: *F. bengalensis,* anti-inflammatory, carrageenan.

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I. INTRODUCTION

Ficus bengalensis Linn. (Moraceae), known as Banyan in English and Bargad in Hindi is a large tree with spreading branches attaining height of 100 ft is well known to ancient Indian traditional medicine and grows almost everywhere in India1. All the parts of the plant are acrid, sweetish and astringent to bowels. Its bark is antidiabetic and also used in piles, gonorrhea, syphilis, dysentery and inflammation of liver. The milky juice is aphrodisiac, tonic and anti-inflammatory. The infusion of the bark is regularly used as tonic and nutrition by tribal of South India2. Bengaloside, a glucoside and leucocynidine derivatives isolated from bark have been reported to possess antidiabetic action3, 4. Two flavonoid compounds dimethyl ether of leucopelargonidine 3-0 alpha L rhamnoside and 5,3 dimethyl ether of leucocyanidine 3-0 alpha L galactosyl celobioside have shown antioxidant effect in hyperlipidemic rats 5.

Inflammation or phlogosis is a pathological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is defense mechanism, the complex events and mediators involved in inflammatory reaction can be induced, maintain or aggravate many diseases 6. It is a complex phenomenon, comprising of biochemical as well as immunological factors. It is recognized by symptoms such as Rubor (redness), Tumor (Swelling), Calor (heat), Dolor (pain) and Functio laesa (Loss of functions) 7. Different degree of inflammation occurs at different conditions and on the basis of that Inflammations are classified as Acute, Sub-acute and Chronic inflammation.

Inspite of the tremendous advances being made in allopathic medicine, no effective anti-inflammatory medicine is available. The available therapeutic agents bring about only symptomatic relief without any influence on the curative process, thus, causing the risks of relapses and danger of untoward effects 8. Recently much attention have been focused on plant derived product for treatment of different disorders. Hence this study was undertaken to assess anti-inflammatory activity of methanolic extract of Ficus bengalensis and further by carry out its fractions and tested against carrageenan and formalin induced inflammation and oedema models in rats.

II. MATERIALS AND METHODS

Formalin and carrageenan was procured from E. Merck India Ltd. Mumbai; Diclofenac was obtained as gift sample from Cadila Pharma Ltd., Ahmeadabad India. All other reagents used for the experiments were of analytical grade.

Preparation of fraction of bark of F. bengalensis

The air-dried coarsely powdered drug 1kg was extracted in Soxhlet extractor with petroleum ether (60□-80□C) for defatation. The defatted powdered drug was then extracted with methanol. The crude methanol extract was fractionated with different solvents of increasing polarity such as toluene, chloroform, ethyl acetate and butanol. Each time before extracting with the next solvent the powdered drug or crude extract were dried in hot air oven below 50□C. All the extracts, after recovering the solvent by distillation, were dried in oven at 50□C. The percentage yields of each extract was calculated and were then subjected to qualitative chemical examination for various phytoconstituents as per method described by Harborne9.

Animals

Wistar albino rats of either sex weighing between 150 and 200 g were used for the hepatoprotective study. The animals were housed in polypropylene cages and maintained at 24±2oC under 12 hr light dark cycle and they were fed ad libitum with standard pellet diet and had free access to water. They were initially acclimatized for the study and the study protocol was

approved by the Institutional Animal Ethics Committee as per the requirements of Committee for the Purpose of Control and Supervision on Animals CPCSEA, New Delhi.

Anti-inflammatory activity

Albino rats weighing between 160-180 gm of either sex were used. They were kept in standard environmental condition and maintained on standard chow diet and water ad libitum. Acute inflammation was induced by 0.1 ml of 1% (w/v) carrageenin into the plantar aponeurosis of the right hind paw of rats 10,11. Suspension of methanolic extract (100,150 and 200 mg/kg) and different fractions prepared using Na-CMC was administered orally 45 minutes before carrageenin injection. Diclofenac sodium (10 mg/kg) was used as reference standard. Paw volume was measured with plethysmometer before and 3 hour after carrageenin injection. The percent inhibition of paw edema was calculated.

Formalin-induced oedema in rat hind paw

0.1 ml of 2% formalin was injected into the subplantar area of right hind paw of ether anaesthetised rat12. All drugs were given orally one hour prior to formalin injection and continued for 7 consecutive days. Paw volume was measured with plethysmometer on day 1 and 7. The percent inhibition of paw edema was calculated.

III. RESULTS

The methanolic extract and its different fractions of F. bengalensis were tested for their antiinflammatory activity by carrageenin induced rat paw edema method. The methanolic extract significantly reduced (%protection >70%) the carrageenin induced rat paw edema ($p< 0.001$) and maximum effect was found at 150mg/kg (Table1). Out of different fractions tested for their antiinflammatory activity ethyl acetate fraction of F. bengalensis at the dose of 100mg/kg was also found to be active and inhibit the paw volume by 64.91% (Table 1). Toluene and butanol fraction, however, did not produce any significant effect.

Table 1. Anti-inflammatory activity of F. bengalensis on carrageenin induced paw oedema in rats:

Treatment	Dose (mg/kg)	Oedema volume ± S.E.M (ml)	% Inhibition
Control saline	2.0 ml	0.57 ± 0.05	--
Diclofenac sodium	10	0.11± 0.03*	80.70
MethanolicExtract of *F. bengalensis*	100	0.15±0.04*	73.68
	150	0.13±0.01*	77.19
	200	0.13±0.01*	77.19
Toluene fraction	25	0.44±0.05	22.80
	50	0.41±0.04	28.07
Ethyl ace. fraction	25	0.20±0.03*	64.91
	50	0.18±0.01	68.42
Butanol fraction	25	0.41±0.06	28.07
	50	0.35±0.05	38.59

Values are mean ± S.E.M.; n=6; * $p<0.001$ vs. control; student t-test.

Similarly the methanolic extract and its different fractions of F. bengalensis were tested for their antiinflammatory activity by formalin induced rat paw edema method. The methanolic extract significantly reduced (%protection >60%) the formalin induced rat paw edema ($p < 0.001$) and maximum effect was found at 150mg/kg (Table2). Out of different fractions tested for their antiinflammatory activity ethyl acetate fraction of F. bengalensis at the dose of 100mg/kg was also found to be active and inhibit the paw volume by 64.91% (Table 2). Toluene and butanol fraction, however, did not produce any significant effect.

Table 2. Anti-inflammatory activity of F. bengalensis on formalin induced paw oedema in rats:

Treatment	Dose (mg/kg)	Oedema volume ± on 7th day S.E.M (ml)	Inhibition (%)
Control saline	2.0 ml	1.10 ± 0.03	--
Diclofenac sodium	10	0.35± 0.03*	68.18
MethanolicExtract of *F. bengalensis*	100	0.52±0.04*	52.72
	150	0.39±0.02*	64.54
	200	0.40±0.01*	63.63
Toluene fraction	25	0.91±0.05	17.27
	50	0.81±0.04**	26.36
Ethyl ace. fraction	25	0.50±0.03*	54.54
	50	0.42±0.01*	61.81
Butanol fraction	25	0.93±0.06	15.45
	50	0.78±0.05**	29.09

Values are mean ± S.E.M.; n=6; * $p<0.001$ vs. control; ** $p<0.05$ vs. Control; student t-test.

IV. DISCUSSION

Majority of the drug discovery in pharmaceutical industry relies on the natural products. Many plants belong to various families have been elucidated for the anti-inflammatory properties13. The present study was carried out to assess anti-inflammatory activity of methanolic extract of Ficus bengalensis and its fractions and tested against carrageenan and formalin induced inflammation and oedema models in rats. Methanol extract was fractionated with different solvents of increasing polarity such as toluene, chloroform, ethyl acetate and butanol and all fractions tested for activity to identify active fraction and subsequently to find lead nucleus.

Carrageenin induced inflammation in rat paw is one of the widely accepted model14 for studying anti-inflammatory properties. The development of carrageenin-induced oedema is biphasic; the first phase is attributed to the release of histamine, 5HT and kinins while second phase is related to the release of prostaglandin 15, 16. Carrageenin induces a protein rich exudates containing large number of neutrophils17. The present investigation revealed that the methanolic extract exhibited significant anti-inflammatory activity, which was comparable to the reference drug diclofenac sodium. When used in a dose of 150 mg/kg, the anti-inflammatory activity was comparable to 10 mg/kg of diclofenac sodium. Recent studies suggest that inflammatory tissue damages is due to the liberation of reactive oxygen species from

phagocytes invading the inflammation sites. Hence the antiinflammatory activity is probably mediated through its free radical scavenging activity 18, 19.

It is well known that inhibition of formalin-induced pedal oedema in rats is one of the most suitable test procedure to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis 20. Injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response 21. Thus formalin-induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. This experiment is associated with the proliferative phase of inflammation. Results with methanolic extract of bark of F. bengalensis and its ethyl acetate fraction [FB1] indicated tested extract and FB1 is quite compatible with those of the standard drug diclofenac. Therefore, the drug appears to be effective against formalin-induced arthritis. These findings justifies the usefulness of FBM and FB1in the treatment of inflammation associated diseases like arthritis.

Phytochemical investigations have revealed that barks of F. bengalensis shows presence of flavonoids, coumarinolignans and tannins. The literature has already documented value of flavonoid 22.

It is thus concluded that methanolic extract of F. bengalensis barks exhibited excellent anti-inflammatory effect against carrageenan and formalin induced inflammation. Further studies in progress in our laboratory for isolation and characterization of phytoconstituents may lead to development of lead nucleus for treatment of inflammation and arthritis.

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