Anti-inflammatory Effect of Ethanolic Extract of Ficus bengalensis Linn. in Carrageenan Induced Paw Edema In Rats

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ABSTRACT

Ficus bengalensis of family Moraceae was extensively utilized by the traditional practitioners for its various ethnopharmacological activities. The aim of this study was to evaluate, experimentally, the anti-inflammatory effect of ethanolic extract of the bark of F. bengalensis in carrageenan induced paw edema in rats at a dose level of 50, 100 and 200 mg/kg, orally. The extract was administered for the anti-inflammatory activity 1 h prior to carrageenan injection in the subplantar region. Paw edema was measured by plethysmometer on 1st and 3rd h, after carrageenan injection. F. bengalensis extracts at all the doses significantly prevented the inflammation in a dose dependent manner which was comparable to that of diclofenac sodium (5 mg/kg, intraperitoneally). The phytochemical analysis of F. bengalensis extracts revealed the presence of antioxidant phytochemicals such as flavonoids and tannins, and thus the anti-inflammatory action of F. bengalensis extracts may be subsequent to its in vivo antioxidant activity. F. bengalensis extracts eliminate the systemic oxidative stress produced by carrageenan injection.

Key words: Anti-inflammatory, antioxidant, Ficus bengalensis.

INTRODUCTION

Ficus bengalensis Linn. (Moraceae) is popularly known as “Banyan.” It is a large tree, widely distributed in tropical and subtropical regions. The bark and aerial roots of this plant are frequently used as a folk medicine, particularly in conditions like ulcer, leprosy, sepsis, diarrhoea, dysentery, diabetes, gonorrhea and piles.[1,2] The milky juice from its aerial roots possesses aphrodisiac and anti-inflammatory activity[3] and its paste is applied externally in conditions like pain, bruises, rheumatism, cracked feet and gum inflammation.[4] Some workers have demonstrated antioxidant effect of F. bengalensis in in vitro studies.[5] Numbers of plants having antioxidant property were found effective in preventing the development of inflammation and thus indicated in inflammatory conditions.[6] The protection offered by such plants has been attributed to their antioxidant property. Hence, it was contemplated that ethanolic extract of F. bengalensis may exhibit anti-inflammatory effect in rats, particularly in view of its traditional use in inflammatory conditions and in vitro antioxidant property.

Therefore, the present study demonstrated the influence of ethanolic extract of the bark of F. bengalensis against carrageenan induced paw edema in rats. Further to delineate the anti-inflammatory effect to the antioxidant activity, oxidative stress parameters were assessed.

MATERIALS AND METHODS

Plant material
The bark of F. bengalensis was collected in July, 1999. The plant material was authenticated and deposited at Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University Campus, Nagpur, Maharashtra, India.

Preparation of ethanolic extract of Ficus bengalensis (FBE)
The dried coarse powder of the bark of F. bengalensis was defatted by extracting with petroleum ether (60-80 °C)
in the Soxhlet apparatus continuously for 72 h, and the phytochemicals were then extracted by using 90%, v/v ethanol. The concentrated ethanolic extract was solvent dried under reduced pressure and subsequently air dried until constant weight (extraction yield 13%, w/w). The extract was suspended in 0.1%, Na-CMC for oral administration.

**Preliminary phytochemical screening**

FBE was subjected to the preliminary phytochemical screening as per standard procedures.[7]

**Animals**

Sprague Dawley rats (220-225 g) of either sex were used. Animals were kept at the departmental animal house at 25 ± 2 °C, under relative humidity of 55-65% and light and dark cycles of 12 h. Animals were provided with a standard pellet diet (Goldmohar brand, Lipton India Ltd.) and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi.

**Doses and treatments**

Diclofenac sodium was a gift sample from Zim Laboratories Ltd., while thiobarbituric acid and carrageenan (Lambda grade) were purchased from Sigma Aldrich, USA. Pyrogallol and trichloroacetic acid were purchased from LOBA Chemicals, India.

**Acute oral toxicity study**

FBE was tested at 2000 mg/kg, orally for its cut off LD$_{50}$ dose as per OECD guidelines. No toxicity of any nature or mortality could be observed in the subsequent 14 days after receiving this dose and hence the maximum employed dose of extract was 200 mg/kg (10% of the cut off level of LD$_{50}$).

**Grouping and treatments**

Sprague Dawley rats (220-225 g) of either sex were divided into five different groups containing 5 animals each. Group I received 0.1 ml of normal saline (in the subplantar region) while group II, III, IV and V received 0.1 ml of 1%, w/v carrageenan (in the subplantar region). During the treatment, group II received vehicle of the extract (5 ml/kg, 0.1% Na-CMC, orally), while group III, IV and V received FBE (50, 100 and 200 mg/kg, orally). Group VI was administered diclofenac sodium (5 mg/kg, intraperitoneally) as standard antiinflammatory agent. The vehicle, extracts and diclofenac sodium were administered 1 h before carrageenan administration.

**Assessment of the antiinflammatory activity in carrageenan induced paw edema**

The increase in the paw volume was recorded on plethysmometer (UGO Basile, Italy) at 1st and 3rd h after administration of carrageenan. The results are expressed in terms of mean increase in paw volume at 1st and 3rd h and antiinflammatory activity was expressed in terms of percent inhibition of paw edema at 3rd h.

**Assessment of oxidative stress parameters in blood**

**Estimation of lipid peroxidation (LPO)**

Malondialdehyde formation was estimated by the method of Stocks and Dormandy, absorbance was measured at 532 nm.[10]

**Estimation of superoxide dismutase (SOD)**

SOD was estimated by the method of Marklund and Marklund.[11]

**Estimation of catalase (CAT)**

Catalase was estimated by Aebi method.[12]

**Statistical analysis**

The data were analyzed by one way ANOVA followed by Dunnett’s multiple comparison test. A difference of $P < 0.05$ was considered significant in all the cases.

**RESULTS**

**Phytochemical screening**

The phytochemical screening of FBE indicates the presence of flavonoids, carbohydrates, glycosides and tannins.

**Assessment of antiinflammatory activity**

One way ANOVA revealed a significant ($p < 0.0001$) influence of FBE on the carrageenan induced inflammation in rat paw. Post hoc Dunnett test indicated that the dose of 50 mg/kg produced significantly less effect while higher doses 100 and 200 mg/kg produced maximum effect when compared to vehicle. This effect of FBE was comparable to that of diclofenac sodium, a standard antiinflammatory agent (Table 1).

**Assessment of in vivo antioxidant activity**

One way ANOVA revealed a significant influence of FBE on oxidative stress parameters in blood. Carrageenan administration increased systemic oxidative stress after 3rd h as shown by increased LPO and activities of SOD and CAT. Post hoc Dunnett test indicated that FBE administration reduced the lipid peroxidation and restored the activities of SOD and CAT at all doses (Figure 1, 2, and 3).
Table 1: Assessment of the antiinflammatory activity of FBE in carrageenan induced paw edema

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean increase in paw volume</th>
<th>Percent inhibition of paw edema at 3rd h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st h</td>
<td>3rd h</td>
</tr>
<tr>
<td>Vehicle</td>
<td>–</td>
<td>0.70 ± 0.02</td>
<td>0.73 ± 0.04</td>
</tr>
<tr>
<td>FBE</td>
<td>50</td>
<td>0.55 ± 0.02*</td>
<td>0.50 ± 0.008*</td>
</tr>
<tr>
<td>FBE</td>
<td>100</td>
<td>0.36 ± 0.01*</td>
<td>0.32 ± 0.002*</td>
</tr>
<tr>
<td>FBE</td>
<td>200</td>
<td>0.28 ± 0.006*</td>
<td>0.22 ± 0.01*</td>
</tr>
<tr>
<td>Diclofenac sodium</td>
<td>5</td>
<td>0.22 ± 0.01*</td>
<td>0.20 ± 0.02*</td>
</tr>
</tbody>
</table>

Each value is presented as mean ± SEM (n = 5 rats; one-way ANOVA followed by Post hoc Dunnett test). *p < 0.05, #p < 0.05, @p < 0.05 compared with the vehicle.

DISCUSSION AND CONCLUSION

The results of the present study showed that FBE treatment prevented carrageenan induced inflammation and development of edema in rat paw. This effect of FBE was very much comparable to that of diclofenac sodium. Since, FBE exhibited its antiinflammatory effect at both 1st and 3rd h, it is possible that FBE might be influencing both the stages of inflammation i.e. release of histamine at 1st h and release of bradykinin and prostaglandins and other inflammatory mediators at 3rd h after administration of carrageenan. In addition, FBE treatment also attenuated the carrageenan induced rise in LPO and increase in SOD and CAT activity suggesting in vivo antioxidant action of FBE against free radicals generated in the process of inflammation. The observed in vivo antioxidant activity of FBE is substantiated by the fact that most of the herbal antiinflammatory agents also possess antioxidant activity.[5,6]

The phytochemical screening of FBE has shown that it contained flavonoids, terpenes and tannins. It has been earlier reported that the bark of this plant contains three flavonoids, two are the forms of leucoanthocyanidin and the remaining is leucoanthocyanin.[13] The antioxidant activities of flavonoids, terpenes and tannins, in general, are well demonstrated[14] and they are often found effective in inflammatory disorders. Further, many of the terpenoids from the other plants sources have been reported to impair the release of autocoids in inflammation.[15]

Therefore, it appears that the observed antiinflammatory effect by FBE is either directly attributed to its terpenoid content or subsequent to the antioxidant effect shown by the flavonoids, terpenes or tannins present in FBE. However, the activity guided phytochemical analysis would only reveal the exact phytochemical responsible for the in vivo antioxidant activity and antiinflammatory effect of FBE.

Concludingly, these preliminary studies revealed that the ethanolic extract of the bark of Ficus bengalensis eliminated the systemic oxidative stress produced by carrageenan and prevented the consequent inflammation in rats.
REFERENCES