ANALGESIC AND ANTI-INFLAMMATORY EVALUATION OF FICUS MICROCARPA L. LEAVES EXTRACT

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ABSTRACT

Ficus Microcarpa L. is a medicinal plant used for the treatment of various body pains in India traditionally. It is also used in various conditions like diabetes, ulcers, burning sensations, haemorrhages, leprosy, itching, liver disease. The methanol extract of its leaves was investigated for its analgesic and anti-inflammatory activities in animal models. The extract at 50, 100 and 200mg/kg body weight reduced significantly the formation of oedema induced by carrageenan and histamine. Statistical analysis of the data was done using one-way analysis of variance (ANOVA) followed by Dunnett’s test and significance determined using P-values <0.05. In the acetic acid-induced writhing model, the extract had a good analgesic effect characterized by a reduction in the number of writhes when compared to the control. Similarly, the extract caused dose-dependent decrease of licking time and licking frequency in rats injected with 2.5% formalin. These results were also comparable to those of diclofenac sodium, the reference drug used in this study. Acute toxicity test showed that the plant may be safe for the pharmacological uses. This study has provided some justification for the folkloric use of the plant in several communities for conditions such as pain, skin allergies and inflammations.

Keywords: Analgesic, Anti-inflammatory, Carrageenan, Diclofenac sodium, Ficus microcarpa extract, Histamine.

INTRODUCTION

Ficus microcarpa L. also known as Ficus Retusa (Moraceae). Ficus microcarpa with common names Chinese or Malayan banyan is a ever green tree reaching height about 15m. It is useful in conditions such as diabetes, ulcers, burning sensations, haemorrhages, leprosy, itching, liver disease, and toothache. The methanolic extract of its leaves was investigated for its analgesic and anti-inflammatory activities in animal models. The extract at 50, 100 and 200mg/kg body weight reduced significantly the formation of oedema induced by carrageenan and histamine. Statistical analysis of the data was done using one-way analysis of variance (ANOVA) followed by Dunnett’s test and significance determined using P-values <0.05. In the acetic acid-induced writhing model, the extract had a good analgesic effect characterized by a reduction in the number of writhes when compared to the control. Similarly, the extract caused dose-dependent decrease of licking time and licking frequency in rats injected with 2.5% formalin. These results were also comparable to those of diclofenac sodium, the reference drug used in this study. Acute toxicity test showed that the plant may be safe for the pharmacological uses. This study has provided some justification for the folkloric use of the plant in several communities for conditions such as pain, skin allergies and inflammations.

MATERIALS AND METHODS

Drugs and chemicals

Methanol (Research-lab fine chem. Industries, Mumbai, India), Diclofenac sodium (Ranbaxy, India). Carrageenan, acetic acid and Tween 80 (Acme chemicals, India). The standard drugs used were Diclofenac sodium and histamine from Ranbaxy India Ltd. All the chemicals and drugs used were of analytical grade.

Plant collection and extract preparation

The leaves of Ficus microcarpa were collected from Mahatma Phule Krushi Vidyapeeth, Rahuri, Maharashtra State, India. The plant was authenticated at the Botanical Survey of India, Pune.

The leaves were air dried at room temperature and were later ground to powder. The coarsely powdered plant leaves (200gm) were extracted with methanol for 5 h. After filtration using Whatman No.1 filter paper, the methanolic extract was evaporated in vaccum below 50 °C. The yield of methanolic extract of Ficus microcarpa was 7.40 % w/w, which was stored in refrigerator for further use.

Animals

The protocol study was approved by Institute Animal Ethical Committee for animal experimentation. (MESCOP-1211/ac/08/CPCSEA). The Albino rats were obtained from National Toxicology Centre, Pune, India and kept in animal house in standard environmental condition of temperature (22±3°C), Humidity (60±5%) and at 12 hr light/dark cycle. During experimental time rats were given standard pellet diet (Prashant Enterprises, Pune, India) and water ad libitum. Male rats (150-200g) of 2-3 month were used.

Preliminary phytochemical screening

The methanolic extract was subjected to qualitative phytochemical screening according to standard methods.

Acute toxicity test:

The acute toxicity of Ficus microcarpa methanol extract was determined in rats according to the method of Hilaly et al. with slight modifications. Rats fasted for 16h were randomly divided into groups of six rats per group. Graded doses of the extract (200, 400, 800, 1600 and 3200mg/kg p.o) were separately administered to the rats in each of the groups. All animals were then allowed free access to food and water and observed over a period of 48h for signs of acute toxicity. The number of death within this period was recorded.

Anti-inflammatory activities

Carrageenan-induced rat paw oedema

The anti-inflammatory activity of the studied compounds on Carrageenan-induced rat paw edema was determined according to the standard method. Five group of rats containing four animal in each group received either plant extract (50, 100, 200mg/kg body weight), Diclofenac sodium (20mg/kg body weight) or vehicle control (0.9% normal saline in 3% Tween 80 (2ml/kg). 1 hour before administration of inflammatory agent, administered orally. Acute inflammation was induced by subplantar administration of 0.1 ml 1% freshly prepared Carrageenan in normal saline that contained Tween 80 in the right paw of rats. Paw volume of rats were measured prior to administration of inflammatory agent and then at predetermined intervals. For Carrageenan the interval was 1 hour
for 6h change in paw volume was measured using micrometer screw gauge and anti-inflammatory activity calculated. Increase in the linear diameter of the right hind paws were taken as an indication of paw oedema. Oedema was assessed in terms of the difference in the zero time linear diameter of the injected hind paw and its linear diameter at time t (i.e. 60, 120, 180min) following carrageenan administration. The anti-inflammatory effect of the extract was calculated by the following equation: anti-inflammatory activity (%)=(1-D/C) x 100, where D represented the percentage difference in paw volume after the extract was administered to the rats and C represents the percentage difference of volume in the control group. The percentage inhibition of the inflammation was calculated from the formula: \( \% \) inhibition = [\( V_c - V_e \) - control - \( V_c - V_e \) treated groups] / \( V_c - V_e \) control x100, where \( V_c \) represents paw volume of the rat before administration of carrageenan, \( V_e \) represents paw volume of the rat after administration of carrageenan at different time intervals.

**Histamine-induced rat paw oedema**

Using the method of Perianayagam et al.

The paw oedema was produced by sub-plantar administration of 0.1% freshly prepared solution of histamine into the right hind paw of rats. The paw volume was recorded before 0 and 1h after histamine injection. Different groups of animals were pretreated with different extracts (50, 100, 200mg/kg or with 2ml/kg of 0.9 % normal saline in Tween 80 (vehicle control) or 20mg/kg Diclofenac sodium (standard drug). The drug and extract were administered orally 1h before eliciting paw oedema. The anti-inflammatory effect of the extract was calculated using the formula for carrageenan-induced paw oedema.

**Analgesic activity**

**Acetic acid-induced writhing response in rats**

Analgesic activity of plant extract was determined according to the method described by Dharmasiri et al. Was used with slight modifications. Different groups of four rats each received orally normal saline solution (2ml/kg) (i.e. control), Diclofenac sodium (20mg/kg), or plant extract (50, 100, 200mg/kg). Thirty minutes later, 0.7 % acetic acid (10ml/kg) solution was injected intraperitoneally to the all animals in the different groups. The number of writhes (abdominal constrictions) occurring between 5 and 20 min after acetic acid injection was counted. A significant reduction of writhes in the control group was considered as an antinociceptive response.

**Formalin test**

Formalin test was conducted as described by Dharmasiri et al. Male rats (n=4/group) were treated respectively with 50, 100 and 200mg/kg of *Ficus microcarpa* extract, 20mg/kg of diclofenac sodium and 2ml/kg of normal saline. Thirty minutes later, the rats were injected with 0.05ml of 2.5% formalin into right hand foot pad, immediately placed in a plastic cage separately; the licking time and frequency of the injected paw were recorded for 30min +1.

**Statistical analysis**

Results were expressed as mean ± S.D. Statistical analysis of the data was done using one-way analysis of variance (ANOVA) followed by Dunnett’s test and significance determined using P-values <0.05.

**RESULTS**

**Phytochemical analysis**

The preliminary phytochemical analysis of the methanolic extract of *Ficus microcarpa* revealed the presence of alkaloid, steroid terpenoids and tannins.

**Acute toxicity test**

Oral administration of graded doses (200, 400, 800, 1600 and 3200 mg/kg p.o.) of the methanolic extract of *Ficus microcarpa* to rats did not produce any significant change in behavior, breathing, cutaneous effect, sensory nervous system responses or gastrointestinal effect during the observation period. No mortality was recorded in any group after 72h of administering the extract to the animals.

**Anti-inflammatory activity**

**Carrageenan-induced paw oedema**

When compared with control, the extract and diclofenac sodium significantly reduced the paw oedema 3h after carrageenan injection. The anti-inflammatory effect of the extract and the reference drug increased with time. This was dose-dependent for the extract (Table 1).

**Table 1: Anti-inflammatory activities of methanol extract of *Ficus microcarpa* leaves and Diclofenac on Carrageenan-induced oedema in the right hind-limb of rats.**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>Extract (mg/kg)</th>
<th>Diclofenac sodium 20mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>1</td>
<td>31.3 ±0.5</td>
<td>27.3 ±0.3</td>
<td>14.3 ±0.5</td>
</tr>
<tr>
<td>2</td>
<td>60.7 ±0.2</td>
<td>33.7 ±0.2</td>
<td>13.9 ±0.6</td>
</tr>
<tr>
<td>3</td>
<td>54.4 ±0.4</td>
<td>29.1 ±0.8</td>
<td>10.4 ±0.8</td>
</tr>
</tbody>
</table>

**Histamine-induced paw oedema**

The effect of the extract (100mg/kg) and the reference drug on histamine-induced oedema was most pronounced 3h after histamine injection, while the 50mg/kg and 200mg/kg doses of the extract showed highest activity at 2h after histamine administration. The anti-histaminic activity of the extract decreased with increase in the dose of the extract (Table 2).

**Table 2: Anti-inflammatory activities of methanol extract of *Ficus microcarpa* leaves and Diclofenac on Histamine-induced oedema in the right hind-limb of rats.**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>Extract (mg/kg)</th>
<th>Diclofenac sodium 20mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>1</td>
<td>25.4 ±0.4</td>
<td>5.2 ±0.2</td>
<td>21.4 ±0.5</td>
</tr>
<tr>
<td>2</td>
<td>18.5 ±0.9</td>
<td>0.7 ±0.4</td>
<td>6.2 ±0.4</td>
</tr>
<tr>
<td>3</td>
<td>17.8 ±0.9</td>
<td>3.2 ±0.5</td>
<td>0.6 ±0.4</td>
</tr>
</tbody>
</table>

**Formalin test**

The methanolic extract of *Ficus microcarpa* and diclofenac sodium induced significant decrease in the number of writhes when compared to the control (Table 3). The extract at 50, 100, 200 and diclofenac sodium at 20mg/kg exhibited higher antinociceptive activity at 98.5, 100, 100 and 96% respectively indicating that the extract has slightly higher antinociceptive than the reference drug used in this study.

**Formalin test in rats**

Treatment with the methanolic extract at 50, 100, 200 and diclofenac at 20mg/kg caused significant decrease in licking time and frequency of licking of the formalin-injected paw of rats (Table 4). The 200mg/kg dose showed the highest effect.
Inflammatory mediators, like histamine, he methanol extract of Ficus microcarpa showed anti-inflammatory activity because ingested in formalin test, the pain in the early phase was due to the direct stimulation of sensory nerve fibers by formalin while the pain in the late phase was due to inflammatory mediators, like histamine, prostaglandins, serotonin and bradykinins. In this study, the extract caused a dose-dependent decrease in licking time and licking frequency by the rats injected with formalin signifying the analgesic effect of the extract.

Diclofenac sodium is cyclooxygenase inhibitor. It inhibits prostaglandin synthesis and somewhat cyclooxygenase-2 selective. The methanol extract has activity which is comparable to Diclofenac can be said to inhibit the cyclooxygenase enzyme but lipooxygenase inhibitors also possess significant anti-inflammatory activity.

In conclusion, since the plant extract reduced significantly the formation of oedema induced by carrageenan and histamine, as well as reduced the number of writhes in acetic acid-induced writhing models and formalin test, the leaves of Ficus microcarpa exhibited anti-inflammatory and analgesic activities. Again, no mortality was recorded in the acute toxicity test, it showed that is safe for use. The study has thus provided some justification for the folkloric use of the plant in several communities for conditions such as pain and inflammations.

Acknowledgement

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REFERENCES


Table 3: Influence of methanol extract of Ficus Microcarpa leaves and Diclofenac on rat writhing reflex induced by acetic acid

<table>
<thead>
<tr>
<th>No. of Writhing/20 min Inhibition (%)</th>
<th>Control 50 100 200 Diclofenac sodium 20mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 50 100 200 Diclofenac sodium 20mg/kg</td>
</tr>
<tr>
<td></td>
<td>54.8±3.3 0.75±0.02 0±0 2.5±2.2 98.6 100 100 95.4</td>
</tr>
</tbody>
</table>

n=4, Mean±SD

Table 4: Analgesic effect of methanol extract of Ficus Microcarpa leaves and Diclofenac on rat using formalin.

<table>
<thead>
<tr>
<th>Duration (sec) Frequency/30min</th>
<th>Control 50 100 200 Diclofenac sodium 20mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 50 100 200 Diclofenac sodium 20mg/kg</td>
</tr>
<tr>
<td></td>
<td>11.9±3.3 5.0±0.2* 4.8±0.7* 4.0±0.5* 4.6±0.6*</td>
</tr>
</tbody>
</table>

Data in Mean±SD, n=4

*significantly different from control at P<0.05.