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Research Article

# 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 extract were reported to have cytotoxic , antifungal, antidiabetic , antibacterial Flavonoids, triterpenoids, acyclic compounds and steroids are the main components found in the leaves of *Ficus microcarpa*[1-3].

In spite of the progress made in medical research during the past decades, the treatment of many serious diseases is still problematic. Chronic inflammatory diseases remain one of the world's major health problems [4]. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair [5, 6]. Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal diseases. The conventional drugs used to ameliorate this phenomenon are either too expensive or toxic and not commonly available to the rural folks that constitute the major populace of the world [7, 8].

This study therefore seeks to examine *Ficus microcarpa* for anti-inflammatory activity and analgesic effects since pain is one of the cardinal signs of inflammation.

## 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* was collected from Mahatma Phule Krushi Vidyapeeth, Rahuri, Mahrashtra 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 coarslely 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 $^{o}$C. The yield of evaporation and solvent removal of methanolic extract of *Ficus microcarpa* was 7.40 % w/w, which was stored in refrigerator for further use [9].

### 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 Center, Pune, India and kept in animal house in standard environmental condition of temperature (22$\pm$ 3$^{0}$C), Humidity ( 60$\pm$ 5$^{0}$C) 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 screenin

The methanolic extract was subjected to qualitative phytochemical screening according to standard methods[10].

### Acute toxicity test*:* [11]

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 sign 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's paw edema was determined according to the standard method [12]. 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 guage 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: [13] % inhibition =[($V_T$-$V_0$) control- ($V_T$-$V_0$) treated groups] / ($V_T$-$V_0$)control*100, where $V_o$ represents paw volume of the rat before administration of carrageenan, $V_T$ 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, 200 mg/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 antinociceptic 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 [14].

**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 Carragennan-induced oedema in the right hind-limb of rats.**

| Time (h) | Control | Extract (mg/kg) | | | Diclofenac sodium 20mg/kg |
|---|---|---|---|---|---|
| | | 50 | 100 | 200 | |
| 1 | 31.3±0.5 | 27.3±0.3 [12.2] | 14.3±0.5 [54.8] | 3.7±0.5 [88.5] | 26.3±0.5 [16] |
| 2 | 60.7±0.2 | 33.7±0.2 [44.8] | 13.9±0.6 [77.5] | 6.6±0.5 [89.5] | 41.7±0.2 [31.4] |
| 3 | 54.4±0.4 | 29.1±0.8 [47.8] | 10.4±0.6[81.4] | 2.8±0.2[95] | 24.2±0.2[56.6] |

Data in Means± SD, n=4

Percentage inhibition of the carragennan-induced inflammation(oedema) produced by test extract and Diclofenac are indicated in parenthesis

**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.**

| Time (h) | Control | Extract (mg/kg) | | | Diclofenac sodium 20mg/kg |
|---|---|---|---|---|---|
| | | 50 | 100 | 200 | |
| 1 | 25.4±0.4 | 5.2±0.2 [79.7] | 21.4±0.5 [60.3] | 10.2±0.2 [66.3] | 19.5±0.6 [24.5] |
| 2 | 18.5±0.9 | 0.7±0.4 [96.8] | 6.2±0.4 [66.5] | 4.5±0.2 [75.9] | 12.6±0.5 [36.5] |
| 3 | 17.8±0.9 | 3.2±0.5 [82.5] | 0.6±0.4 [96.6] | 12.8±0.6 [28.1] | 3.4±0.4 [56.6] |

Data in Means± SD, n=4

Percentage inhibition of the Histamine-induced inflammation (oedema) produced by test extract and Diclofenac are indicated in parenthesis

**Analgesic activity**

**Acetic acid-induced writhing in rats**

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 96.1% 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.

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

| | Control | Extract (mg/kg) 50 | 100 | 200 | Diclofenac sodium 20mg/kg |
|---|---|---|---|---|---|
| **No. of Writhing/20 min** | 54.8±3.3 | 0.75±0.02 | 0±0 | 0±0 | 2.5±2.2 |
| **Inhibition (%)** | 0 | 98.6 | 100 | 100 | 95.4 |

n=4, Mean±SD

**Table4: Analgesic effect of methanol extract of *Ficus Microcarpa* leaves and Diclofenac on rat using formalin.**

| | Control | Extract (mg/kg) 50 | 100 | 200 | Diclofenac sodium 20mg/kg |
|---|---|---|---|---|---|
| **Duration (sec)** | 11.9±3.3 | 5.0±0.2* | 4.8±0.7* | 4.0±0.5* | 4.6±0.6* |
| **Frequency/30min** | 24.6±2.6 | 20.6±2.2* | 11.8±1.7* | 9.8±0.6* | 13.8±1.9* |

**Data in Mean±SD, n=4**
***significantly different from control at P<0.05.**

**Discussion**

Inflammation is the response of living tissue to injury. Which involve activation of various enzyme, mediators release, cell migration, tissue breakdown and repair[15]. The present study showed the anti-inflammatory activity of the methanol extract of *Ficus microcarpa* in a number of experimental models. Carrageenan induced paw edema is suitable experimental animal model for evaluation anti- edematous effect of natural product [16]. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation [17]. Carrageenan oedema is a multimediated phenomenon that liberates various mediators.

And this is involve three phases, in first phase (1 hr after Carrageenan induce) involves the release of serotonin and histamine from mast cells, in second phase (2hr) is provided by kinins and the third phase (3hr) is mediated by prostaglandins, the cycloxygenase product and lipoxygenase products[18]. Since carrageenan model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation [19, 20]. The results of this study are an indication that *Ficus microcarpa* can be effective in acute inflammatory disorders.

The extract also caused pronounced reduction in the oedema produced by the histamine. This result tends to suggest that the anti-inflammatory activity of the extract is possibly backed by its anti-histamine activity. The antihistaminic effect of the extract decreased with increase in the dose of extract. Histamine is an important inflammation mediator, potent vasodilator substance and also increases the vascular permeability [21, 22].

Since the extract effectively suppressed the oedema produced by the histamine, it showed that the extract exhibited anti-inflammatory action by inhibiting the synthesis, release or action of inflammatory mediators such as histamine, serotonin and prostaglandins.

With respect to the acetic acid-induced abdominal writhing which is the visceral pain model [23] the result has shown that all the doses produced significant analgesic effect. This could be concluded that its anti-inflammatory effect as, as in the visceral pain model, the processor releases arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which play a role in the nociceptive mechanism [24].

Thus the results obtained for the writhing test are similar to those obtained for odematogenic test using carrageenan. Therefore, an anti-inflammatory substance may also be involved in the peripheral analgesic activity because inhibition of the acute inflammation by this extract led to their inhibitory effect on pain development.

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. [25] 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 cycloxygenase inhibiter. It inhibits prostaglandin synthesis and somewhat cycloxygenase-2 selective. The methanol extract has activity which is comparable to Diclofenac can be said to inhibit the cycloxygenase enzyme but lipoxygenase 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.

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