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Evaluation of anti-inflammatory activity on the leaves of *Filicium decipiens* in experimental animal models

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ABSTRACT

The effects of *Filicium decipiens* (Family: Sapindaceae) leaf extracts on different models of acute inflammation were studied. Investigations were performed using different phlogistic agents-induced paw edema viz., Carrageenan-induced paw edema and Dextran-induced paw edema in rats. Various extracts (ethanol and aqueous) of *Filicium decipiens* leaves at a dose of 250 mg/kg and 500 mg/kg orally were tested. Diclofenac sodium at the dose of 10mg/kg was used as standard. Both the extracts showed significant activity (* $p < 0.05$ & ** $p < 0.01$) compared with the control in carrageenan-induced rat paw edema model and where as ethanolic extract (500mg) showed a significant reduction (68.42%) in dextran-induced rat paw edema model. Thus it is revealed from the screening model that the ethanol and aqueous extract of this plant possesses acute anti-inflammatory activity.

Key words: *Filicium decipiens* and Anti-inflammatory.

INTRODUCTION

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [1]. Drugs which are in use presently used for the management of pain and inflammatory conditions are very expensive in cost and toxic effects. On the Contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations [2]. It is

therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [3].

Filicium decipiens (Family: Sapindaceae), whose common name is Japanese Fern Tree, was given its name because of the large fern like leaves. The leaves are compound and very large. Each leaf consist of 12 to 16 leaflets. Each leaflet is 4 to 6 inches in length and relatively narrow. The Japanese Fern Tree is a fairly slow grower, reaching a top size of 35 feet by 35 feet. It is evergreen, and will tolerate almost any soil conditions. Moisture requirement are average, and it is draught tolerant after it becomes established [4]. Studies undergone in the plant were comparatively low and the studies undergone showed that the plant constitutes saponins [13] and glycosides [14]. Anti-inflammatory activities of many plants have been attributed to their high sterol/ triterpenoid saponin content [12]. As there is no reference in literature regarding *Filicium decipiens* in their anti-inflammatory aspects, it was considered worthwhile to study the anti-inflammatory activity of ethanolic and aqueous extracts of leaves of *filicium decipiens* in rats.

EXPERIMENTAL SECTION

Plant Materials

The fresh leaves are collected from Botanical garden, Osmania University, Hyderabad, India in the month of December 2010 and authenticated by Department of Botany, Osmania University, Hyderabad. Voucher specimen number of the plant is FD-170 stored in the herbarium of St.Mary's college of pharmacy, Secunderabad for the further reference.

Preparation of methanolic and aqueous extracts

The freshly collected leaves was shade dried and coarsely powdered. The powder was defatted with petroleum ether (60-90 °c) then successively extracted with ethanol and distilled water with using soxhlet extractor. The ethanolic and aqueous extracts were dried under reduced pressure using a rotary vacuum evaporator (Buchi US). The percentage yield was found to be about 9% w/w for ethanolic extract and 12% w/w for aqueous extract.

Drugs and Chemicals

All the drugs used in this study were of pharmaceutical grade. Carrageenan and Dextran was supplied by Sigma Chemicals Pvt Ltd Diclofenac Sodium was gifted by Dr. Reddy's Laboratories, Hyderabad, India.

Animals

Male and female albino Wistar rats weighing about (150-250gm) were selected and kept under the standard conditions. They were randomly distributed into groups, housed in cages and maintained under standard conditions at $26 \pm 2^\circ\text{C}$ with the free access to food and water. All the protocols were performed under the guidance from the institutional animal ethical committee.

Acute toxicity activity

Acute oral toxicity study was performed as per OECD-423 guidelines [7]. Wistar rats (n = 5) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg / kg body weight by gastric intubation and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg / kg body weight.

Determination of Anti-Inflammatory Activity**Carrageenan induced rat paw oedema model**

The rats were divided into six groups containing five rats in each group (one control, one standard and four test groups). Acute inflammation was induced according to oedema assay [5, 6]. The extracts were suspended in 2.0 % tween 80 and administered orally (200-400 mg/kg/b.w) to rats 1 hour before Carrageenan injection. Diclofenac Sodium (10 mg/kg b.w) is given to standard group. Carrageenan was prepared as 1% w/v solution in 0.9 % w/v NaCl & injects 0.1 ml just underneath the plantar region.

Control group 1: Carrageenan + 2% Tween 80(10 ml /kg b.w)

Standard group 2: Carrageenan + DiclofenacSodium (10 mg/kg b.w)

Test group 3: Carrageenan + Ethanolic extract (250 mg/kg b.w)

Test group 4: Carrageenan + Ethanolic extract (500 mg/kg b.w)

Test group 5: Carrageenan + Aqueous extract (250mg/kg b.w)

Test group 6: Carrageenan + Aqueous extract (500mg/kg b.w)

The paw volume was measured by the movement of water in Plethysmometer after the application of carrageenan after 3 hours. Reduction in the paw volume is compared with the vehicle treated controlled animals with that of the test groups and the anti-inflammatory activity was carried on the basis of the percentage(%) of inhibition of edema. The percentage of inhibition of edema was calculated by using the formula % inhibition of edema = $(V_c - V_t / V_c) \times 100$ Where V_t = Paw volume in test group animals and V_c = Paw volume in control group.

Dextran - induced rat paw oedema model:

The experiment was carried out by the same procedure as done in Carrageenan induced model, but instead of Carrageenan, here 0.1 ml of Dextran (1.0 % w/v in normal saline) was used as to produce oedema in rats. As on the details with the previous experiment the ethanolic extract (500mg) showed the maximum activity(65.20%) as compared with that of the control group in Carrageenan induced rat paw oedema model and therefore the test drug can be particularly screened for this model to have an comparison studies between these groups.

Control group: Dextran + 2% Tween 80 (10 ml /kg b.w)

Standard group: Dextran + Diclofenac Sodium (10mg/kg b.w)

Test group1: Dextran + Ethanolic extract (500mg/kg b.w)

Statistical analysis

All the data are expressed as mean \pm S.E.M. Statistical significance was determined by one way ANOVA (Analysis of Variance) followed by Dunnet multiple comparison test by using the Graphpad Instant version 3.01 $P < 0.01$ was regarded as significant

Table 1: Anti-inflammatory activity of *filicium decipiens* extracts on Carrageenan induced paw edema in rats

Group / Treatment	Dose (mg/kg, p.o)	Mean Paw edema (ml) + S.E.M after 3 hours	Percentage of inhibition (%)
Group 1 / Control	-----	1.09 \pm 0.03	-----
Group 2 /Diclofenac sodium	10	0.28 \pm 0.04	74.35 **
Group 3 / Ethanol	250	0.47 \pm 0.02	56.95 *
Group 4 / Ethanol	500	0.38 \pm 0.07	65.20 **
Group 5 / Aqueous	250	0.61 \pm 0.04	44.13 *
Group 6 / Aqueous	500	0.45 \pm 0.01	58.79 *

*Results are mean \pm S.E.M. (n=5) * $P < 0.05$ & ** $p < 0.01$ compared to control*

RESULTS AND DISCUSSION

All the extracts were tested for the anti-inflammatory activity using carrageenan induced edema models and the results are tabulated in table 1.

Among them only ethanolic extract (500mg/kg b.w) in the test groups shows the maximum activity (69.52%) compared with the control group in reducing the oedema by using carrageenan induced oedema model, therefore the ethanolic extract (500mg) was screened using Dextran - induced oedema model to find out the conclusive results in anti inflammatory activity. Carrageenan induced rat paw edema is a suitable experimental animal model for evaluating the anti-edematous effect of natural products [8], and this is believed to be triphasic, the first phase (1hr after carrageenan challenge) involves the release of serotonin and histamine from mast cells, the second phase (2hr) is provided by kinins and the third phase (3hr) is mediated by prostaglandins, the cyclooxygenase products and lipoxygenase products [9]. The metabolites of arachidonic acid formed via the cyclooxygenase and lipoxygenase pathways represent two important classes of inflammatory mediators, prostaglandins (products of the cyclooxygenase pathway) especially prostaglandin E2 is known to cause or enhance the valuable signs of inflammation, similarly, leukotriene B4 (product of lipoxygenase pathway) is a mediator of leukocyte activation in the inflammatory cascade[10]. The Ethanolic extract(500mg) effectively suppressed the dextran-induced rat paw oedema (Table 2), but the effect was less than that of Diclofenac sodium(Standard drug) .

Table 2 : Anti-inflammatory activity of *filicium decipiens* (ethanolic extract) on Dextran induced paw oedema model on rats

Group / Treatment	Dose (mg/kg,p.o)	Mean paw edema \pm S.E.M after 3 hours	Percentage of Inhibition (%)
Control	-----	0.95 \pm 0.06	-----
Standard / Diclofenac sodium	10mg	0.22 \pm 0.04	76.84**
Group 4 / Ethanol	500mg	0.30 \pm 0.03	68.42**

Results are mean \pm S.E.M. (n=5) *P<0.05 & **p<0.01 compared to control

The dextran-induced oedema is a well known experimental model in which the oedema is a consequence of liberation of histamine and serotonin from the mast cell [11]. The results of present study determines that *filicium decipiens* possess significant anti-inflammatory activity. Further detailed investigation is underway to investigate the exact phytoconstituents, which are responsible for the anti-inflammatory activity.

CONCLUSION

It may can be concluded that both the extracts of *filicium decipiens* has shown the anti-inflammatory activity against carrageenan induced model and where as the ethanolic extract(500mg) of *filicium decipiens* shows the moderate activity in Dextran induced oedema model. The ethanolic extract showed better activity profile compared to the aqueous extract in terms of anti-inflammatory activity. This study demonstrates the efficacy of *filicium decipiens* as an anti-inflammatory agent. Further studies are required to determine the active constituents responsible for its anti-inflammatory activity.

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