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Research Article

# ANTI-INFLAMMATORY ACTIVITY OF *FICUS DALHOUSIAE* MIQ ROOTS ETHANOLIC EXTRACT IN WISTAR ALBINO RATS

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**ABSTRACT**

**Objective:** The aim of the present investigation was to evaluate the anti-inflammatory activity of *Ficus dalhousiae* Miq roots ethanolic extract (FDREE) in Wistar albino rats using carrageenan and formalin induced paw edema model.

**Methods:** Plant material was collected from Tirupati, A.P, during the month of June 2014.The roots were made free from dust and foreign material and dried under shade at room temperature. After a week, the roots were powdered and passed through a sieve. The powder was weighed (500 g) and was extracted by successive solvent extraction process. The total yield of the ethanolic extract was 17.6%. Phytochemical screening was carried out for the detection of the phytoconstituents by simple qualitative methods. The dosing was designed as per the acute toxicity study reported earlier.The anti-inflammatory activity was performed by carrageenan and formalin induced paw edema model at three different doses, 150 mg/kg, 300 mg/ kg and 600 mg/kg. Wistar rats weighing (130-150 g) of either sex were used for the study.

**Results:** There was a significant reduction of elevated paw volume in the test groups observed in both carrageenan and formalin induced paw edema models. The percentage inhibition of inflammation was also high in the test groups compared with the negative control.

**Conclusion:** FDREE exhibited anti-inflammatory activity in both acute and subacute experimental models which provides the evidence of its use as a potent anti-inflammatory drug.

**Keywords:** *Ficus dalhousiae* roots ethanolic extract, Carrageenan, Formalin, Intraperitonial.

## INTRODUCTION

Inflammation is considered as the response of the body to injury and danger. It is the central communication and regulatory process that senses and controls threat, damage and healing, which are the critical aspects in the maintenance of integrity of an organism [1]. This process occurs as a defensive response, which induces profound physiological adaptations triggered in an attempt to limit tissue damage and remove the pathogenic insult. Such mechanisms involve a complex series of events including dilatation of arterioles, venules and capillaries with increased vascular permeability, exudation of fluids, including plasma proteins and leucocyte migration into the inflammatory area [2]. However, there are several clinical conditions where inflammation becomes chronic with excessive production of macroscopic-derived mediators which may lead to collateral damage to normal cells, resulting in diseases including atherosclerosis, bowel disease, rheumatoid arthritis, glomerulonephritis and septic shock [3].

The classical anti-inflammatory agents glucocorticoids and nonsteroidal anti-inflammatory drugs (NSAIDs) can only alleviate symptoms without, altering the course of the disease [4]. The current anti-inflammatory therapy aims to control the cardinal signs of inflammation, antagonizing or blocking key pro-inflammatory mediators that are released at the beginning of an acute inflammatory response [4]. However, many of the commonly used anti-inflammatory agents have become less acceptable due to serious adverse reactions such as gastric intolerance, bone marrow depression, water and salt retention, resulting from prolonged use [5]. Hence, it is important to search for substances' that can promote resolution of inflammation, homeostatic and modulators efficient and which are tolerated by the body [4].

Plants are an important source of biologically active natural products and are considered a promising avenue for the discovery of new drugs due to easy access and relatively low cost, as they are grown abundantly in nature [6,7]. The development of standardized herbal medicines with proven efficacy and safety can be considered as an important source for increasing the access of people toward medicine and offers new therapeutic options [8]. *Ficus dalhousiae* Miq. (Moraceae) known as Kal Aal, Pei-Aal and Soma-valka to the locals is a traditional medicinal plant found in Tamil Nadu and Kerala states of India. Literature indicates ethno medicinal use of this plant for hepatic and skin disorders [9]. The present study was undertaken to investigate anti-inflammatory activity of *F. dalhousiae* roots ethanolic extract (FDREE).

## METHODS

Plant material was collected from Tirupati, A.P, during the month of June 2014. The authentication of plant material was previously done by Department of Botany, Osmania University, Hyderabad - 500 007, India. The plant was given Voucher No. 0949.

### Chemicals

All the solvents and chemicals required for extraction, preliminary phytochemical screening and pharmacological evaluation were of analytical grade. Carrageenan was obtained from Himedia Laboratories Pvt. Ltd., Mumbai; Distilled water and sodium chloride were obtained from Stangen Fine Chemicals, Hyderabad and Baxter (India) Pvt. Ltd, Tamil Nadu respectively. Formalin was procured from S.D Fine Chem. Ltd, Mumbai. Indomethacin, aspirin (ASA) were obtained from a local drug store, Hyderabad.

### Experimental Animals

Healthy Wistar albino rats weighing about (130-150 g) of either sex were procured from Sainath Agencies, Bapujinagar, Musheerabad, Hyd-48 Reg. No. 282. The animals were maintained under standard conditions of relative humidity and temperature. The animals

were acclimatized for 10 days under laboratory conditions before carrying out the experiments. The animals were housed in the animal house approved by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA)-Registration number-1534/ PO/A/11/CPCSEA.

**Method of preparation of plant extract**

The collected roots were washed thoroughly under running water, cut into smaller pieces and air dried for 8 days. Then the dried roots were coarsely powdered using grinder and were continuously extracted in a soxhlet apparatus (Borosil, Mumbai, India) by different solvents in the increasing order of polarity. The extracts were filtered through a filter paper (Whatman No. 1) and evaporated under reduced pressure in a rotary evaporator (Roteva-Equitron, Medical Instruments, Mumbai, India). The obtained extracts were stored in amber colored glass bottles for further processing [10].

**Phytochemical screening**

Phytochemical screening was carried out for the detection of tannins, alkaloids, flavonoids, glycosides, sterols and saponins by simple qualitative methods [11].

**Evaluation of anti-inflammatory activity**

The anti-inflammatory activity was determined by two experimental models namely carrageenan-induced paw edema and formalin induced paw edema. The dosage of the test drug was designed based on the oral acute toxicity studies carried out on the plant [12]. The experimental animals were divided into five groups in both the models with six animals in each group.

**Carrageenan-induced paw edema**

Acute inflammation was produced by sub-plantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats, 1 hr after oral administration of the standard and test drug. The paw volume was measured using plethysmometer (UGO Basile S.R.L. Samitek Instrument, Italy) at 0, 1, 2, 3, 4 hrs after the carrageenan injection. FDREE (150, 300 and 600 mg/kg. p.o.) were administered to rats 1 hr before carrageenan administration. Control rats received 1% w/v NaCl (2 ml/kg b.w, p.o), Standard group was given indomethacin suspended in 2% gum acacia at a dose of 5 mg/kg, p.o. Mean increase in paw volume was noted and percentage of inhibition was calculated [13].

$$\text{Percentage inhibition} = \frac{\left(\left[C_t - C_0\right]_{control} - \left[C_t - C_0\right]_{treated}\right)}{\left(\left[C_t - C_0\right]_{control}\right)} \times 100$$

Where, $C_t$ = Mean paw volume for each group at time t, and
$C_0$ = Mean paw volume for each group before carrageenan injection.

**Formalin induced paw edema**

Acute inflammation was induced by sub-plantar injection of 0.1 ml of 2% formalin to the 1 hr after oral administration of FDREE (150, 300 and 600 mg/kg, b.w, p.o, ASA (100 mg/kg) to the test groups and standard group respectively. The volume of the paw was determined at 0, 1, 2, 3, 4, 24 hrs following the injection of formalin. The changes in the paw volume were measured plethysmographically, and percentage inhibition was calculated [14].

**Statistical analysis**

The values were expressed as mean±standard error mean. P<0.05 was considered significant, denoted by symbol (*). The data was analyzed by one-way analysis of variance followed by Dunnets multiple comparison using GraphPad Prism Version 6 for Windows, GraphPad Software, San Diego California USA.

## RESULTS

**Phytochemical screening**

The phytochemical screening of ethanolic extract of *F. dalhousiae* roots showed the presence of alkaloids, flavonoids, glycosides, tannins, and terpenoids.

**Carrageenan induced paw edema model**

The test doses FDREE 300 mg/kg, 600 mg/kg and standard drug indomethacin 5 mg/kg produced significant (p<0.01) anti-inflammatory activity throughout the experiment. The test dose FDREE 150 mg/kg could not reduce the paw volume from the beginning of the 1st hr. The reduction in paw volume in terms of percentage inhibition was found to be 36.84-66.66%, 36.84-40%, 42.10-53.33% in indomethacin 5 mg/kg, FDREE 300 mg/kg and FDREE 600 mg/kg respectively from 1st to 4th hr. The maximum reduction in paw volume in terms of percentage inhibition was in the last hour of the experiment. The results are given in Table 1.

In the formalin induced paw edema model, the test doses FDREE 150 mg/kg, 300 mg/kg, 600 mg/kg and standard drug ASA 100 mg/kg showed significant (p<0.05-p<0.01) results. The test dose 150 mg/kg couldn't show its effect at the end of the 3rd hr FDREE 600 mg/kg and ASA 100 mg/kg showed similar effects all over the experimental period. The significance level of FDREE 300 mg/kg was reduced from p<0.01 to p<0.05 at the last hour of the experiment i.e. at 24 hrs. The reduction in paw volume in terms of percentage inhibition was found to be 33.33-57.14%, 30.30-47.64%, 36.36-57.14% in ASA 100 mg/kg, FDREE 300 mg/kg and FDREE 600 mg/kg respectively from 1st to last hour. The results are given in Table 2.

## DISCUSSION

Inflammation is a complex pathophysiological response to different stimuli. It can be treated and resolved by acting on the different mediators, enzymes, and pathways implicated in the process. This includes the arachidonate metabolism, inhibiting either certain transcription factors or the production and/or scavenging of the free radicals produced during the process, and by acting on the cells implicated in the process, such as macrophages and lymphocytes. By activating the cyclooxygenase, the levels of prostaglandins, especially prostaglandin E2, increases markedly and its production provokes inflammation [15], pain and fever [16]. Therefore, it can be assumed that some active metabolites of the extract in this study could inhibit cyclooxygenase activity. The most widely used primary test to screen anti-inflammatory agents is to measure the ability of a compound

**Table 1: Paw volumes of rats in different experimental groups**

| Treatment and dose (mg/kg) | 0 hr (after carrageenan injection) | 1 hr | 2 hrs | 3 hrs | 4 hrs |
|---|---|---|---|---|---|
| Saline 1 ml | 0.40±0.0055 | 0.31±0.014 | 0.31±0.0091 | 0.29±0.0095 | 0.27±0.010 |
| Indomethacin 5 | 0.30±0.0076** | 0.23±0.0087** | 0.21±0.0088** | 0.19±0.0034** | 0.16±0.0030** |
| % Inhibition | - | 36.84 | 47.36 | 52.94 | 66.66 |
| FDREE 150 | 0.35±0.012* | 0.29±0.011 | 0.29±0.0076 | 0.26±0.0060 | 0.24±0.0094 |
| % Inhibition | - | 5.2 | 5.2 | 11.76 | 13.33 |
| FDREE 300 | 0.35±0.010* | 0.23±0.0081** | 0.22±0.0074** | 0.21±0.013** | 0.20±0.0081** |
| % Inhibition | - | 36.84 | 42.10 | 41.17 | 40 |
| FDREE 600 | 0.29±0.017** | 0.22±0.0060** | 0.21±0.0071** | 0.19±0.010** | 0.18±0.014** |
| % Inhibition | - | 42.10 | 47.36 | 52.94 | 53.33 |

FDREE: *Ficus dalhousiae* roots ethanolic extract, *,**: p<0.001

**Table 2: Paw volumes of rats at different time intervals**

| Treatment and dose (mg/kg) | 0 hr (after formalin injection) | 1 hr | 2 hrs | 3 hrs | 4 hrs | 24 hrs |
|---|---|---|---|---|---|---|
| Saline 1 ml | 0.45±0.0088 | 0.45±0.013 | 0.44±0.010 | 0.42±0.0094 | 0.40±0.0087 | 0.33±0.012 |
| ASA 100 | 0.35±0.014** | 0.33±0.011** | 0.31±0.0096** | 0.29±0.010** | 0.25±0.011** | 0.20±0.015** |
| % Inhibition | - | 33.33 | 37.5 | 40 | 50 | 57.14 |
| FDREE 150 | 0.41±0.0071* | 0.41±0.0076* | 0.39±0.011* | 0.38±0.0084* | 0.36±0.0060 | 0.30±0.0042 |
| % Inhibition | - | 9 | 12.5 | 10 | 10.7 | 9.5 |
| FDREE 300 | 0.36±0.010** | 0.34±0.0094** | 0.32±0.010** | 0.30±0.011** | 0.28±0.011** | 0.22±0.0096* |
| % Inhibition | - | 30.30 | 34.37 | 36.66 | 39.28 | 47.61 |
| FDREE 600 | 0.34±0.066** | 0.32±0.0065** | 0.29±0.010** | 0.28±0.010** | 0.27±0.021** | 0.20±0.022** |
| % Inhibition | - | 36.36 | 43.73 | 43.33 | 42.85 | 57.14 |

FDREE: *Ficus dalhousiae* roots ethanolic extract, ASA: Aspirin, *,**: p<0.001

to reduce local edema induced in rat paw following the injection of irritants such as carrageenan [17].

The results from the present study showed that the extract of FDREE exhibited anti-inflammatory activity in various degrees. Carrageenan-induced hind paw edema can be regarded as standard experimental model for acute inflammation. Carrageenan is considered as a phlogistic agent of choice for testing anti-inflammatory drugs as it is devoid of systemic effects [18]. Carrageenan model of inflammation is said to be biphasic, with the first phase attributed to the release of histamine, serotonin and kinnins in the 1st hr; while the second phase is attributed to the release of prostaglandins and lysosome enzymes in the second to the 3rd hr [19]. The most widely used method for screening of newer anti-inflammatory drugs measures the ability of the drug to reduce local edema induced the paw of rats by injecting an irritant [20]. Inflammation is the result of concerted participation of a number of proliferative factors like vasoactive, chemotactic, etc. at different stages and also there are many targets for the study of anti-inflammatory activity [21]. The ability of the extract to inhibit carrageenan induced paw edema suggests that it possesses a significant effect against acute inflammation. It is, therefore, interesting that the extract behaved similar to NSAIDs in this study. The phytochemical screening of the ethanolic root extract showed the presence of flavonoids and tannins. The flavonoids and tannins might be responsible for the observed anti-inflammatory effect.

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