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Research Article

ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *HIBISCUS PLANTIFOLIUS*

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ABSTRACT

Objective: The objective of the present study was to carry out anti-inflammatory activity of methanolic extract of *Hibiscus plantifolius* (MEHP) belonging to the family Malvaceae.

Methods: The shade dried stem part of *H. plantifolius* (1 kg) was powdered and extracted with methanol using soxhletion. The extract was concentrated using rotary evaporator under reduced pressure at 40°C, till free from the solvents and thereby providing crude methanol extract which was subsequently employed for further studies. Anti-inflammatory effect was studied by carrageenan-induced paw edema model in rats at dose level of 50, 150, and 300 mg/kg. Acute oral toxicity study was also studied.

Results: The results indicate that MEHP, 300 mg/kg, exhibited significant inhibition ($p<0.001$) of increase in paw edema at 4th h.

Conclusion: The results of the experimental study confirmed that MEHP possesses significant anti-inflammatory activity.

Keywords: *Hibiscus plantifolius*, Anti-inflammatory, Soxhletion.

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INTRODUCTION

Inflammation is a complex reaction to injurious agents such as microbes, usually necrotic cells that consist of vascular response, leading to accumulation of fluids, migration, and activation of leukocytes and systemic reactions. Inflammation is fundamentally a protective response, the ultimate goal of which is to rid the organism of both the initial causes of cell injury such as microbes and toxins and consequence of injuries such as necrotic cells and tissue. Although the process of inflammation is brought about by vascular as well as cellular events, the former appears to contribute maximum for the pathogenesis of acute inflammation. This complex phenomenon involves endogenous chemical mediators such as histamine, 5-hydroxytryptamine, various chemotactic factors, bradykinin, leukotrienes, and prostaglandins [1].

Many nonsteroidal anti-inflammatory drugs such as aspirin, phenylbutazone, and ibuprofen are in clinical use, but all these are not completely devoid of adverse effects. Hence, the search for safer and better anti-inflammatory agents continues to be an area of great interest due to the non-availability of a safer and more effective anti-inflammatory agent. This has led to increase in demand for natural products with anti-inflammatory activity having fewer side effects. In the traditional system of medicine, many plant-based drugs and formulations are in use since ancient times. Many herbs and plant products have been claimed to have a significant anti-inflammatory action. However, not much work has been reported on the pharmacological evaluation of such plant-based products for the anti-inflammatory effects claimed in traditional medicine [2].

Among these plants, *Hibiscus plantifolius* [3] (Maple-Leaved Mallow) is a species of flowering tree in the mallow family, Malvaceae, that is native to India and Sri Lanka. In Sri Lankan texts, the plant is widely known by its synonym *H. eriocarpus*. The tree is about 8 m tall. Leaves are cordate at base, hairy, and trilobed. Flowers show axillary panicles where flowers show typical *Hibiscus* flower colors, pink with dark center. Fruit is a capsule. Common names for Hibiscus plantifolius in kannada is Bili daasavaala, Daasaala, Daasaani and in telugu is Kondabenda, Kondagogu.

METHODS

Procurement and authentication of plant

H. plantifolius was identified and authenticated by P. Satynarayana Raju, Plant Taxnomist, Department of Botany and Microbiology, Acharya Nagarjuna University.

Plant material

1 kg of the stem of *H. plantifolius* were collected from the Thirumala forest in Andhra Pradesh State, India, in the months of June–July 2017. The stem of *H. plantifolius* was washed and allowed to dry for 15 days. The dried stem was then ground to fine powder using the laboratory Hammermill. Powdered samples were stored desiccators until required for extraction.

Preparation of *H. plantifolius* extract

The powdered materials of *H. plantifolius* were extracted with methanol using Soxhlet apparatus [4] for 18 h. The extract was concentrated using rotary evaporator till free from the solvents and obtained yield was 25 g/kg, respectively.

Animals

Wistar Albino rats of either sex weighing between 100 and 200 g were used for this purpose. The animals were housed in polypropylene cages and maintained at 24±20 under 12 h light-dark cycle and were fed *ad libitum* with standard pellet diet and had free access to water maintenance, and the use of animals as per the experiment was approved by the Institutional Animal Ethics Committee (010/IAEC/NCPA/B.PHARMACY/2018-19). Wistar rats were randomly divided into five groups six of each and each rat was treated with oral administration for 6 days as follows [5]:

Group I: Received 10% of dimethyl sulfoxide (DMSO) solution;
Group II: Received diclofenac 10 mg/kg bd. wt.

Table 1: Effect of MEHP on carrageenan-induced hind paw edema in Wistar albino rats

S. no	Treatment	Before treatment (0 h)	After treatment (h)		
			1	3	5
1	10% of DMSO	0.52±0.05	1.22±0.09	1.7±0.13	2.32±0.1
2	Diclofenac (10 mg/kg bd. wt.)	0.53±0.08	0.75±0.08	0.8±0.08	0.73±0.05 [a***]
3	MEHP (50 mg/kg bd. wt.)	0.63±0.03	1.08±0.06	1.33±0.11	2.12±0.08
4	MEHP (150 mg/kg bd. wt.)	0.6±0.03	0.87±0.02	1.07±0.08	1.57±0.21
5	MEHP (300 mg/kg bd. wt.)	0.6±0.04	0.82±0.07	0.97±0.06	1.12±0.2 [b**]

Data were expressed as mean±SEM. Significant at [a***]p<0.001, [b ***]p<0.01 compare with compared before treatment group rats, DMSO: Dimethyl sulfoxide, MEHP: Methanolic extract of *H. plantifolius*, *H. plantifolius: Hibiscus plantifolius*

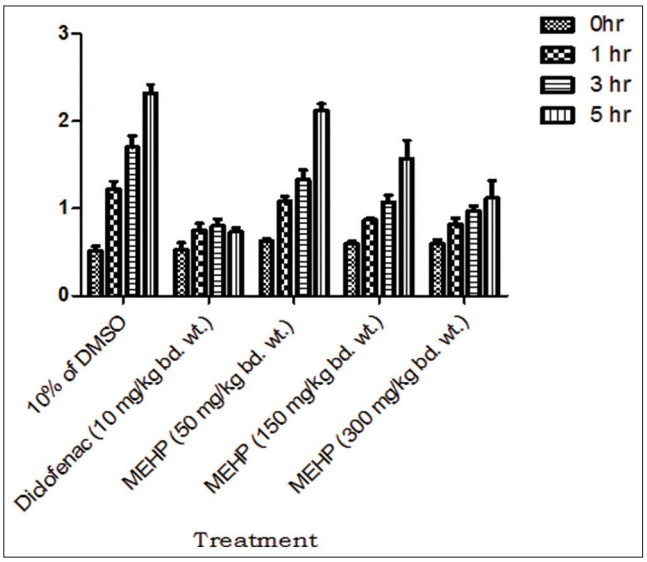

Fig. 1: Effect of methanolic extract of *Hibiscus plantifolius* on carrageenan-induced hind paw edema in Wistar albino rats

Groups III: Received methanolic extract of *H. plantifolius* (MEHP) (50 mg/kg bd. wt.)
Group IV: Received MEHP (150 mg/kg bd. wt.)
Group V: Received MEHP (300 mg/kg bd. wt.).

Carrageenan-induced edema in rat hind paw

The anti-inflammatory activity of the *B. serrata* was determined by the method of. All group rats were injected with 1% carrageenan (in 1% carboxymethylcellulose) solution into the subplanter region of rat right hind paw. The first group referred as control received 10% DMSO, the second group served as standard received ibuprofen (10 mg/kg/bw) through oral gavage, and the third, fourth, and fifth groups received low dose (50 mg/kg/bw), middose (150 mg/kg/bw), and high dose (300 mg/kg/bw) of *H. plantifolius* through oral gavage, respectively. Before 1 h of injecting of carrageenan, the rats were treated with different doses of *H. plantifolius*, ibuprofen, and 10% DMSO [6]. The volume of paw edema was measured by dislocation of the water column in a plethysmometer [7] immediately after carrageenan injection at 0, 1, 2, 3, 4, and 5 h. The average paw volume was measured and compared with control and standard groups. Reduction in the paw volume in *H. plantifolius* pretreated groups compared with the control animals was considered as anti-inflammatory response (Table 1).

RESULTS

Carrageenan-induced hind paw edema is an appropriate examination for appraising anti-inflammatory drugs and has commonly been used to evaluate the anti-edematous activity of natural products [8]. Oral treatment of animals with MEHP produced considerable inhibition of carrageenan-induced hind paw edema. As shown in Fig. 1, MEHP at dose of 300 mg/kg evidently reduced the edema formation of the hind paw induced by carrageenan at different time schedule. MEHP and reference drug diclofenac (10 mg/kg) produced an inhibitory activity on the paw edema formation even at 4 h after treatment.

DISCUSSION

Inflammation is complicated biological and biochemical processes consist of vascular tissues and non-specific reactions activated by natural immune responses against irritants, infection, injury, and injured cells. The microcirculation is the central playground where the course of inflammatory events was assessed and examined. Inflammation contains a lengthy sequence of molecular reactions and cellular actions, which are intended to renovate a tissue from simple skin cut or to cure numerous burn wounds. An inflammatory process in cellular and tissue levels consist of a chain of events with dilation of arterioles and venules, increased blood vessel permeability, and blood flow with infiltration of leukocytes into the tissues. Medicinal plants showed essential roles as foundations of effective anti-inflammatory agents. According to the World Health Organization, nearby three-quarters of the world's inhabitants depend on traditional medicines for their healthiness.

In our work, MEHP at doses of 50, 150, and 300 mg/kg of MEHP treatment inhibited edema formation in the phase of carrageenan induction with a similar effect to cyclooxygenase (COX) antagonist (nonsteroidal anti-inflammatory) used as reference drug. COX, an inducible enzyme found in activated inflammatory cells, plays a crucial role in cytokine production and prostanoid mediator release. The inhibition of COX protein expression has been used to evaluate the anti-inflammatory effects of compounds *in vivo* and *in vitro* [9,10]. These data show that possibly the mechanism of action of MEHP is involved with inactivation of COX in carrageenan-induced paw edema. The anti-inflammatory potential of medicinal plants has been reported in plants *Solanum nigrum* [11], *Phyllanthus amarus* [12], *Syringa patula* [13], *Plumeria acuminate* [14], and *Pistia stratiotes* [15]. As seen in this study, MEHP at a high dose has an inhibitory effect on edema formation in carrageenan-induced rat paw edema model.

CONCLUSION

Nature has been a source of medicinal medicine. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since the ancient times. Previous research on *H. plantifolius* exhibited antioxidant activity and the present research revealed the anti-inflammatory activity due to the presence of flavonoids, tannins, and phenolic compounds which was present in MEHP. It is an easily available plant for natural remedies.

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AUTHORS' CONTRIBUTIONS

K. Sowjanya conducted the experiments and also prepared the manuscript. S. Swati was involved in the planning of the experimental work and assisting the manuscript preparation. Both the authors have

read and approved the content of the manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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