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Original Article

Anti-inflammatory activity of methanolic extract of *Hibiscus schizopetalus* (Mast) Hook

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ABSTRACT

The present study was aim to offer scientific proof to the ethnobotanical usage of the medicinal plant in the management of inflammation. *Hibiscus schizopetalus* (Mast) Hook belongs to family Malvaceae, and known as Fringed Hibiscus. The methanolic extract different morphological parts of *H. schizopetalus* (HFE and HLE) were examined for anti-inflammatory and total phenolic content. The anti-inflammatory activity was evaluated using Carrageenan induced paw edema in rats. While phenolic content was determined by Folin-Ciocalteau. Total phenolic content of HFE and HLE was found to be 51.4 ± 0.21 mg (GAE/g) and 33.6 ± 0.22 mg (GAE/g) respectively. HFE and HLE showed significant anti-inflammatory activity in dose dependent manner. At a dose of 200 mg/kg, p.o. maximum effect was observed and was comparable ($P< 0.05$) to that of indomethacin (standard, 10 mg/kg). Outcomes of the analysis suggested that the activity of extracts of *H. schizopetalus* was due to the prevention of prostaglandins and cessation of inflammatory events. Therefore, this study provides a support for the plant in the management of inflammation related disorders.

Keywords: *Hibiscus schizopetalus* (Mast) Hook, anti-inflammatory activity, phenolic content, methanolic extract, Carrageenan induced

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INTRODUCTION

Modern drugs having active components marked on one specific pathway, on the other hand herbal medicine work on a classical approach. The medicinal plant contains number of different components and molecules, and these molecules work synergistically on the targeted elements of complex cellular pathway [1]. For centuries medicinal plant are used as a source of diversity of biologically active substances that are utilized as crude material or as a pure compound for the treatment of numerous disorders. Due to less side effects consumption of herbal medicine become more popular as compare to the allopathic medicine. For the development of effective and compelling therapeutic agents these medicinal plants play an imperative role [2].

The native and indigenous people of different regions of Pakistan have been using medicinal plants as a major source of their healthcare system [3].Several experimental procedures of inflammation are used for assessing the potency of drug. The management and controlling of inflammation and its related dieses is a real problem in the rural community. The peoples of these areas used drug that are of natural origin such as material produced from these medicinal plants.
Hibiscus schizopetalus (Mast) Hook belongs to family Malvaceae, and known as Fringed Hibiscus. *H. schizopetalus* is found in various countries of the world like Pakistan and Tropical Africa. In Pakistan, it is available in Karachi districts. The infusion of flower is used to treat cold and cough [4]. Analgesic, antipyretic, urease inhibition and antioxidant activities have been previously reported [5-6]. The objective of this work is to evaluate the anti-inflammatory potential of *H. schizopetalus* because after the vast literature search we have found that no systemic approach has been made to evaluate its anti-inflammatory potential. The present study also involves determination of total phenolic content of *H. schizopetalus.*

MATERIAL AND METHODS

Plant material and preparation of extract: *H. schizopetalus* flower and leaves were collected from the premises of University of Karachi. It was authenticated by Prof. Dr. Ghazala H. Rizwani (Meritorious), Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Karachi. The specimen voucher (No. 82) has been preserved in the herbarium of the Department, Faculty of Pharmaceutical Sciences, Karachi University for future reference

Flower and leaves of *H. schizopetalus* (1 kg) were air dried in shade separately and pulverized coarsely. The plant material was soaked in methanol at room temperature for 7 to 10 days. After this period, the extract was filtered and the residue was evaporated under vacuum using rotary evaporator (Buchi Rotavapor R-200) at 40 ± 2°C. The dried extract 73.8 g and 50.1 g respectively was stored at 4 °C.

Chemicals: Absolute Methanol (Merck, Germany), Indomethacin (B.D.H. laboratory supplies, UK), Carrageenan (MP Biomedicals, LLC, USA), Folin–Ciocalteu reagent (B.D.H. Laboratory supplies, UK), Gallic acid (B.D.H. Laboratory supplies, UK), Sodium carbonate (Merck, Germany).

Experimental animals: Adult albino rats weighing 120-160 gm of either sex were used for the experiment. Animals were kept on a 12 h of dark/light cycle at ambient temperature and fed with standard laboratory diet (PCSIR Laboratories, Karachi, Pakistan) and water. All animals were fasted overnight before evaluation of activity. The experiment was approved by the institutional animal ethical committee.

Total phenolic contents: For the determination of phenolic content of extract HFE and HLE of *H. schizopetalus* the reaction mixture contained .05 ml Folin-Ciocalteu, 2.5 ml sodium carbonate, and different concentration of gallic acid (1, 2.5, 5, 10,25, 50, 100 µg/ml) or 1.0ml sample extracts. For 40 minutes the reaction mixture was placed in the dark and the absorbance was recorded at 750 nm against blank [7]. The total phenolic content was expressed based on gallic acid equivalent (GAE). The formula used for the calculation is

$$C\ (GAE) = \frac{c \times V}{M}$$

(C is total phenolic content in mg/g; c is concentration of gallic acid established from calibration curve; V is volume of extract; M weight of plant extract)

Anti-inflammatory activity

Carrageenan induced paw edema

Albino rats of either sex weighing 150-200 grams were divided into eight groups of six animals each. Group I served as control and administered with normal saline. Group II received indomethacin (10 mg/kg, body weight). While group III- VIII orally administered with extracts of *H. schizopetalus* (50 mg/kg, 100 mg/kg and 200 mg/kg). After one hour of the administration of the extracts (HFE, HLE) and indomethacin, 0.1 ml of 1% w/v carrageenan solution in normal saline was injected into the sub plantar tissue of the hind paw of the rat. The paw volume of the rats were measured in the digital plethysmograph (Ugo basile, Italy), at 0 min., 30 min., 60min., 90 min., 120min., and 150 min [8-9].

Percentage inhibition was calculated by the following formula

$$\text{Anti} - \text{inflammatory activity } (\%) = \frac{Vc - Vt}{Vc} \times 100$$

Vt= percentage difference in increased paw volume after administration of test drug to animal Vc= difference of increased volume in the control group

Statistical Analysis: The results are expressed as Mean ± SEM. The data were analyzed by ANOVA followed by LSD multiple comparison tests using SPSS software No. 20. A level of $P < 0.05$ was considered as statistically significant.

RESULTS

Total phenolic content: Total phenolic content of HFE and HLE was found to be 51.4 ± 0.21 mg (GAE/g) and 33.6 ± 0.22 mg (GAE/g) respectively and was determine from regression equation of calibration curve ($y = 0.0093 + 0.051$; $r^2 = 0.995$) showed in Figure 1 and Figure 2.

Anti-inflammatory activity: The anti-inflammatory effect of HFE and HLE in carrageenan- induced paw edema model was presented in Figure 3 and Figure 4. It is clear that the injection of carrageenan in vehicle treated control group causes substantial increase in the paw volume (0.79 ml, 4h), whereas time and dose dependent reduction in the paw volume were observed in the HFE and HLE treated rats. Maximum response was observed 3 h after the oral administration of HFE and HLE at 50, 100 and 200 mg/kg doses respectively and was comparable to that of the standard drug indomethacin (10 mg/kg).

DISCUSSION

The present study was based to establish the pharmacological effect of methanolic extract of flower and leaves of *H. schizopetalus* extract (HFE and HLE). The total phenolic content was estimated in both the extracts, in term of gallic acid equivalent (GAE). The floral extract was richer in phenolic content than leaves extract (HLE). The phenolic content represents the major group of constituent and function as a strong scavenger of free radicals. These are found to be involved in the etiology of various diseases i.e., cancer, diabetes, inflammatory disorders etc. Moreover, the helpful property of these constituents is dependent on the nature; chemistry and amount present [10-12]. Since, the study revealed the higher content of phenolic compounds in HFE and HLE respectively. Hence, it was thought meaningful to evaluate the anti-inflammatory activity of phenol rich extract of *H. schizopetalus*.

The basic characteristics of inflammation include swelled and red color patches on the skin that are triggered by several factors and it involves very complex mechanism [13]. The anti-inflammatory activity of flower and leaves extract of *H. schizopetalus* was evaluated in carrageenan induced paw edema using animal model. This method is a well-established procedure to assess the anti-inflammatory effects of substances that of natural origin as well as of synthetic source. Edema that is formed in paw of animal by the administration of carrageenan is a biphasic event; the initial phase and the second phase. Initial phase (1hr -1.5 hr) is a non-phagocytic and it is followed by the second phase or late phase which is 2 to 5 hours long [14-15]. During the initial phase different mediators are released i.e., histamine, serotonin, and bradykinin [16].The second phase is due to the overproduction of prostaglandin [17]. The maximum anti-inflammatory response obtained 3 h after HFE and HLE treatment, suggested that the activity may be due to inhibition of PGs secretion or their synthesis. The results of this study are an indication that *H. schizopetalus* can be effective in acute inflammatory disorders.

CONCLUSION

It is concluded that although *H. schizopetalus* is an ornamental plant but has great medicinal significance as proved by the research findings. Future study would be conducted for isolation and identification of compounds and purification of the active principles of the plant responsible for the observed pharmacological effects.

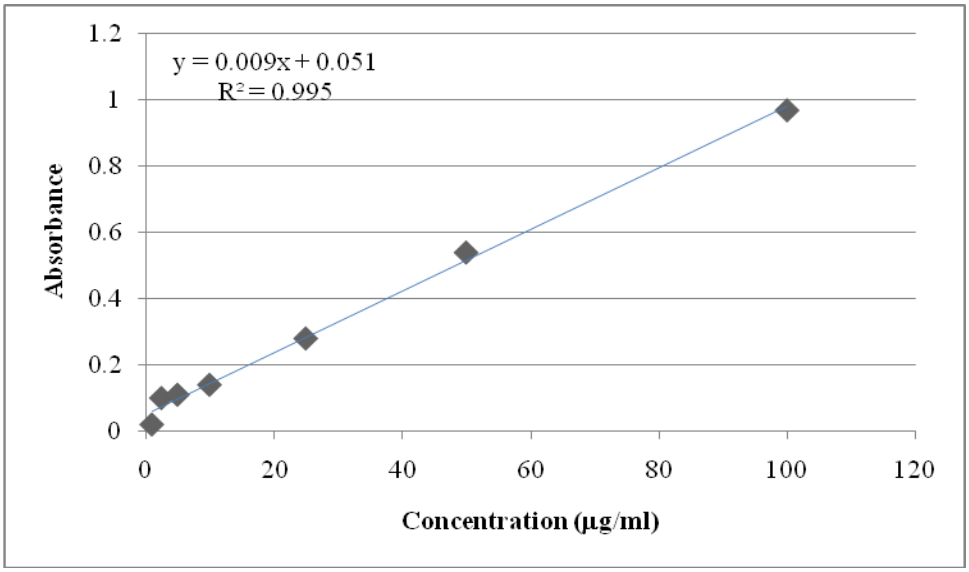

Figure 1. Calibration curve for Gallic acid

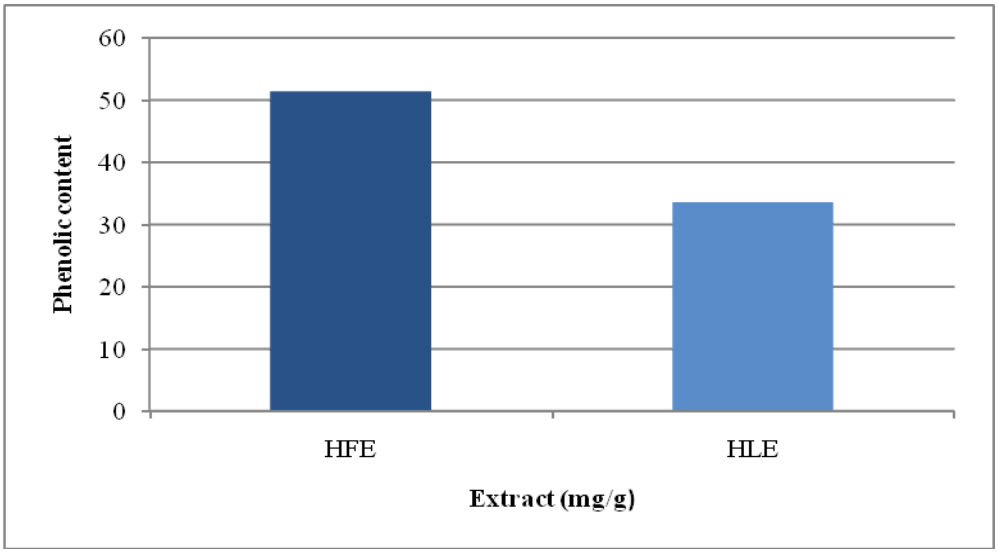

Figure 2. Total phenolic contents of *H. schizopetalus* extract (HFE and HLE)

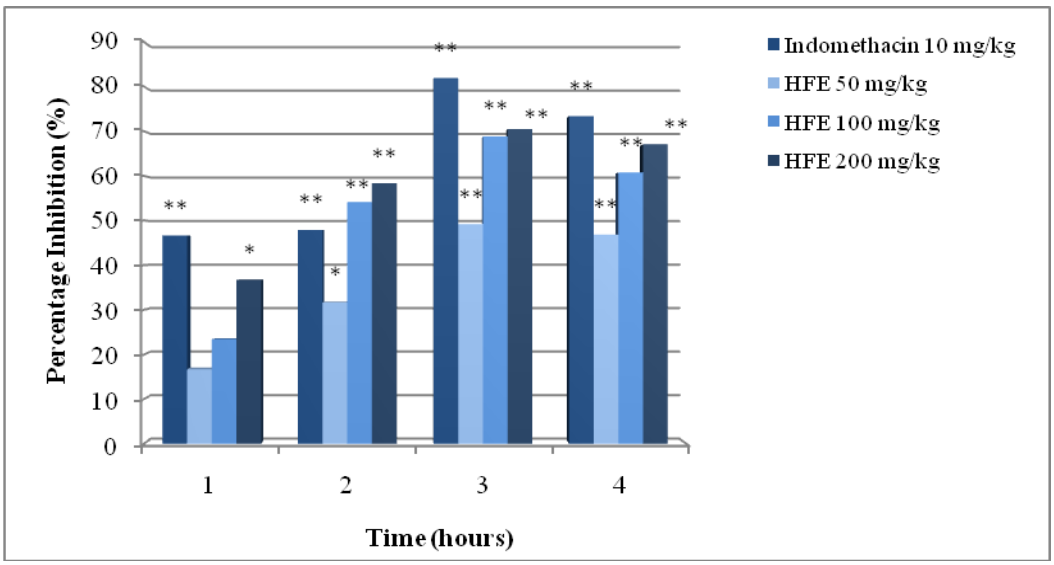

Figure 3. Anti-inflammatory activity of *H. schizopetalus* extract (HFE). Asterisks in the figure indicated statistically significant values from control. **P*<0.05, ***P*<0.01

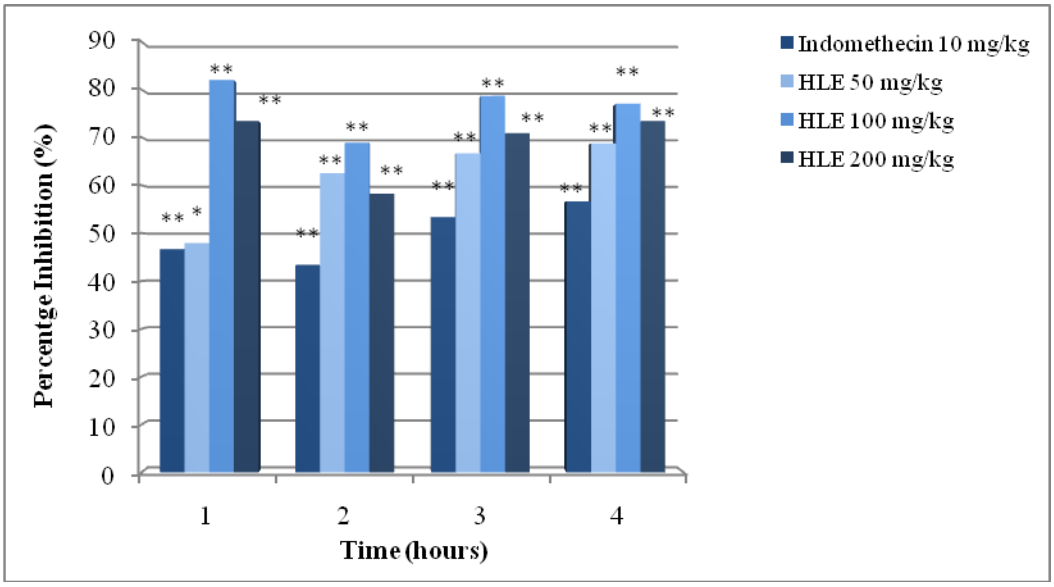

Figure 4. Anti-inflammatory activity of *H. schizopetalus*extract (HLE). Asterisks in the figure indicated statistically significant values from control. **P*<0.05, ***P*<0.01

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