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Research Article

ANALGESIC AND ANTIPYRETIC ACTIVITIES OF *HIBISCUS SCHIZOPETALUS* (*MAST.*) HOOK

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ABSTRACT

Analgesic and antipyretic effects of methanolic extracts of flower and leaves of *Hibiscus schizopetalus (Mast.)* Hook (Malvaceae) was investigated at doses 50, 100 and 200 mg/kg body weight, using tail-flick test, tail immersion test and yeast-induced pyrexia tests, respectivley. Flower and the leaves extracts were found to be non-toxic up to doses of 5 g/kg, body weight and did not cause any mortality of the tested animals. Oral administration of *Hibiscus schizopetalus (Mast.)* Hook methanolic extracts of flower (HFE) and leaves (HLE) respectively produced significant (P < 0.05-0.001) prolongation of the reaction time. Moreover, in tail immersion test, HFE and HLE (50, 100, 200 mg/kg, body weight) respectively produced significant (P < 0.05-0.001) dose dependent analgesic effect at all the tested doses when compared to the control group. Yeast-induced pyrexia in rats, HFE and HLE significantly (P < 0.05-0.001) reversed hyperthermia. The results of pharmacological tests performed in the present study suggest that HFE and HLE possess potent analgesic and antipyretic effects.

Keywords: *Hibiscus schizopetalus (Mast.)* Hook, Malvaceae, Analgesic, Tail-flick test, Tail immersion test, Yeast-induced pyrexia, Rats

INTRODUCTION

Medicinal plants can grow everywhere in the world, ranging from wild plant to trees and shrubs. Depending upon the plant and its bioactive phytochemicals, different plant parts such as bark, root, flower, leaves are used for medicinal purposes. For many years, natural products have contributed enormously to the development of important therapeutic drugs that are used in modern medicine[1]. There is a need to search new plant derived pharmacologically active agents that contribute in the discovery of clinically useful drugs that helps in improving disease conditions from everyday aliments to usual problems. About 25% of all modern drugs that are used to treat disorders are derived directly or indirectly form higher plants[2]. Due to having adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates[3].

Pain is an unpleasant sensation it is usually beneficial to man (or animal). It is mainly a protective mechanism for the body, occurs whenever any tissues are being damaged, and it causes the individual to react to remove the pain stimulus[4]. Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states[5]. When body temperature becomes high, the temperature regulatory system, which is governed by a nervous feedback mechanism, dilates the blood vessels and increases sweating to reduce the temperature. When the body temperature becomes low, hypothalamus protects the internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism and dehydration[6].

The genus Hibiscus comprises about 275 species in the tropics and subtropics. With attractive and colorful flowers, plants of Hibiscus are widely planted as ornamentals and are used in traditional medicine[7]. *Hibiscus schizopetalus* belongs to the family Malvaceae[8] and it is one of the least examined specie of this genus. It is a shrub with spreading or usually drooping branches found in east of tropical Africa. It is also a common ornamental shrub cultivated in Pakistan. Coral Hibiscus, Chinese Hibiscus, Japanese lantern, Fringed Hibiscus (English), Tanglong (Malay), Arana (Spanish) are its common names. From April to September it bears red or orange-red flowers, drooping with deeply fringed petals. It is used as male parent in the crosses with Hibiscus rosa-sinensis Linn and its varieties. Colombians use the infusion of flower to treat cold and cough[9].

MATERIALS AND METHODS

Plant material

Flowers and leaves of *Hibiscus schizopetalus* was collected from the premises of University of Karachi, Pakistan, in summer season in the month of July, 2009. The plant was identified and authenticated by Prof. Dr. Suriya, Chairperson Department of Botany, University of Karachi, Pakistan. Voucher specimen (No. 082) was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan.

Preparation of plant extracts

Both morphological parts of plant were washed with distilled water to remove dirt and dried under shade separately. 1000 g of leaves and 500 g of flowers were soaked in methanol for percolation at room temperature for 7 to 10 days. After filtration, the solvents were removed at 40 ± 2°C under reduced pressure on a rotary evaporator (Buchi, Switzerland). The yield of flower (HFE) and leaves (HLE) extracts obtained were 8.02% (40.1 g), 7.38% (73.8 g) respectively. For the experiments the both extracts were administered by oral route at 50, 100 or 200 mg/kg, body weight of the animal in a final volume of 10 ml in 0.9 % saline.

Experimental animals

Adult albino rats weighing 120-160 g and Swiss mice weighing 20-25 g of either sex were used for the experiments. They were housed in standard cages at a temperature of 27± 2°C. The animals were exposed to the alternate cycle of 12 h of darkness and light and fed with standard laboratory diet (PCSIR Laboratories, Karachi, Pakistan) and water. The animals were fasted for at least 12 h before each experiment. The set of rules followed for animal experiment were approved by the institutional animal ethical committee.

Chemicals

Absolute Methanol (Merck, Germany), Diclofenac sodium (Merck, Germany), Brewer's yeast (Rossmoor Food Products, Karachi, Pakistan), Aspirin (Sigma Chemical, USA).

Acute Toxicity

Acute toxicity of methanolic extracts of flower and leaves of *H. schizopetalus* was evaluated using the experimental model[10].The animals were divided into control and test groups each group containing six mice. The control group received normal saline while test groups were treated with graded doses of the extracts (50 mg/kg - 5000 mg/kg, body weight) respectively. The mice were closely observed for the toxic signs and behavioural changes for first 3 h of extract administration. Behavior parameters of animals observed were hyperactivity, movement, sense of pain and touch, convulsion, phonation, aggression, increased or decreased respiration, laccrimation, social interaction, defecation and urination. All animals were observed for mortality and toxicity for one week.

Evaluation of analgesic activity of the extracts

Tail-flick Test in rats

The method was used with slight modification[11] using the tail –flick apparatus (Ugo-Basile, 37360). Rats were divided into eight groups of six animals weighing between 120-160 g. Group I served as control and orally administered with normal saline. While group II were orally administered with diclofenac sodium (50 mg/kg, body weight). Rats in group III-VIII were orally administered with HFE and HLE (50,100 and 200 mg/kg, body weight) respectively .The rats were placed on the tail-flick unit, so that beam of light focused onto the rat's tail at a point midway along the tail surface and the latency for the animal to flick the tail out of the beam was measured automatically. The reaction time was determined at 30, 60, 90, 120, 150 min after treatment. Baseline was considered as reaction time before administration of extracts or reference drug. The cut-off time was taken as 10 s to prevent tissue damage. Tail flick antinociceptive index (TFAI) was calculated from the expression:

TFAI = reaction time-baseline/cut-off baseline

Tail Immersion Test in mice

Mice were divided into eight groups of six animals weighing between 20-25 g .The lower two- thirds of the tail of the mice was immersed in a water bath (Buchi water bath) maintained at a temperature of 50 ± 0.5 °C[12].The time spent by the animal before reacting to pain considered as the reaction time and was measured by stop watch with a cut-off time of immersion set as 10 s to avoid tissue injury. After the oral administration of HFE and HLE (50,100 and 200 mg/kg, body weight) respectively to the test groups and diclofenac sodium (50 mg/kg, body weight) and normal saline to the control groups, the reaction time was measured at 30, 60, 90, 120, 150 min.

Evaluation of antipyretic activity of the extracts

Yeast-Induced Pyrexia in rats

Rats were divided into eight groups of six animals in each weighing between 120-155 g .The normal body temperature of each rats was measured rectally by digital thermometer (TMP, 812 RS) at predetermined interval. After measuring the rectal temperature, animals were given of 15% (w/v) of brewer's yeast in 0.9% saline solution at a dose of 10 mg/kg body weight near the groin region of the rats[13]. After the administration of the injection, the site was messaged in order to uniformly spread the suspension beneath the skin. After 18 hour rats were again restrained as described previously in individual cages for recording of their rectal temperature. Animals showing increase of at least 0.5 °C rise in temperature were used for the experiment. Then HFE and HLE were administered orally (50, 100 and 200 mg/kg, body weight) to six groups respectively. Other groups were administered orally with saline and aspirin (100 mg/kg, body weight). The rectal temperature of the groups was then recorded at 1 h interval up to 24 h after yeast injection.

Statistical Analysis

The results were expressed as mean ± S.E.M. The statistical evaluations were made using ANOVA followed by LSD post hoc multiple comparison tests, in order to compare more than two groups. All data were processed with SPSS software version no. 19. $P \leq 0.05$ was considered as significant.

RESULTS

Acute toxicity

Flower and the leaves extracts were found to be non-toxic up to doses of 5 g/kg, body weight and did not cause any mortality of the tested animals.

Analgesic activity of the extracts

The effect of extracts on tail-flick response showed in Table 1. The oral administration of HFE and HLE (50, 100, 200 mg/kg, body weight) respectively caused significant prolongation (P < 0.05-0.001) of the reaction time after 30 minutes administration when compared to the control group. The prolongation in the reaction time showed central analgesic activity of the extracts. The Tail-flick antinociceptive index (TFAI) of both the extracts was showed in Fig 1 (A) and Fig 1 (B) when compared to standard drug Diclofenac sodium.

The response of HLE and HFE on tail immersion test was showed in Fig 2. It was observed that oral administration of HFE and HLE (50, 100, 200 mg/kg, body weight) respectively produced significant dose dependent analgesic effect at all the tested doses when compared to the control group (P < 0.05-0.001).

Table 1: Analgesic effects of extracts by tail-flick test

Treatment Groups	Dose (mg/kg)	Reaction Time (min)					
		0	30	60	90	120	150
Control	-	1.28±0.04	1.25±0.04	1.26±0.04	1.21±0.04	1.28±0.03	1.26±0.03
Diclofenac sodium	50	1.51±0.10	4.53±0.32***	5.61±0.40***	6.40±0.33***	7.31±0.27***	5.78±0.39***
HFE	50	1.51±0.27	3.73±0.18***	4.50±0.18***	4.90±0.25***	3.80±0.09***	3.80±0.18***
	100	1.75±0.11	3.95±0.28***	5.01±0.37***	6.25±0.31***	4.91±0.38***	4.41±0.30***
	200	1.93±0.24	3.85±0.56***	4.45±0.48***	6.55±0.36***	5.11±0.48***	4.68±0.41***
HLE	50	1.65±0.21	2.65±0.19**	4.25±0.33***	4.80±0.28***	5.56±0.41***	4.15±0.19***
	100	1.33±0.08	2.68±0.18**	3.96±0.38***	4.88±0.41***	5.63±0.41***	4.63±0.30***
	200	1.78±0.15	2.80±0.3***	3.58±0.36***	4.90±0.35***	5.81±0.41***	5.16±0.32***

Animal were pretreated by oral administration of HFE, HLE (50 mg/kg, 100 mg/kg, 200 mg/kg, body weight), Diclofenac sodium (50 mg/kg, body weight) and normal saline. Values were expressed as mean ± S.E.M (n=6). Statistical significance were calculated by ANOVA followed by LSD post hoc test when compare to the control * p < 0.05; ** p < 0.01; *** p < 0.001.

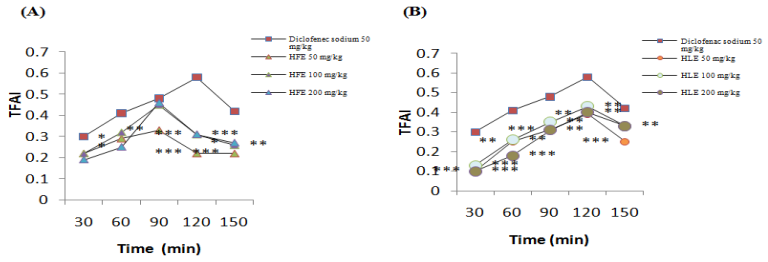

Fig. 1: Antinociceptive Index of extracts.

Animal were pretreated by oral administration of HFE, HLE (50 mg/kg, 100 mg/kg, and 200 mg/kg, body weight), Diclofenac sodium (50 mg/kg, body weight) and normal saline. TFAI was calculated as described on method. Values were expressed as mean ± S.E.M (n=6). Statistical significance were calculated by ANOVA followed by LSD post hoc test when compare to diclofenac sodium * p < 0.05; ** p < 0.01; *** p < 0.001.

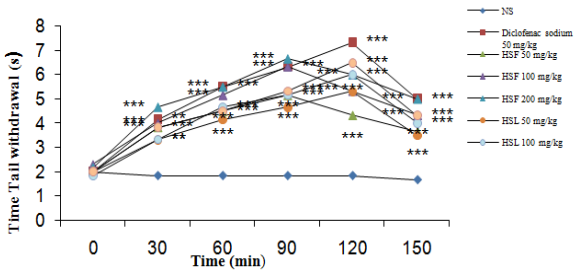

Fig. 2: Analgesic effect of *Hibiscus schizopetalus (Mast.)* Hook extracts by tail immersion response.

Animal were pretreated by oral administration of HFE, HLE (50 mg/kg, 100 mg/kg, and 200 mg/kg, body weight), Diclofenac sodium (50 mg/kg, body weight) and normal saline (NS). Values were expressed as mean ± S.E.M (n=6). Statistical significance were calculated by ANOVA followed by LSD post hoc test when compare to the control * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Anti-pyretic activity of the extracts

The antipyretic effect of extracts on yeast-induced pyrexia was shown in Table 2. The subcutaneous injection of brewer's yeast markedly increased the rectal temperature after 19 h of administration. The oral administration of HFE and HLE (50, 100, 200 mg/kg, body weight) respectively caused significant reduction in temperature when compare with the control (P < 0.05-0.001). HFE (50 mg/kg, body weight) produced anti-pyretic response in non-dose dependent manner. While dose dependent anti-pyretic response was observed with HLE when compared with standard drug aspirin (100 mg/kg, body weight).

Table 2: Antipyretic effects of leaf and flower extracts of *Habiscus schizopetalus* in rats

Groups	Dose (mg/kg)	Rectal temperature (°C)						
		Initial	19 h	20 h	21 h	22 h	23 h	24 h
Control	-	37.76±0.13	38.83±0.24	39.03±0.22	39.15±0.23	39.31±0.24	39.30±0.21	39.50±0.18
Aspirin	100	37.50±0.15	38.88±0.29	38.41±0.28*	38.20±0.29**	37.95±0.23***	37.66±0.20***	37.46±0.19***
HFE	50	35.88±0.24	37.30±0.19***	37.51±0.17***	37.30±0.16***	37.16±0.16***	37.05±0.16***	36.88±0.16***
	100	37.08±0.07	38.40±0.18	38.46±0.16*	38.28±0.17**	38.16±0.16***	38.00±0.15***	37.83±0.12***
	200	37.55±0.09	38.76±0.09	38.21±0.07**	37.90±0.12***	37.70±0.14***	37.61±0.13***	37.46±0.11***
HFE	50	37.06±0.20	38.51±0.17	38.40±0.15*	38.31±0.17**	38.31±0.17***	38.16±0.18***	38.08±0.17***
	100	37.33±0.12	38.36±0.10	38.25±0.14**	38.05±0.08***	37.76±0.06***	37.61±0.05***	37.50±0.06***
	200	36.86±0.22	38.06±0.23*	37.86±0.23***	37.66±0.24***	37.50±0.21***	37.20±0.20***	37.03±0.20***

Animal were pretreated with 15% (w/v) of brewer's yeast in 0.9% saline solution at a dose of 10 mg/kg body weight near the groin region of the rats. After 18 h rectal temperature of animals were recorded. Animals showing raise of temperature were oral administered with HFE, HLE (50 mg/kg, 100 mg/kg, and 200 mg/kg, body weight), Aspirin (100 mg/kg, body weight) and normal saline. Values were expressed as mean ± S.E.M (n=6). Statistical significance were calculated by ANOVA followed by LSD post hoc test when compare to the control * $p < 0.05$; ** $p < 0.01$; *** $p <$ 0.001.

DISCUSSION

The herbal medicine consists of natural plant substances which are used for prevention and treatment of ailments. This practice has existed since prehistoric times and flourishes today as the primary form of medicine. The nature has bestowed upon us a very rich botanical wealth and over 80,000 species of plants are in use throughout the world. Since the use of herbal drugs remains a good alternative to allopathic agents, with fewer side effects[14]. According to WHO still about 80% of the world population relies mainly on plant based drugs[15].

The analgesic effects of *H. schizopetalus* were tested in two different models of analgesia: the tail-flick test in rats and tail immersion test in mice. The tail-flick and tail immersion responses predominantly considers being selective for centrally acting analgesics. The Tail-flick response is believed to be a spinally mediated reflex, which is mediated by a supraspinal inhibitory mechanism[16-18]. Inhibition of the release and synthesis of prostaglandin has been shown to be the major mechanism by which NSAIDs produce analgesia or reduce inflammation. By this action, NSAIDs reduce the sensitization of afferent neuron by prostaglandins to the algesic actions of bradykinin and other pain provoking stimuli[19-22]. The analgesic effect of HFE and HLE extracts are dose dependent. The effect produced may be through inhibiting the synthesis of prostaglandin by inhibiting cyclooxygenase. Prolongation of reaction time in tail-flick and tail immersion test confirmed that HFE and HLE extracts possesses central analgesic action.

Fever occurs when there is a disturbance in the hypothalamic thermostat that led to rise of body temperature[23]. Pyrexia is the body's natural function to create an environment where infectious agents or damaged tissues cannot survive. Normally, the infected or damaged tissue initiates the enhanced formation of pro inflammatory mediators (cytokines, such as interleukin 1β, α, β, and TNF- α), which increase the synthesis of prostaglandin E2 (PgE2) near hypothalamic area and thereby trigger the hypothalamus to elevate the body temperature[24]. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis[25]. Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature[26]. The HFE at a dose of 50 mg/kg, body weight produced effective antipyretic activity by reducing elevated temperature in yeast-induced model. While the HLE showed maximum activity at a dose of 200 mg/kg, body weight. The present results show that HFE and HLE possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats, and its effect is comparable to that of aspirin (100 mg/kg, body weight).

CONCLUSION

The results obtained suggested that the methanolic extracts of flower and leaves of *Hibiscus schizopetalus (Mast.)* Hook endowed with analgesic and antipyretic properties, which are mediated through central inhibitory mechanism. The active compound in the extracts responsible for the observed analgesic and antipyretic are needed to be identified.

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Conflict of Interest

The authors declare that there are no conflicts of interests in this study.

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