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RESEARCH ARTICLE

EVALUATION OF ANALGESIC ACTIVITY OF *Hibiscus Schizopetalus* BY CENTRAL AND PERIPHERAL MODELS

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ABSTRACT

Objective: The present study was aimed to evaluate the central and peripheral analgesic activity of ethanolic extracts of Hibiscus schizopetalus (EEHS) (Mast) Hook (Malvaceae) in Wistar rats. **Methods:** Rats weighing about 180 to 200 g were made into 4 groups of 6 animals each. Aspirin 100mg/kg was used as standard drug. Analgesic activity was evaluated by physical, chemical and thermal methods such as tail clip test, acetic acid induced writhing test and hot plate test at the doses 200 and 400 mg/kg of EEHS respectively. **Results:** The EEHS was found to be non-toxic up to doses of 2000 mg/kg. The EEHS revealed the presence of flavonoids, alkaloids, terpenes, saponins and glycosides in the phytochemical evaluation. The EEHS treated rats showed significantly increased basal reaction time in hot plate test ($p<0.001$) and in tail clip test ($p<0.01$) respectively, while in acetic acid method significant ($p<0.001$) reduction in a number of writhing was observed. The results were obtained in a dose-dependent manner**. Conclusion:** Preliminary phytochemical screening of the EEHS which may be responsible for analgesic activities through both by central and peripheral models.

Keywords: Ethanol, Hibiscus schizopetalus, Analgesic activity.

INTRODUCTION

*Hibiscus*is a genus of flowering plants in the mallow family, Malvaceae. It is quite large, containing several hundred species of about 275 species that are native to warm temperature, sub-tropical and tropical regions throughout the world. These plants are more colorful and attractive, they are planted as ornamentals. *Hibiscus schizopetalus* (Mast) Hook belongs to the family Malvaceae [1]. It is one of the least examined species of this genus. *Hibiscus schizopetalus* is a shrub with spreading or usually drooping branches found in Kenya, Tanzania, and northern Mozambique. Japanese lantern, Fringed Hibiscus, Tanglong (Malay), Arana (Spanish) are its common names. It is a seasonal bloomer. This plant is synonymous with and formerly known as Hibiscus rosa-sinensis var.schizopetalus. Colombians use the infusion of the flower to treat cold and cough [2].

Pain is a very critical situation associated with many medical, physical and biochemical condition of health. It is described as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [3, 4]. Pain perception involves two components, the nociceptive component, and affective component. Pain is mainly a protective mechanism for the body and it causes the individual to react to remove the pain stimulus [5]. By using non-pharmacologic approaches or by administration of diversity of drugs pain relief can be achieved [6].

Analgesic drugs are used in single or in combination to affect peripheral or central nervous system (CNS) to decrease pain sensation [7].At present, the most widely used medication for the management of pain is opioids and NSAID. However, the clinical uses of these drugs are accompanied with unpleasant side effects such as hepatic failure, gastrointestinal events, renal dysfunction, respiratory depression and addiction [8].Therefore, the search for new analgesic agents with lesser side effects and more efficacies is of great interest. Plants are used extensively as raw drugs for many formulations in traditional as well as in modern medicines

Literature studies show that methanolic extract from *H. schizopetalus* has anti-hyperglycemic, hypo-lipidemic, anti-oxidant and urease inhibition activity which could support the use of the plants by traditional healers to treat various diseases. On the other hand, studies on phytochemical constituents in leaf and flower of *H.schizopetalus* shows that it contains carbohydrates, alkaloids, flavonoids, steroids, terpenes, saponins and glycosides [9, 10]. Another study shows the heavy metal contents present in *H.schizopetalus* have been determined by Atomic absorption spectroscopy [11].

However, the analgesic activities of the *H. schizopetalus* have not been scientifically tested and validated with this background the purpose of the present study was to determine the analgesic effects of *H. schizopetalus* on wistar rats.

MATERIALS AND METHODS

Chemicals

Ethanol, Acetic acid and Aspirin (Sigma Aldrich Chemicals Ltd., New Delhi) were used. All other chemicals and reagents unless specified were of analytical grade.

Plant collection and extract preparation

The whole plant of *H. schizopetalus* was collected from Chennai, Tamil Nadu (India) during January 2016 and the plant was identified and authenticated by Dr. P. Jeyaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu. The voucher specimen number was PARC/2016/3248. The whole plant was washed and dried under shade, pulverized and subjected to distillation in a Soxhlet apparatus using 95% ethanol for 72 hrs. Then, the extract was tested on the screening of analgesic activity.

Animals

Wistar rats (180 to 220g: n=24 rats) were procured from King Institute of Preventive Medicines, Chennai and divided into 4 groups of 6 animals each. The rats were housed in colony cages at an aberrant temperature of $25^{o}C \pm 2^{o}C$ with 12 hours light/dark cycle. The animals had free access to standard pellet diet and drinking water. Behavioral studies were carried out in a quiet room between 9.00 am to 11.00 am to provide circadian variation. The study was approved by Institutional Animal Ethical Committee, and work was carried out as per CPCSEA Guidelines, New Delhi.

Table.1: Experimental design.

Groups	Treatment
Group: 1	Normal saline (0.9% Nacl., p.o)
Group: 2	EEHS (200mg/kg., p.o)
Group: 3	EEHS (400mg/kg., p.o)
Group:4	Aspirin (100mg/kg., p.o)

Toxicological evaluation

Acute toxicity studies were carried out using acute toxic class

method as per OECD guidelines 423 [12]. Wistar rats (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept fasting for overnight providing only water, after which the extract were administered orally at the dose level of 5mg/kg by oral feeding needle and observed for 14 days. Mortality was not observed, the procedure was repeated for further higher dose such as 50, 300, 2000mg/kg body weight [13].

Phytochemical evaluation

The plant extracts were qualitatively tested for the presence of secondary metabolites such as carbohydrates, alkaloids, flavonoids, fixed oils, terpenes, steroids, saponins, tannins, glycosides, gums and mucilage by using standard methods [14].

Evaluation of Analgesic activity

Physical method (Haffner's tail clip test)

A metal artery clip was applied to root of the mice tail to induce pain. A sensitivity test was carried out and animals that were not attempted to dislodge the clip within 10 sec were discarded. The responsive mice were allotted to 4 groups of 6 animals each. The tail clip was applied 60 min after oral administration of EEHS (200 and 400 mg/kg, per oral) or 30 min after intra-peritoneal administration of aspirin (100 mg/kg). Distilled water (10 mL/kg) was served as the control. A cut-off time was determined by taking the average reaction time plus 3 times the standard deviation of the combined latencies of the control mice at all time periods. Any reaction time of the test animals which is greater than the cut-off time is called a positive response indicative of analgesic activity. The length of time until response indicates the period of greatest activity after dosing [15].

Chemical Method (Acetic acid induced Writhing test)

The acetic acid induced writhing method was an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. The EEHS was given orally by means of feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intraperitonally to each of the animals of the group. After an interval of fifteen minutes, this was given for absorption and no writhing was counted for 5 minutes. Then every mouse of all groups was observed carefully to count the number of writhing which made within 15 minutes [16]. The following formula was used to calculate percentage inhibition [17].

$$\% \text{ inhibition} = \frac{(\text{No. of writhes in control group} - \text{No. of writhes in test group})}{(\text{No. of writhes in control group})} \times 100$$

Thermal Method (Hot Plate Test)

This is one of the most commonly used methods for evaluating central analgesic activity of a drug [18]. In this method heat was used as a source of pain. Rats were being divided into 4 groups of six animals each. After 1 hour, animals were individually placed on a hotplate maintained at a temperature of 55± 0.5^{o}C, and not more than 15 seconds (cut off time) on the hotplate, in order to avoid damage to the paws. The time taken to flick or lick the hind paw or jumping response was considered as the reaction time of the particular animal. The reaction time was recorded at 0, 15, 30, 45, 60, 90, and 120 min. An analgesic increases the reaction time. Percent decrease in reaction time was taken as index of pain perception at each interval.

$$\% \text{ Protection} = \frac{(\text{Drug latency} - \text{Baseline latency})}{(\text{Baseline latency})} \times 100$$

Statistical Analysis

The results were reported as the mean ± SEM one way ANOVA followed by Dunnet's test was used for comparison. Difference were considered significantly at p<0.05.

RESULTS

Effect of EEHS on acute toxicity

No mortality was observed in rats treated with EEHS up to the dose of 2000 mg/kg. All the animals were alive, healthy and active during the observational period.

Effect of EEHS on phytochemical evaluation

The EEHS showed the presence of carbohydrates, alkaloids, steroids, terpenes, saponins, glycosides, flavonoids and tannins.

Effect of EEHS on Tail clip test

During the training session (Day 1) of the tail clip test, there were no significant differences between any groups. However, the animals treated with EEHS in this test have shown positive response by increasing the basal reaction time at 30, 60 and 90 minutes post administration compared to control rats. As compared to the control group, the treatment of EEHS at doses 200 and 400mg/kg produced a significant (p<0.05) and (p<0.01) increase in latency time of animals. Aspirin treated animals showed significant (p<0.001) increase in basal reaction time when compared to all other groups. According to the results obtained, the rats treated with EEHS of 200mg/kg and 400mg/kg both showed an increase in latency time in a dose dependent manner.

Effect of EEHS on acetic acid induced writhing method

In the present study the effect of EEHS on visceral pain was tested in the acetic acid induced writhing method. The EEHS at the doses of (200mg/kg and 400mg/kg) showed significant (p<0.001) reduction in the number of writhings compared to control rats. The EEHS at the dose of 200mg/kg and 400mg/kg produced 39.2% and 53.4% inhibition of writhings movements respectively when compared with the control group. The aspirin treated rats showed 68.45% inhibition of writhing movements (Table-3). The peripheral analgesic activity of EEHS at 400mg/kg was effective when compared to that of EEHS at 200mg/kg.

Effect of EEHS on Hot plate method

The effect of EEHS on somatic pain was tested in the hot plate method. Results of analgesic study showed that EEHS increased basal reaction time in hot plate. The treatment of EEHS at doses of 200 and 400mg/kg produced a significant (p<0.05), (p<0.01) increased in the basal reaction time of rats respectively (Table-4). The increase in reaction time of EEHS was dose dependent. Aspirin treated rats showed highly significant (p<0.001) increase in reaction time. However, the reaction time for EEHS groups of both the doses remained significantly greater than that of the control group (p<0.01).

DISCUSSION

Mortality of rats was not observed in toxicity studies on administration of EEHS up to a dose of 2000 mg/kg. Preliminary qualitative phytochemical screening reveals that the fraction shows the presence tannins, flavonoids, saponins and terpenes in *H.schizopetalus.* Several reports have shown that the analgesic property of plant drugs were due to the presence of the flavonoids, triterpenoids, tannins and other polyphenolic compounds [19, 20].It has been shown that the flavonoids have considerable anticonvulsant and analgesic effects [21, 22]. Moreover, tritrepenoids, flavonoids and tannins are known to inhibit prostaglandin synthesis and the chronic phase of pain could be attributed to inhibition of prostaglandin release due to the presence of these components [23, 24]. Since the phytochemical testing of EEHS has reported to contain flavonoids, saponins or phenolic compounds which may be responsible for the analgesic activity of this plant.

Analgesic activity of EEHS was studied using physical, chemical and thermal induced pain method. These include the tail clip test as physically induce pain model, acetic acid induced writhing test as chemically induced pain model, whereas hot plate latency test as thermally-induced pain model.

The acetic acid induced writhing response is a sensitive method to evaluate peripherally acting analgesics. EEHS exhibited significant inhibition of acetic acid induced writhing, which is a visceral pain. Intraperitoneal injection of acetic acid produces pain through activation of chemosensitive nociceptors [25], or irritation of the visceral surface, which lead to the liberation of histamine, bradykinin, prostaglandins and serotonin [26]. Intra-peritoneal injection of acetic acid cause writhing by increasing the high level of prostaglandin especially PGE2 and PGF2 as well as lipoxygenase products in peritoneal fluids [27]. Prostaglandin activates peripheral nociceptors to induce abdominal constrictions by increasing the capillary permeability [28, 29]. The analgesic effect occurs due to its action on visceral receptors that is sensitive to acetic acid by inhibiting the transmission of painful messages to the central level [30]. The effect produced by EEHS may be through inhibiting the synthesis of prostglandin by inhibiting cyclooxygenase enzyme.

An agent that causes a prolongation of the hot plate latency using thermally-induced pain in mice must be acting centrally is an established fact [31]. Tail clip method is a well-established method for measuring the central analgesic effect of drugs through opioid receptor. Thermally-induced pain and physically induced pain in mice is type of somatic pain which measures the complex response to a non inflammatory, acute nociceptive input used for studying central nociceptive activity [32].The brain and spinal cord play an important role in central pain mechanism. The noxious stimuli cause the release of endogenous opioids in a number of CNS regions [33]. When the peripheral sensory fibers respond to noxious stimuli substance P is released [34]. The dorsal part of the spinal cord was rich with substance P, endogenous opioids, somatostatine, and other inhibitory hormones which transmit the information through the dorsal root ganglion to the dorsal horn of the spinal cord [35].Thus, the analgesic effect exerted by EEHS may be through inhibiting the substance P. Thus, EEHS has shown inhibition of both the types of pain. The analgesic effect of the plants in all models reveals that they had dual action through the central and peripheral mechanism.

CONCLUSION

The EEHS has been proven the analgesic properties which were mediated through both by central and peripheral inhibitory mechanism. The phytochemical constituents such as flavonoids, terpenes, alkaloids were observed in the EEHS was responsible for the analgesic activity.

Table 2: **Effect of ethanolic extract of *Hibiscus schizopetalus* (EEHS) on Tail clip test.**

Treatment	Latency time (sec)			
	0 min	30 minutes	60 minutes	90 minutes
Normal saline	11.5 ± 2.6	11.6 ± 1.24	11.33 ± 0.8	12.1 ± 0.7
Aspirin 100mg/kg	10.17 ± 1.6	17.66 ± 0.54 ***	21.16 ± 2.3 ***	27.21 ± 0.9 ***
EEHS 200mg/kg	11.1 ± 0.1	13.4 ± 5.3 **	15.6 ± 2.6 **	20.2 ± 6.7 ***
EEHS 400mg/kg	10.19 ± 9.1	15.4 ± 0.5 ***	19.3 ± 1.2 ***	21.6 ± 8.2 ***

Values are expressed as mean ± SEM. * p<0.05, ** p< 0.01, *** p<0.001 is considered significant when compared with control rats by Two-way ANOVA.

Table 3: **Effect of ethanolic extract of *Hibiscus schizopetalus* (EEHS) on Acetic acid induced writhing test.**

Treatment	No. of. Writhings	% Inhibition
Normal saline	43.2 ± 0.13	-
Aspirin 100mg/kg	16. 8 ± 0.27 ***	62.71%
EEHS 200mg/kg	26. 3 ± 0.25 ***	39.2%
EEHS 400mg/kg	20. 2 ± 0.50 ***	53.48%

Values are expressed as mean ± SEM. * p<0.05, ** p< 0.01, *** p<0.001 is considered significant when compared with control rats by one-way ANOVA followed by Dunnet's test.

Table 4: **Effect of ethanolic extract of *Hibiscus schizopetalus* (EEHS) on Hot plate test.**

Treatment	Reaction Time (s)			% Protection
	Before drug treatment	1 h	3 h	
Normal Saline	8.1 ± 0.6	7.8 ± 1.9	8.4 ± 0.5	-
Aspirin 100mg/kg	9.4 ± 3.7	14.2 ± 1.3 ***	21.1 ± 2.3 ***	89 %
EEHS of 200mg/kg	9.4 ± 0.6	10.1 ± 0.6 **	13.9 ± 9.9 ***	36 %
EEHS of 400mg/kg	9.9 ± 2.3	13.4 ± 1.4 ***	17.1 ± 2.5	65 %

Values are expressed as mean ± SEM. * p<0.05, ** p< 0.01, *** p<0.001 is considered significant when compared with control rats by Two way ANOVA.

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