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EVALUATION OF ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *HOLARRHENA ANTIDYSENTERICA* LEAVES BY TAIL IMMERSION AND HOT PLATE ASSAY METHODS

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ABSTRACT

Objective: The alcoholic extracts of seeds and barks of *Holarrhena antidysenterica* have been reported to show antidysenteric, antibacterial, astringent, anthelmintic, stomachic and febrifugal properties. We aimed to evaluate analgesic potential of this plant using methanolic extract of leaf. **Methods:** In the present study, methanolic extract of *Holarrhena antidysenterica* leaves was obtained by maceration. Then, analgesic activity of the extract was investigated in mice at three different doses of 100, 200 & 300 mg/kg by using the conventional tail immersion and hot plate assays. **Results:** In the tail immersion assay, significant analgesia was shown by all doses at 30 & 60 min study period which was comparable to the reference standard ($P<0.01$). Similar to this, in hot plate assay basal latency times of response were significantly increased by all doses at 30 min study period. However, all doses showed gradual decrease in analgesia after 90 min in both tests. These results demonstrate possible indication of the medicinal use of the leaves of this plant along with mostly reported seeds and barks parts. **Conclusion:** The methanolic extract of leaves possess significant analgesic potential.

KEYWORDS: *Holarrhena antidysenterica*; leaf extract; analgesic activity.

INTRODUCTION

Holarrhena antidysenterica (L.) Wall. (Apocynaceae) known as “Kurchi” in Bengali and Tellicherry Bark in English is a plant being utilized from antiquity till to date for medicinal

purposes. The plant possesses its medicinal values due to the presence of various types of the chemical constituents that have alsovalidated the traditionally claimed properties associated with the plant viz. analgesic, antibacterial, anti-diarrhoeal, anti-amoebic, anti-inflammatory and anti-haemorrhoidal activities.[1,3] The major known constituents found in various parts of this plant are steroidal alkaloids, flavonoids, triterpenoids, phenolic acids, tannin, resin, coumarins, saponins and ergosterol.[1] The crude aqueous and alcoholic extracts of stem bark of this plant contains a number of therapeutically important alkaloids like holadysenterine, conessine, isoconessimine and kurchessine, conkurchineconamine, connessimine, kurchine, conarrhinine and holarrhinene.[4,5] Moreover, it has been shown that the crude aqueous and alcoholic extracts of stem bark also exert anti-bacterial activity against known enteric pathogens.[6,7] Seeds are also used in the treatment of diarrhea and dysentery and are reported to contain antidysentericine alkaloid.[2,5,8] More recently, some other properties viz. antimalarial, anti-diabetic, anti-oxidant anti-urolithic, anti-mutagenic, CNS-stimulating, Angiotensin-converting-enzyme inhibitory and acetylcholinesterase inhibitory activity have also been discovered from the various plant parts.[9,14] Most of the reported activities are been investigated using the alcoholic extract of stem bark and seeds of this plant. The objective of the present study was to evaluate analgesic property of this plant using crude methanolic extract (ME) of leaves.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *H. antidysenterica* were collected from Gazipur, Bangladesh and identified at Bangladesh National Herbarium Institute, Mirpur, Dhaka (accession code 38157). The collected leaves were washed with tap water and shade dried for about ten to fourteen days. Then air-dried leaves were grounded to fine powder by a grinder and preserved in an air-tight container at room temperature.

Preparation of plant extracts

About 250 g of fine powder of the plant leaves were weighed and soaked in 750 mL of methanol (Sigma Aldrich, Germany) (20% w/v) into an Erlenmeyer flask. The solution was covered, occasionally shaken and allowed to stand for 3 days at room temperature. After filtration through Whatman filter paper (No.1), the solvent was dried by using a rotary evaporator under reduced pressure at a temperature below 55°C.

Experimental animals

Swiss albino mice (25-30g) of both sexes were purchased from Pharmacology Laboratory, Jahangirnagar University, Savar, Dhaka and were kept in animal house for adaptation under standard conditions (relative humidity 55-65%, at 21.0±2.0°C and 12 h light/dark cycle) with free access to water and standard food. Animals were not allowed to food four hours before the experiment. For screening of analgesic activities by both hot plate and tail flick assays, mice were grouped into three groups. The first group served as control, the second group as reference standard and the third group received ME at different doses (100, 200 and 300 mg/kg; p.o) as test groups. All experimental procedures involving animals were conducted in accordance to ethical guidelines of Jagannath University and approved by the Institutional Ethical Committee of Jagannath University.

Drugs and chemicals

Pentazocine (Beximco, Bangladesh), sodium carboxy methyl cellulose (CMC) (Sigma, Germany) and 0.9% saline (Opsonin, Dhaka) were used in this study. All the solvents were purchased from Active Fine Chemicals Ltd (Bangladesh). The ME of leaves of *H. antidysenterica* (100, 200 and 300 mg/kg) were prepared in two per cent sodium CMC suspension before oral administration (p.o). Pentazocine was mixed in saline before intra peritoneal administration (i.p.). Two per cent sodium CMC suspension prepared in distilled water was used in the control group.

Tail immersion test

It involves immersing the tail in water at a predetermined temperature.[15] Sample having analgesic activity increases the time of tail flicking. The cut-off time for the screening and test were 5 sec and 10 sec, respectively. The test animals were divided into five groups of six mice in each group. The animals were pre-treated with drugs 30 minutes before submerging the distal part of tail (2-3 cm) in a water bath (55 ± 0.5°C). The animals received pentazocine (10.0 mg/kg, i.p.) as reference standard. The time of tail flicking from water was recorded before and after 30, 60 and 90 minute of drug treatment. A positive analgesic response was to be considered for the increase in response time compared with the control.

Hot plate test

The analgesic activity of the ME of the leaves was evaluated by using Eddy's hot plate method,[16] with minor modification. In this study, mice were divided into five groups of six mice in each group. The group receiving pentazocine (10 mg/kg, i.p.) was termed as

reference standard. The hot plate (Ugo Basile, Models-DS 37) was maintained at 50±0.5°C and animals were placed into the hot plate chamber. The time of latency period between placing the animals on the hot plate surface and licking back paw or jump off was recorded and used as the index of anti-nociception. The latency of time response was determined before and after 30, 60 and 90 minutes of treatment. The animals were pretreated with drugs 30 minutes before the experimental set up. In order to minimize the damage on the animal paws, the cut-off time was taken as 20 sec.

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's*t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Tail immersion test

The ME of leaf of *H. antidysenterica* showed highly significant increase in heat tolerance compared to control ($P < 0.001$) at all doses (100, 200 and 300 mg/kg, p.o.) within 30 min and reaching to maximum at 60 min and then decreased after 90 min observation period (**Table 1**).

The highest nociception inhibition of stimulus was observed at a dose of 200 mg/kg at 60 minutes.

Table 1: Analgesic effects of *Holarrhena antidysenterica* methanolic leaf extract in tail immersion assay.

Substance/dose	Basal reaction time (seconds) Mean ± SEM			
	0 min	30 min	60 min	90 min
Control (saline) l0 mL/kg bw p.o	1.4 ±0.1	1.9±0.17	2.1±0.19	2.1±0.19
Standard(Pentazocine) 10 mg/kg bw i.p	3.0±0.34**	5.2±0.26***	6.9±0.39***	7.3±0.26***
Test Sample (ME)				
100 mg/kg bw p.o	4.2±0.16***	4.5±0.17**	4.6±0.16*	3.9±0.21
200 mg/kg bw p.o	4.1 ±0.22***	4.6 ±0.19**	4.8±0.19**	4.2±0.23
300 mg/kg bw p.o	4.0 ±0.07***	4.3±0.12**	4.7±0.19*	4.3±0.15

Basal reaction times are presented as mean ± standard error mean (SEM); n = 6. *n* = 6 mice in each group. 0 min means before drug administration and 30 min, 60 min and 90min indicate 30, 60 and 90 min after drug administration, respectively. Values are significantly different from the control (Dunnett's *t*-test) (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Hot Plate test

In the hot plate assay method**,** the ME of leaves of *H. antidysenterica* at all doses (100, 200, and 300 mg/kg, p.o.) showed significant increase in response time at 30 minute compared to control (*P<0.001*). In this period, the extract, at the dose of 300 mg/kg showed the highest response. However, other doses of the extract did not show any significant increase in the mean latent period of response time compared to control (*P* > 0.05) (**Table 2**).

Table 1: Hot plate assay of *Holarrhena antidysenterica* methanolic leaf extract in term of latent period of response (jump off time).

Substance/dose	Latent period of response time (seconds), Mean ± SEM			
	0 min	30 min	60 min	90 min
Control (saline) l0 ml/kg bw p.o	10.2±0.80	10.5±0.67	12.6±0.81	13.8±0.86
Standard (Pentazocine) 10 mg/kg bw i.p	11.7 ±0.85	10.1±1.31	14.4±0.51*	16.0 ±0.41**
Test Sample (ME)				
100 mg/kg bw p.o	11.8±0.66	16.0 ±0.72***	14.4±1.69*	12.9 ±0.62
200 mg/kg bw p.o	11.7±0.70	15.3 ±1.13***	14.2 ±0.80	10.8 ±1.35
300 mg/kg bw p.o	11.1 ±1.40	18.0 ±0.83***	14.6 ±0.29*	12.2 ±0.31

Latency time values are presented as as mean ± standard error mean (SEM); n = 6. *n* = 6 mice in each group. 0 min means before drug administration and 30 min, 60 min and 90 min indicate 30, 60 and 90 min after drug administration, respectively. Values are significantly different from the control (Dunnett's *t*-test) (**P <0.05, **P <0.01, ***P <0.001*).

DISCUSSION

The tail immersion and hot plate methods have been found to be suitable for assessing centrally acting analgesics.[17] because it is evident from experimental data that time of paw licking in the animals exposed to thermal stimuli (mice, rats) characterizes the phenomenon of pain perception at thalamic level, serving therefore to determine the analgesic action of the investigated substances. The ME of *H. antidysenterica* leaves at all three doses of 100, 200, and 300 mg/kg displayed significant to moderate analgesic effects on mice in both models of thermal stimuli. In the tail immersion assay, the extract increased the stress tolerance capacity of the animals; thus indicating the possible involvement of a higher center for this effect. The optimum response found during 30 to 60 minute could be due to maximum systemic absorption of the drugs. Likewise, due to metabolism the effects were fading away with increasing study period to 90 minute. The effectiveness of the extract is further evident by the evaluation in hot plate study. These effects could involve compromised prostaglandin pathways with sensitization of thermal nociceptors by the sensory nerves. Moreover, this

property could be justified by the presence of the reported phytoconstituents present in the methanol extract like alkaloids, flavonoids, steroids, tannins and phenolics.

CONCLUSION

The ME of *Holarrhena antidysenterica* leaves showed potential analgesic property at the three different doses used in these studies.

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