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In vivo Anti-diarrheal and CNS Depressant Activities of *Hemigraphis hirta* (Vahl) T. Anders.

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Abstract

Hemigraphis hirta has been used by folk practitioners to alleviate symptoms of several diseases, although the pharmacological activities of this plant have not been thoroughly explored. The current study was designed to assess the anti-diarrheal and CNS depressant activities of *H. hirta* in mice model. For both assays, the experimental mice received the methanolic crude extract and its petroleum ether soluble fraction at dose of 200 and 400 mg/kg body weight which are denoted as CME 200, CME 400, PESF 200 and PESF 400, respectively. Both fractions remarkably attenuated castor oil-induced diarrheal effect in a dose-dependent manner and the results were comparable to standard loperamide (89.47%). Among all, PESF 400 exhibited statistically significant ($p < 0.01$) anti-diarrheal activity as demonstrated by 78.95% inhibition of defacetion. Compared to reference drug diazepam, all the tested samples considerably shortened the time for onset of sleep and prolonged the duration of phenobarbitone-induced sleep in mice. The results of our present study, being reported for the first time, demonstrate that the methanol extract of leaves of *H. hirta* and its organic soluble partionates possesses significant anti-diarrheal and CNS depressant properties. However, this preliminary screening requires further detailed investigation to confirm these findings as well as to isolate and characterize the bioactive compounds.

Key words: *Hemigraphis hirta*, antidiarrheal activity, CNS depressant activity, buripana.

Introduction

A substantial portion of the world population is reliant on plant-based medicines for their treatment for various diseases. As reported by World Health Organization (WHO), around 80% population in developing countries fundamentally depends on plant-based medicines for their primary health care (Tabassum *et al*., 2019; Kayser *et al*., 2019). In drug development, the use of plant materials is increasing due to their therapeutic values over synthetic drugs. As a tropical country, Bangladesh is a worthy storehouse of numerous families of medicinal plants, and there are around 600 plants known to have medicinal value (Yusuf *et al*., 1994).

Hemigraphis hirta, a member of Acanthaceae family, is locally known as buripana and borati gas, and generally regarded as a medicinal plant in the native area of Bangladesh. This plant has been shown to possess antidiarrheal, antishigellotic, analgesic and antipyretic acitivties and hence used by the native physicians (Alam *et al*., 2002). Several medicinal botany books described *H. hirta* as a potential treatment option for abdominal pain, glossitis, stomatitis, acute wounds and helmintic infections (Nasir *et al*., 1974; Kirtika *et al*., 1994). Although *H. hirta* is extensively used by folk practitioners, the pharmacological activities of this plant have not been elaborately explored and hence the current study was

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designed to evaluate the CNS depressant and antidiarrheal activities of *H. hirta.*

Materials and Methods

Collection of plant materials: For this present investigation, fresh leaves of *Hemigraphis hirta* were collected from Narail, Khulna, Bangladesh in November 2018 and were washed thoroughly. The leaves were then dried at room temperature for several days and pulverized into a coarse powder with the help of electric grinding machine.

Extraction of plant material: Approximately 250 g of the powdered leaves of *H. hirta* was soaked in methanol (2.5 liter) in a clean, flat bottomed glass container and kept at room temperature for 15 days with occasional shaking and stirring. The whole mixture was filtered by fresh cotton plugs and Whatman filter paper number 1. A rotary evaporator was used to concentrate the methanolic extract (CME) and followed by the petroleum ether (PESF) partition was partitioned by slightly modification of Kupchan method (VanWagenen *et al*., 1993).

Experimental animals: Forty eight Swiss albino mice (20-25 g weighed) of either sex and aged 4-5 weeks were used for the evaluation of antidiarrheal and CNS-depressant activity which were collected from the Animal Resources Branch of the International Centre (ICDDR,B). The mice were housed in polyvinyl cages and kept at room temperature (24 ± 2ºC) for at least 3-4 days ahead of the experiment and fed with icddr,b formulated food. Standard protocols were followed to minimize the pain and stress of the experimental mice.

Drugs and chemicals: Loperamide, phenobar-bitone-sodium and normal saline (0.9% NaCl) were obtained from Square Pharmaceuticals Limited, Bangladesh. All other analytical graded reagents and solvents were received from Phytochemical Research Lab of State University of Bangladesh.

Anti-diarrheal activity: The castor oil-induced diarrheal method, as described by Shoba and Thomas (2001) was used to evaluate the anti-diarrheal activity of CME and PESF (Shoba and Thomas, 2001; Bashar *et al*., 2019; Razan *et al*., 2016). To assess the antidiarrheal activity, the animals were divided into six groups each containing four mice. Individual treatments were provided to each group followed by oral administration of castor oil after 1 hr. Among six groups, Tween 80 (1%) in saline of 10 ml/kg of body weight and loperamide, at a dose of 50 mg/kg body weight, were used for the negative and positive control group, respectively. On the other hand, other four test groups received the CME, PESE at doses of 200 and 400 mg/kg body weight. During the experiment, each animal was placed in an individual pre-cleaned cage, where the floor was lined with blotting paper. The total numbers of defecating episodes were counted per hr and this was continued for four hrs. The following percent inhibition of defecation was calculated by using the following formula:

The percent inhibition of defecation

$$= (MC - MT)/MC \times 100$$

where, MC = Mean defecation of control and MT = Mean defecation of test sample.

CNS depressant activity: Evaluation of the CNS depressant activity of methanolic crude extract of *H. hirta* and its petroleum-ether fraction were assessed as described previously (Williamson and Fitter, 1996). Phenobarbitone was used to induce sleep in mice. To evaluate the CNS depressant activity, twenty-four mice were divided into six groups each with four mice. In this assay, two groups received the test samples (i.e. CME and PESE at 200 and 400 mg/kg b.w.) while the positive and negative control received standard diazepam (1 mg/kg b.w., i.p.) and normal saline, respectively. About 30 min later, phenobarbitone sodium (25 mg/kg body weight) was administered to all the groups of mice to induce sleep. During the study period, the animals were observed for the latent period (time between phenobarbitone administration to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex) to induce sleep.

Results and Discussion

The primary aim of the current study was to investigate the anti-diarrheal and CNS depressant

activity of methanolic crude extract of *H. hirta* as well as its petroleum ether soluble fraction. The results have been illustrated in tables 1 and 2.

During the screening for anti-diarrheal activity, the methanolic crude extract and petroleum-ether soluble fraction of *H. hirta* at doses of 200 and 400 mg/kg b.w. exhibited remarkable anti-diarrheal effect in in dose-dependent manner. As shown in table 1. PESF 400 exhibited statistically significant ($p < 0.01$) anti-diarrheal activity by 78.95% inhibition of defecation, whereas CME 200 and 400 and PESF 200 showed the reduction of diarrheal faeces by 61.40%, 64.91% and 61.40%, respectively in comparison to the standard loperamide (89.47%).

Table 1. Anti-diarrheal activity (in terms of % reduction of diarrheal episodes) of methanolic extract of *H. hirta* and petroleum-ether soluble fraction.

Animal group	Dose (mg/kg b. w.)	Number of diarrheal feces (Mean ± SEM)	% Reduction of diarrhea
Saline	-	14.25±1.03	-
Loperamide	50	1.50±0.50	89.47***
CME	200	5.50±2.18	61.40*
	400	5.00±2.48	64.91*
PESF	200	5.50±2.25	61.40*
	400	3.00±2.38	78.95**

All values are expressed as mean ± SEM; n = 4, *p < 0.05, **p< 0.01, ***p < 0.001 indicate significant compared to control.

Table 2. CNS depressant activity of methanolic extract of *H. hirta* and petroleum ether partitionate.

Animal group	Dose (mg/kg)	Average onset of sleep in minute (Mean ± SEM)	Average total sleep in minute (Mean± SEM)
Saline	-	39.25±4.35	91.75±10.18
Diazepam	1	11.25±1.49***	265.25±5.25***
CME	200	19±2.68**	128.00±9.13*
	400	15.75±1.38**	132.00±7.79*
PESE	200	23.25±3.35*	124.75±8.51*
	200	22.00±3.24*	156.75±13.12**

All values are expressed as mean ± SEM; n = 4, *p < 0.05, **p< 0.01, ***p < 0.001 indicates significant compared to control

There are several mechanisms by which castrol oil can induce diarrhea. Ricinoleic acid, the hydrolytic product of castor oil can stimulate the secretion of fluid and electrolytes of the small intestine which eventually speed up the intestinal transit (Ammon *et al*., 1974; Moniruzzaman *et al*., 2019). The plant extractives of *H. hirta* might have exerted its anti-diarrheal activity by directly inhibiting the effect of the ricinoleic acid on prostaglandin E2 receptors (Tripathi, 2008). On the other hand, there are some evidences that medicinal plants containing flavonoids, tannin, saponins, alkaloids and terpenoids are known to exhibit antidiarrheal effects (Teke *et al*., 2010; Mukherjee *et al*., 1998; Galvez *et al*., 1993; Otshudi *et al*., 2000). Therefore, these constituents could be responsible for the anti-diarrheal activity of *H. hirta.*

Compared to the reference drug diazepam, the methanolic crude extract of leaves of *H. hirta* and its petroleum ether soluble fraction considerably shortened the time for onset of sleep as well as prolonged the duration of phenobarbitone sodium-

induced sleeping time in mice (Table 2). The current study has established the central nervous system depressant properties of leaves of *H. hirta.* It is possible that, substances that have CNS depressant activity mostly act by potentiating GABAergic inhibition in the CNS. This GABAergic inhibition may be due to direct activation of GABA receptor by the extract of *H. hirta* (Shams-Ud-Doha *et al*., 2013). On the other hand, *H. hirta* may act by enhancing the affinity for GABA or an increase in the duration of the GABA-gated channel opening (Barria *et al*., 2013; Staley *et al*., 1995).

Conclusion

The present study revealed the profound anti-diarrheal and CNS depressant properties of the methanol extract and its petroleum-ether partionates of leaves of *H. hirta* for the first time. However, we performed a preliminary screening which will require further detailed investigation to confirm these findings as well as to isolate and characterize the bioactive compounds.

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