**IJP (2025), Vol. 12, Issue 3** **(Research Article)**

Received on 05 March 2025; received in revised form, 18 March 2025; accepted, 26 March 2025; published 31 March 2025

# ANALGESIC AND CNS DEPRESSANT ACTIVITIES OF THE METHANOLIC EXTRACT OF *HEMIGRAPHIS HIRTA*

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**Keywords:**

*Hemigraphis hirta*, Phytochemical, Analgesic, CNS depressant

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**ABSTRACT:** *Hemigraphis hirta*, a member of the Acanthaceae family, is locally known as buripana and borati gas and widely considered a medicinal plant in its native Bangladesh. It has been utilized for treating abdominal pain, glossitis, stomatitis, acute wounds, and helmintic infections. Analgesic activity was studied in rats using acetic acid induced writhing method. Diclofenac sodium25 mg/kg and vehicle served as standard and control respectively. Two doses 200 mg/kg and 400 mg/kg of plant extract exhibited significant ($P<0.05$) analgesic activity in acetic acid induced writhing method in comparison to control.CNS depressant activity was studied in mice by open field and hole cross methods respectively. In both models, the standard drug used was Dizepam2 mg/kg. The two doses of plant extract exhibited significant CNS depressant activity in open field ($P<0.05$) and hole cross tests in comparison to control. In conclusion *H. hirta* possesses analgesic and CNS depressant activities.

**INTRODUCTION:** Medicinal plants are generally defined as plants that have secondary metabolites with therapeutic potential. Since the beginning of time, people have used plant components to treat a variety of illnesses. Every culture has identified the therapeutic properties of specific medicinal plants, subsequently passing this knowledge on to the following generation. The World Health Organization (WHO) reports that 80% of people worldwide rely on herbs for their fundamental medical requirements [1]. There are 34 species of plants in the genus *Hemigraphis*, which is native to tropical Asia and belongs to the Acanthaceae family. Some species are currently classified under the genus Strobilanthes because of their similarities.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.12(3).228-33

Article can be accessed online on: www.ijpjournal.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.IJP.12(3).228-33

The grayish green, occasionally reddish-purple-tinged leaves of *Hemigraphis* are its defining characteristic. It is a prostrate plant with rooted stems and spreading branches [2]. *Hemigraphis hirta*, belonging to the Acanthaceae family, is widely recognized as a medicinal plant in its home region of Bangladesh. It is a soft herb with a stem 15-45 cm long, creeping in grass. It has small leaves, 1.25-2.5 cm long, ovate and crenate. Heads 2-6-flowered, axillary.

Bracts 1.25 cm long, elliptic; sepals linear, or in fruit subspathulate; corolla 1.25 cm, subequal, pale lavender blue. The capsule is 1.25 cm long and 12-seeded. This plant has been shown to possess antidiarrheal, antishigellotic, analgesic and antipyretic acitivties and hence used by the native physicians [3]. Several medicinal botany books described *H. hirta* as a potential treatment option for abdominal pain, glossitis, stomatitis, acute wounds, and helmintic infections [4, 5]. Previous phytochemical studies on *H. hirta* revealed the presence of squalene, lupeol, and β-sitosterol [6] and stigmasterol and n-hentriacontanol [7].

## MATERIALS AND METHODS:

**Chemicals and Reagents:** Methanol and acetic acid were obtained from Marc, Germany. Tween 80, Diclofenac Sodium and Diazepam from Square Pharma Ltd. Distilled water, which had been used in this experiment, was laboratory prepared.

**Test Animals:** Swiss-albino mice of both sexes, aged 4-5 weeks weighing 18-30 g were procured from the International Center for Diarrheal Diseases and Research, Bangladesh (ICDDR, B), Dhaka, Bangladesh. ICDDR, B formulated rodent pellet food were fed to the animals. They also had free access to the tap water. The mice were kept in a polyvinyl cage with a layer of soft husk.

**Collection and Identification of the Plant:** *Hemigraphis hirta* was collected from Mirpur, Dhaka during the month of August,2023. The plant was identified by the taxonomist of Bangladesh National Herbarium and a voucher specimen of this plant was deposited (Accession no. DACB 92793).

**Extraction and Preparation of Plant Materials:** The leaves of the collected plants were thoroughly cleaned under running water. Small pieces of leaf material were cut up and allowed to air dry for 22 days in a shaded area. The dried leaves were ground into a 400-gram powder.

The dried plant material was ground into a fine powder using an electric blender and stored in little plastic containers labeled with paper. The Soxhlet extraction method was used for around 20 hours to prepare the crude plant extract with methanol. For later usage, the crude extract was stored in sealed vial at 4°C in the refrigerator.

**Phytochemical Screening:** Standard phytochemical screening test was carried out to detect the presence of secondary metabolites to relate the analgesic and CNS depressant of *H. hirta* leaves extracted with the presence or absence of these active constituents. Thus, the test for alkaloids, saponins, flavonoids, protein, lead acetate, glycosides, and carbohydrates were performed using standard test procedures [8, 9].

**Analgesic Activity:** Analgesics are substances that, without substantially changing consciousness, selectively reduce pain by acting on peripheral pain mechanisms or the central nervous system. Tail-flick and hot plate are two of the several methods available for evaluating central analgesic activity while peripheral analgesic activity was tested by an acetic acid. This study used the acetic acid-induced writhing test to evaluate the potential analgesic effects of the methanolic extract from the *H. hirta* plant.

**Acetic Acid Induced Writhing Method:** The acetic acid-induced writhing assay [10] is employed for the evaluation of peripherally acting analgesics. 20-40g Swiss Albino mice were used. The test involves injecting an intraperitoneal 0.6% acetic acid solution and then watching the animal for a particular body concentration known as writhing.

All the samples/drugs were administered 30 minutes before the injection of acetic acid. The mice were then observed for a period of 20 min and the numbers of writhes are recorded for each animal. Diclofenac sodium was used as the positive control. The % inhibition of writhing was calculated as following equation:

% Inhibition = Average writhing of control- Average writhing of sample / Average writhing of control × 100%

**CNS Depressant Activity:** Numerous experimental models, such as the open field test, hole cross test, thiopental sodium-induced sleeping test, forced swimming test, tail suspension test, etc., can be used to assess a test compound's central nervous system (CNS) function. The open field and hole cross test were used in this work to assess the extract from the *H. hirta* plant's possible CNS depressive action. The typical dosage of diazepam (standard) in this case was 1 mg/kg body weight.

**Open Field Test:** The test animals' locomotor activity was assessed in a specially designed open field. The field was surrounded by a wall of 40 cm height. The field was a combination of several alternate black and white squares separated by black borders.

The animals were divided into control, positive control and three test groups containing four mice each. The test groups received extracts at the doses of 200 and 400 mg/kg body weight orally whereas the control group received distilled water (0.1 mL/mouse, p.o.). Diazepam (1 mg/kg, i.p.) is used as positive control group.

Following the intraperitoneal administration of the test samples, mice were kept in the field. At intervals of 0, 30, 60, 90, and 120 minutes, the animals visited the squares, and the number of squares visited was counted for three minutes [11].

**Hole Cross Test:** For this test, a customized case (30 × 20 × 14 cm) with a wooden divider in the center was utilized.

The center of the cage included a 3 cm diameter hole that was 7.5 cm high. The animals were divided into control, positive control, and two test groups containing four animals in each.

The test groups received extracts at the doses of 200 and 400 mg/kg body weight orally and the control group received distilled water (0.1 mL/mouse, p.o.). The standard drug diazepam (1 mg/kg, i.p.) was used as positive control group.

The mice were passed through the hole from one chamber to another and the number of passages was counted for 3 min at 0, 30, 60, 90, and 120 min intervals respectively [12].

**Statistical Analysis:** Where applicable, the data was expressed as mean ± SEM. The level of significance was verified by using Student's t-test and one-way ANOVA followed by Dunnett's multiple tests, as required and $p < 0.05$ was considered as statistically significant.

## RESULT AND DISCUSSION:

**Phytochemicals Present in the Plant:** Preliminary phytochemical screening for secondary metabolites was carried out to detect the presence or absence of different phytoconstituents from methanolic leaf extract of *H. hirta* **Table 1.**

The presence of carbohydrates, glycosides, lead acetate, protein and alkaloids were confirmed through qualitative color changes of test reagents which will give a clue to the possible mechanisms of analgesic and CNS depressant effects of the extract.

**TABLE 1: DIFFERENT PHYTOCHEMICALS FOUND IN THE *H. HIRTA* PLANT EXTRACT**

| Phytoconstituents | Observation |
|---|---|
| Carbohydrates | + |
| Glycosides | + |
| Saponin | - |
| Flavonoid | - |
| Lead acetate | + |
| Protein | + |
| Alkaloid | + |

**Analgesic Activity:**

**Acetic Acid Induced Writhing Method:** The effect of methanolic extract of *H. hirta* on writhing response in mice is shown in **Table 2.** Two doses of 200 mg/kg and 400mg/kg of methanol extracts were subjected to evaluation for analgesic activity by acetic acid induced writhing method using mice as animal model.

Diclofenac sodium (25 mg/kg) was taken as standard drug with the inhibition of the writhing response 73.49%. The methanolic extract at two doses caused dose dependent and showed significant analgesic activity as compared to standard drug.

**TABLE 2: ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *HEMIGRAPHIS HIRTA* LEAVES ON ACETIC ACID INDUCED WRITHINGS IN MICE**

| Treatment Groups | Dose mg/kg, Route of admin. | Writhing counting | | | | No of writhing (Mean± SEM) | % of writhing | % of Inhibition |
|---|---|---|---|---|---|---|---|---|
| Control (1% Tween 80) | 10, ml/kg, p.o. | 42 | 40 | 41 | 43 | 41.5±0.65 | 100 | ----- |
| Diclofenac sodium | 25, i.p. | 6 | 8 | 13 | 17 | 11±2.48** | 26.50 | 73.49 |
| Methanolic extract | 200,p.o. | 29 | 14.5 | 31 | 22.5 | 17±3.72** | 40.96 | 59 |
| Methanolic extract | 400,p.o. | 2 | 21 | 4 | 10 | 9.25±4.27** | 22.28 | 77.71 |

Number of writhing are presented as (mean ± standard error of mean). **p<0.01, vs control (Dennett's t test)

**CNS Depressant Activity:**

**Open Field Test:** From the initial observation period (0 min) to the 5th observation period (120 min), the methanolic extract significantly reduced the locomotor activity in mice at the doses of 200 and 400 mg/kg body weight ($p < 0.05$) **Table 3.** As anticipated, mice treated with diazepam (2 mg/kg) demonstrated a noticeable decrease in locomotion from the 2nd to the 5th observation period.

**TABLE 3: CNS DEPRESSANT ACTIVITY OF METHANOLIC EXTRACT OF *HEMIGRAPHIS HIRTA* LEAVES BY OPEN FIELD TEST IN MICE**

| Groups and Doses | Mean movements (no.) before and after drug administration | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Control | 111.25±3.50 | 105.75±1.89 | 91.5±1.94 | 86.75±1.93 | 86.25±1.11 |
| Diazepam 2mg/kg | 113±2.62 | 57.83±9.68** | 46.67±11.28** | 37.83±11.67** | 26.67±14.33** |
| Methanolic extract 250mg/kg | 71.5±8.33 | 17±4.60** | 16.25±9.84** | 10.75±3.79** | 5.25±2.39** |
| Methanolic extract 500mg/kg | 69.25±8.61 | 36.5±9.51** | 31.75±6.64** | 42.25±13.19** | 29±14.15** |

**Hole Cross Test:** Methanolic extract of *H. hirta* showed significant decrease of movement at the doses of 200, and 400 mg/kg body weight from its initial value at 0 min to 120 min ($p < 0.05$). The number of hole crossed from one chamber to another by mice of the standard drug diazepam (2 mg/kg,) is decreased from 0 min to 120 min **Table 3.** The methanol extract showed dose dependent activity and maximum depressive effect was observed at 5th observation period.

**TABLE 4: CNS DEPRESSANT ACTIVITY OF METHANOLIC EXTRACT OF *HEMIGRAPHIS HIRTA* LEAVES BY HOLE CROSS TEST IN MICE**

| Group and Dose | Mean hole cross (no.) before and after drug administration | | | | |
|---|---|---|---|---|---|
| | 0 min | 30 min | 60 min | 90 min | 120 min |
| Control | 22.5±2.10 | 12±0.82 | 11.75±0.85 | 7.25±0.85 | 5.5±1.56 |
| Diazepam 2mg/kg | 15±1.09 | 7.34±0.76 | 4.67±0.61 | 3.83±0.75 | 2.83±0.59 |
| Methanolic extract 250mg/kg | 6.25±0.47 | 4.75±1.11 | 2±0.71 | 2.75±0.25 | 2.5±1.04 |
| Methanolic extract 500mg/kg | 4.75±1.11 | 8.75±1.43 | 4.75±0.85 | 4.5±1.44 | 4.25±1.38 |

**DISCUSSION:** Many plant metabolites effectively treat a variety of illnesses. Even now, a large portion of the global population still gets their medicine from plant sources [13]. The writhing test, a chemical pain model that has been shown to be helpful for examining peripheral antinociceptive activity, was used to measure the analgesic activity [14–15]. In both animal models of pain, the methanol extract of *H. hirta* showed a considerable antinociceptive effect that was dose dependent. It is thought that acetic acid raises the levels of PGE2 and PGF2α in peritoneal fluid [16]. Mice that received intraperitoneal acetic acid (0.6%) experienced localized inflammation as a result of prostaglandin and leukotriene biosynthesis. It has been suggested that the pain experienced when acetic acid is administered intraperitoneally is caused by the biosynthetic prostaglandins, specifically prostacyclin and prostaglandin E [17]. Like other non-steroidal anti-inflammatory medications, diclofenac sodium prevents prostaglandin biosynthesis, which stops experimental animals like mice from writhing. Assuming that *H. hirta* leaf extract prevents mice from writhing, it's plausible that the extract works via a similar mechanism as diclofenac sodium. In this investigation, we used the acetic acid-induced writhing method to examine the analgesic effects of two doses of *H. hirta* extracts [18, 19, 20]. According to the study, the methanol extracts significantly reduced writhing at doses of 200 and 400 mg/kg body weight. The study found that raising the dose can raise the percentage of writhing inhibition. Results of two doses were also comparable with those of standard drug- diclofenac sodium.

Depression is an episodic and unpredictable illness [21]. Worldwide 264 million individuals suffer from depression [22]. It is already expected to constitute the second largest source of global burden of disease after heart disease in 2020 [22]. The etiology, development, and treatment of depression are not fully explained by the monoaminergic hypothesis of depression [23]. The pathophysiology of depression is thought to entail oxidative stress, according to the most widely recognized theory of depression [24]. The open field and hole cross tests, two neuropharmacological models, were used to examine the CNS depressing effect of *H. hirta*. These methods are frequently applied to conventional models of neuropharmacological action assessment. The methanolic extract of *H. hirta* decreased the frequency and amplitude of movements in the open field and hole cross tests in mice. The results of these study provided evidence

that the extract reduced locomotor activity confirming its CNS depressant effects. Locomotor activity is considered as an index of alertness and a reduction of it is an indicative of CNS depressant activity [25]. It is a measurement of the level of excitability of the CNS [26], this decrease in spontaneous motor activity could be attributed to the CNS depressant effect of the plant extract [27]. Both tests significantly decreased locomotion in mice. Gamma amino butyric acid is the major inhibitory neurotransmitter in the central nervous system [28], which is involved in the physiological functions related to the psychological and neurological disorders as epilepsy, depression, parkinson syndrome, and alzheimer's disease [29].

Diverse drugs might modify the GABA system, at the level of the synthesis of it by potentiating the GABA-mediated postsynaptic inhibition through an allosteric modification of GABA receptors. It directly increases in chloride conductance or indirectly by potentiating GABA-induced chloride conductance with simultaneous depression of voltage activated $Ca^{2+}$ channel like barbiturates [30]. Therefore, it is predictable that the extract may act by potentiating GABAergic inhibition in the CNS via membrane hyper polarization leading to a reduction in the firing rate of critical neurons in the brain or it may be due to direct activation of GABA receptors [31]. It may also be due to enhanced affinity for GABA or an increase in the duration of the GABA-gated channel opening [32]. We suspect that more in-depth, sophisticated research may be done in the future to examine the plant's analgesic and central nervous system (CNS) depressive properties as well as its active ingredients.

**CONCLUSION:** The plant extract's qualitative phytochemical screens identified a number of significant secondary metabolites, including proteins, alkaloids, carbohydrates, glycosides, and lead acetate. Based on the current study's findings, it can be said that the plant extract has remarkable analgesic and central nervous system depressant properties, which supports the plant's traditional use in treating inflammatory and painful conditions. The current work was an initial attempt that would need more thorough research, including the characterization of active ingredients, as well as preformulating experiments to establish a possible dosage form.

**ACKNOWLEDGEMENT:** Authors are grateful to Primeasia University, Department of Pharmacy, Dhaka, Bangladesh, for providing the necessary facility to carry out the study.

**CONFLICTS OF INTEREST:** The authors declare 'no conflict of interest'.

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**How to cite this article:**
Begum T, Nasrin M, Ahsan M and Dash PR: Analgesic and CNS depressant activities of the methanolic extract of *Hemigraphis hirta*. Int J Pharmacognosy 2025; 12(3): 228-33. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.12(3).228-33.