

Evaluation of Analgesic and Central Nervous System Depressant Effects of *Holarrhena antidysenterica* Stem on Swiss Albino Mice Model

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Abstract

Plants are astonishing reservoir of bioactive natural compounds; many of these are able to exhibit outstanding biological activities in animals. *Holarrhena antidysenterica* is widely used as traditional medicine. Aim of the present study was to investigate the possible analgesic and central nervous system (CNS) depressant effects of methanol (MHAS), petroleum ether (PHAS), chloroform (CHAS), dichloromethane (DHAS) and aqueous (AHAS) extracts of the *H. antidysenterica* stem. The analgesic effect was evaluated by acetic acid induced writhing and hot plate methods at 50 and 100 mg/kg dose. The CNS-depressant effect was assessed by using open field, hole cross and head deep tests at 100 and 200 mg/kg dose. All the extracts had exhibited significant ($P^b < 0.01$, $P^a < 0.001$) analgesic and CNS depressant effects at dose dependant manner. CHAL showed maximum analgesic and CNS-depressant effect. It had shown 71.34% inhibition of abdominal writhing and 70.39% elongation of paw licking time at 100mg/kg dose. In CNS-depressant study, it exhibited 88.51% inhibition in open field (locomotion), 83.20% inhibition in hole cross (locomotion) and 85.15% inhibition (head deeping) in head deep test at 200mg/kg dose. Among the five extracts CHAL is a potent analgesics and CNS-depressant agent.

Key words: *Holarrhena antidysenterica*; Traditional medicine; Analgesic effect; CNS-depressant effect; Writhing; Paw licking

Introduction

Nociception is a noxious stimulus that mediates through neural path. Mechanical, thermal, or chemical stimuli, for example, activate primary afferent nociceptors in the peripheral and central nervous system. After any stimulation, a nociceptor transmits signal to the brain via the spinal cord which causes perception of pain (Yogesh *et al.*, 2015). Anxiety is associated with psychological and physiological state marked by cognitive, somatic, emotional and behavioral elements. Together, these components provoke a disagreeable emotion associated with fear, worry as well as restlessness. Therefore, it can be an obstacle in everyday life (Xueli *et al.* 2014). So, there is a need to develop

new analgesic and CNS-depressant drugs as the currently available drugs are associated with severe side effect and many patients are resistance to these (Jang *et al.*, 2013).

Holarrhena antidysenterica is a shrub. It is commonly known as kurchi and belongs to the Apocynaceae family (Sujan *et al.*, 2009). Traditionally, it is used in the treatment of amoebic dysentery, diarrhoea, asthma and bronchopneumonia. In addition, it has been reported to possess anti-helminthic, appetizing, anti-diarrhoeal and astringent effects (Gaurav *et al.*, 2011). It is distributed in Asia, tropical areas of Africa, Madagascar, India, Philippines and Malayan Peninsula. In Bangladesh it grows in Chittagong and Sylhet hilly area (Nahar

et al., 2015 and Jalalpure et al., 2006). Various bioactive compounds like polyphenols, flavonoids, tannins, alkaloids etc are present in the plant (Karunakar et al., 2014). Among the alkaloids, conessine, conamine, conkurchine, connessimine, kurchine, conarrhinine, holarrhinene, isoconcessimine and holarrifine-24-ol is isolated from bark of it. Medicinal plants are believed to be cost-effective and harmless source of novel biochemical constituents with strong therapeutic properties (Hammad et al., 2015). Therefore, the present study was undertaken to investigate analgesic and CNS depressant activities of the *H. antidysenterica* stem extracts that may be safe and cost effective for the treatment of diseases associated with pain and anxiety.

Materials and Methods

Plant materials: For the investigation, *Holarrhena antidysenterica* stem were collected from Comilla Hill tract, Bangladesh in September, 2014 and identified by an expert of the Bangladesh National Herbarium, Dhaka, where a voucher specimen has also been retained with accession no. DACB-41637. The collected stems were cleaned, dried for one week in air, and then pulverized into a coarse powder using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until further analysis.

Extract preparation: Approximately 500g of powdered material was placed in each of five clean, flat-bottomed glass containers and soaked in methanol, petroleum ether, chloroform, dichloromethane and water and kept for 7 days. Then extraction was carried out using Ultrasonic Sound Bath accompanied by sonication (40 minutes). The entire mixture then underwent a coarse filtration by a piece of clean, white cotton material. The extract then was filtered through Whatman filter paper and was dried to obtain the methanol (15 g), petroleum ether (6 g), chloroform (4 g), dichloromethane (10 g), and aqueous (13 g) extracts. The gummy extracts were transferred to a closed container for further use and storage.

Drugs and chemicals: Methanol, petroleum ether, dichloromethane, and chloroform were purchased from Active Fines, Bangladesh. Acetic acid and Tween-80 were purchased from Merck, Germany. Diazepam and diclofenac sodium (DS) were collected from Square Pharmaceuticals Ltd. Bangladesh. All chemicals in this investigation were of analytical reagent grade.

Animals: Swiss albino mice of either sex weighing approximately 25-30 g were used for this experiment. The mice were purchased from the animal research branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The set of rules followed for animal experiment were followed in accordance with international guidelines for care and use of laboratory animals (Zimmermann et al., 1983).

Evaluation of analgesic effect: The analgesic effect of the extracts (MHAS, PHAS, CHAS, DHAS and AHAS) was evaluated by using acetic acid-induced writhing and hot-plate test that were previously described by Koster et al., 1959 as well as Eddy et al., 1953, respectively. Briefly, 72 mice (Swiss albino) were divided into 12 groups. Then, mice of specific group were feed with vehicle (2% acacia), diclofenac sodium (10mg/kg), and the extracts (50 and 100mg/kg, b.w.). 30 minutes later, 0.1ml acetic acid solution (0.7%) was injected intraperitoneally in each mice and after 5 minutes of it writhing number was counted for 20 minutes. Percent reduction of writhing, an index of analgesia, was calculated as: $[(N_c - N_t) / N_c] \times 100$, where, N_c = writhing number of control group, N_t = writhing number of treated group.

Similarly, 72 mice were divided into 12 groups of 6 mice in each and were feed with vehicle, diclofenac sodium, and the extracts (50 and 100mg/kg, b.w.). After 30 minutes of feeding each mice was placed on a hot plate ($55 \pm 5^\circ$), and heat induced reaction time (licking or jumping of mice) was measured at 0, 30, 60, 90 and 120 minutes of the experiment. A cut off period of 20 s was maintained to avoid paw tissue damage.

Percentage of the maximal possible effect (%MPE) was calculated as: %MPE = [(Post drug latency–pre drug latency) / (Cut off period–pre drug latency)] × 100.

Evaluation of CNS depressant effect: This effect was evaluated by applying open field (Gupta et al., 1971), hole cross (Takagi et al., 2015) and head deep (Dorr et al., 1921). tests. Shortly, 72 mice were divided in to 12 groups and feed with vehicle (2% acacia), diazepam (2mg/kg), and the extracts (100 and 200mg/kg, b.w.). 30 minutes later, each mice was placed in a open field box, a hole cross box, and a head deep box where number of mice movement (open field), passes through hole (hole cross) and head deeping were counted for 5 minutes at 0, 30, 60, 90, and 120 minutes of the experiment. Reduction of mice locomotion indicates CNS-depressant potential of the extracts. Percentage inhibition of locomotion was calculated at 120 min by the following formula: % inhibition = [(N₀-N_s)/N₀] × 100, where, N₀ is the average number of movements or passes or head deeping in control group and N_s is the average number of movements or passes or head deeping in treated group (extract or standard drug).

Statistical analysis: All the values were expressed as the mean ± SEM (Standard Error Mean)

of experiment (n = 6 mice per group). The analysis was done in SPSS statistical package, version 15.0. P^b<0.01, P^a<0.001 were considered to be statistically significant compared to vehicle control group. ANOVA followed by Dunnett's test was done by the SPSS software.

Results

Analgesic effect: All the extracts had significantly (P^b < 0.01, P^a < 0.001) reduced the writhing response, and increased reaction time (licking or jumping of mice) as dose dependant manner. In writhing test, DS the standard drug, showed 85.70% inhibition and the extract CHAS showed 60.80% (50mg/kg) as well as 71.34% (100mg/kg) inhibition whereas AHAS showed the lowest effect with 25.05% (50mg/kg) as well as 36.20% (100mg/kg) inhibition. In hot plate test, DS had shown 39.24%, 63.71%, 76.52% and 57.20% MPE at 30, 60, 90, 120 minute. Among the five extracts, CHAS had shown the highest effect with 34.38%, 60.38%, 70.39% and 55.07% MPE at 30, 60, 90, 120 minute. The order of writhing inhibition and MPE is DS> CHAS>MHAS>PHAS>DHAS>AHAS (Tables 1 and 2).

Table 1. Analgesic effect of *H. antidysenterica* stem extracts in acetic acid-induced writhing test.

Sample	Dose mg/kg, p.o.	Writhing number	Inhibition of writhing (%)
Control (vehicle)	0.1 ml/mice	74.45 ± 6.32	00
DS	10 mg/kg	10.65 ± 0.36 ^a	85.70
MHAS	50	34.15 ± 2.31 ^a	54.13
	100	28.76 ± 3.14 ^a	61.37
PHAS	50	46.35 ± 4.36 ^a	37.74
	100	38.53 ± 4.12 ^a	48.25
CHAS	50	29.18 ± 3.10 ^a	60.80
	100	21.34 ± 2.35 ^a	71.34
DHAS	50	52.28 ± 5.14 ^a	29.78
	100	45.15 ± 3.37 ^a	39.36
AHAS	50	55.80 ± 4.45 ^a	25.05
	100	47.5 ± 3.98 ^a	36.20

Each value is presented as mean ± SEM (n = 6); p^a< 0.001 compared with the control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

Table 2. Analgesic effect of *H. antidysenterica* stem extracts in hot plate test.

Sample	Dose mg/kg, p.o.	Response time (s) (%MPE)				
		0 min	30 min	60 min	90min	120 min
Vehicle	0.1 ml/mice	6.11 ± 0.28	7.45 ± 0.37	7.75 ± 0.10	7.89 ± 0.56	6.46 ± 0.17
DS	10	6.80 ± 0.12	11.98 ± 0.86 ^a (39.24)	15.21 ± 1.27 ^a (63.71)	16.90 ± 2.78 ^a (76.52)	14.35 ± 1.46 ^a (57.20)
	50	6.48 ± 0.12	8.88 ± 0.47 (17.75)	10.53 ± 1.54 ^a (29.96)	12.85 ± 1.45 ^a (47.12)	11.67 ± 0.54 ^a (38.39)
MHAS	100	6.98 ± 0.40	9.58 ± 2.20 ^b (19.97)	12.27 ± 0.87 ^a (40.63)	13.35 ± 2.16 ^a (48.92)	12.87 ± 1.59 ^a (45.24)
	50	6.11 ± 0.25	8.26 ± 1.13 (15.48)	9.38 ± 1.29 ^b (23.54)	11.22 ± 1.84 ^a (36.79)	9.15 ± 0.47 ^b (21.89)
PHAS	100	5.59 ± 0.47	9.03 ± 1.71 ^b (23.87)	10.35 ± 1.88 ^a (33.03)	12.16 ± 1.98 ^a (45.32)	11.52 ± 1.01 ^a (41.15)
	50	5.47 ± 0.23	8.20 ± 0.55 (18.79)	12.22 ± 2.66 ^a (46.46)	14.21 ± 1.15 ^a (60.15)	12.42 ± 0.58 ^a (47.83)
CHAS	100	5.31 ± 1.10	10.36 ± 1.90 ^a (34.38)	14.18 ± 0.70 ^a (60.38)	15.65 ± 1.83 ^a (70.39)	13.40 ± 1.75 ^a (55.07)
	50	6.18 ± 1.12	8.15 ± 0.52 (14.25)	9.30 ± 1.36 ^b (22.58)	10.85 ± 1.07 ^a (33.79)	9.52 ± 1.80 ^b (24.17)
DHAS	100	5.18 ± 1.10	8.85 ± 0.12 (24.76)	9.89 ± 1.80 ^b (31.78)	11.75 ± 2.21 ^a (44.33)	10.25 ± 1.10 ^a (34.21)
	50	6.59 ± 0.42	7.10 ± 0.37 (3.80)	8.54 ± 1.18 ^b (14.54)	9.12 ± 1.63 ^b (18.87)	8.26 ± 1.22 (12.45)
AHAS	100	5.37 ± 0.20	7.98 ± 0.16 (17.84)	9.10 ± 1.62 ^b (25.50)	10.11 ± 1.36 ^a (32.40)	10.18 ± 1.75 ^a (32.88)

Each value is presented as mean ± SEM (n = 6); p^b < 0.01, p^a < 0.001 compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0.

Table 3. CNS-depressant effect of *H. antidysenterica* stem extracts by the open field test.

Sample	Dose mg/kg, b.w.	Number of movements					Inhibition (%) after 120 min
		0 min	30 min	60 min	90 min	120 min	
Control	0.1 ml	271.60±7.70	269.26±8.43	267.10±9.29	264.36±7.60	264.23±8.13	-
Diazepam	2	282.30±15.69	152.0±9.12 ^a	85.40±8.45 ^a	28.32±4.24 ^a	25.20±3.32 ^a	90.46
	100	269.78±10.25	195.26±4.74 ^a	136.20±9.12 ^a	75.37±5.73 ^a	43.18±3.39 ^a	83.66
MHAS	200	280.10±7.30	180.55±8.13 ^a	110.10±8.05 ^a	45.76±4.52 ^a	36.20±4.18 ^a	86.30
	100	268.32±6.22	221.45±7.22 ^b	144.78±5.23 ^a	82.35±7.32 ^a	62.45±9.21 ^a	76.37
PHAS	200	273.80±3.89	210.20±5.34 ^b	123.20±7.34 ^a	68.65±6.42 ^a	46.30±8.80 ^a	82.48
	100	266.75±7.25	174.63±3.29 ^a	105.20±45.0 ^a	52.5±5.35 ^a	42.35±3.45 ^a	83.97
CHAS	200	274.58±8.5	167.5±12.5 ^a	92.50±7.25 ^a	34.0±3.20 ^a	30.36±2.50 ^a	88.51
	100	269.84±9.61	210.23±8.50 ^b	167.23±5.23 ^a	91.20±7.73 ^a	70.46±5.20 ^a	73.33
DHAS	200	273.82±10.5	193.28±4.17 ^a	135.48±7.14 ^a	75.65±3.17 ^a	53.32±7.48 ^a	79.82
	100	258.93±8.30	230.35±6.72	182.36±8.94 ^a	112.52±7.71 ^a	83.30±7.18 ^a	68.47
AHAS	200	276.20±5.13	212.36±9.33 ^b	152.47±7.90 ^a	98.12±7.92 ^a	61.37±5.26 ^a	76.77

Each value is presented as the mean ± SEM (n = 6); p^b < 0.01, p^a < 0.001 compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0.

Table 4. CNS-depressant effect of *H. antidysenterica* stem extracts by the hole cross test.

Sample	Dose mg/kg, b.w.	Number of movements					Inhibition (%) after 120min
		0 min	30 min	60 min	90 min	120 min	
Control	0.1ml	12.25±3.09	12.78±1.20	12.10±1.41	11.23±2.86	11.25±1.10	
Diazepam	2	14.20±1.63	4.32±0.47 ^a	2.65±1.88 ^a	1.10±0.25 ^a	1.60±0.05 ^a	88.73
	100	15.43±3.39	5.72±1.39 ^a	4.76±0.60 ^a	3.78±0.35 ^a	3.10±0.63 ^a	79.91
MHAS	200	14.20±2.30	4.90±1.60 ^a	3.80±1.29 ^a	3.02±0.20 ^a	2.70±0.24 ^a	80.99
	100	13.28±2.50	10.59±1.25	6.65±1.10 ^a	4.58±0.34 ^a	3.75±0.24 ^a	71.76
PHAS	200	14.60±2.45	8.40±1.47 ^a	5.29±1.23 ^a	3.65±0.20 ^a	3.20±0.35 ^a	78.08
	100	13.5±0.55	5.30±1.16 ^a	4.50±0.54 ^a	3.50±0.35 ^a	2.50±0.10 ^a	81.48
CHAS	200	12.5±0.35	4.40±1.05 ^a	3.25±0.22 ^a	2.45±0.15 ^a	2.10±0.18 ^a	83.20
	100	13.35±2.25	8.58±2.15 ^a	6.95±0.54 ^a	5.87±0.25 ^a	4.83±0.20 ^a	63.82
DHAS	200	12.45±2.50	7.18±0.20 ^a	5.10±0.38 ^a	4.90±0.45 ^a	3.75±0.10 ^a	69.88
	100	10.0±2.08	9.30±0.60 ^a	7.33±0.62 ^a	6.20±0.35 ^a	5.33±0.80 ^a	46.70
AHAS	200	14.66±1.65	7.0±0.94 ^a	6.30±0.24 ^a	5.67±0.40 ^a	4.80±0.62 ^a	67.26

Each value is presented as the mean ± SEM (n = 6); p^b< 0.01, p^a< 0.001 compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0

Table 5. CNS-depressant effect of *H. antidysenterica* stem extracts by head deep test in mice.

Sample	Dose mg/kg, b.w.	Number of movements					Inhibition (%) after 120min
		0 min	30 min	60 min	90 min	120 min	
Control	0.1ml	98.0±5.73	95.65±8.78	94.20±5.68	94.33±8.35	93.36±7.60	--
Diazepam	2	95.0±6.38	56.33±5.65 ^a	34.34±3.29 ^a	15.55±0.47 ^a	11.0±2.97 ^a	88.42
	100	85.40±5.54	51.60±5.37 ^a	35.78±5.12 ^a	24.73±2.55 ^a	18.28±1.10 ^a	78.59
MHAS	200	89.25±4.78	40.36±4.58 ^a	27.74±4.88 ^a	21.46±2.45 ^a	15.36±2.60 ^a	82.79
	100	90.12±6.36	76.38±5.37 ^b	69.23±6.87 ^a	58.65±5.47 ^a	42.25±4.18 ^a	53.12
PHAS	200	84.67±8.55	68.12±6.12 ^a	58.12±5.69 ^a	43.36±3.25 ^a	34.29±3.50 ^a	59.50
	100	86.38±6.20	38.50±6.25 ^a	28.24±2.90 ^a	18.35±2.89 ^a	15.75±1.27 ^a	81.77
CHAS	200	85.47±8.60	33.12±5.12 ^a	22.37±2.47 ^a	16.67±1.23 ^a	12.69±1.10 ^a	85.15
	100	98.30±7.58	69.78±5.55 ^a	53.45±5.14 ^a	41.26±7.61 ^a	30.27±2.86 ^a	69.21
DHAS	200	96.35±5.65	57.0±9.82 ^a	42.15±7.01 ^a	34.16±8.46 ^a	24.0±5.42 ^a	75.09
	100	97.47±6.57	63.0±6.39 ^a	47.58±4.25 ^a	34.33±4.45 ^a	24.38±5.14 ^a	74.99
AHAS	200	86.66±5.36	48.36±6.68 ^a	38.80±2.25 ^a	26.37±2.54 ^a	17.53±1.04 ^a	79.77

Each value is presented as mean ± SEM (n = 6); p^b< 0.01, p^a< 0.001 compared with the vehicle control group. ANOVA followed by Dunnett's test is done in SPSS version 15.0.

CNS depressant effect: All the extracts had shown significant (P^b<0.01, P^a<0.001) reduction of mice locomotion dose dependently. Among the five extracts, CHAS had shown maximum effect in the three experiments. After 120min of treatment, it had shown 88.51% and 83.97% inhibition of movement in open field, 81.48% and 83.20% inhibition in hole cross as well as 81.77% and 85.15% inhibition in head deep test at 100 and 200 mg/kg dose,

respectively. The order of CNS depressant effect of the extracts was CHAS> MHAS> PHAS> DHAS> AHAS (Tables 3, 4 and 5).

Discussion

Pain and inflammation are associated with the pathophysiology of various clinical conditions such as arthritis, cancer, vascular diseases, asthma, multiple sclerosis, colitis, inflammatory bowel

disease and atherosclerosis. Many natural products are used in traditional medical systems to relieve the symptoms from pain and inflammation (Marrassini *et al.*, 2010). Acetic acid-induced writhing and heat-induced hotplate tests are simple, reliable and well recommended protocols in evaluating medicinal agents for their analgesic property. Analgesics can act both on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptors site of pain, while centrally acting analgesics not only raise the threshold for pain, but also alter the physiological response to pain and suppress the patient's anxiety and apprehension (Chandann *et al.*, 2011). Abdominal writhing is associated with local peritoneal receptor. This behavior results from the activation of acid-sensitive ion channels (ASICs) and transient receptor potential vanilloid-1 (TRPV1) localized in afferent primary fibers (Luize *et al.*, 2012). Acetic acid causes inflammatory pain by inducing capillary permeability and liberating endogenous substances that excite pain nerve ending (Chandann *et al.*, 2011). Here the pain sensation is generated by producing localized inflammatory response due to release of free arachidonic acid from tissue phospholipids via cyclo-oxygenase (COX) and producing prostaglandin specifically PGE2 and PGF2 α . The level of lipoxygenase products may also increases in peritoneal fluids (Hiron *et al.*, 2010). More specifically acetic acid injection induces a release of TNF- α , interleukin-1 β (IL-1 β) and interleukin-8(IL-8) by resident peritoneal macrophages and mast cells, prostanoids and bradykinin (Luize *et al.*, 2012). These prostaglandin and lipoxygenase products are responsible for inflammation and pain. Substance(s) inhibiting the writhing response will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Pedro *et al.*, 2012) [20]. Non steroidal anti-inflammatory drugs (NSAIDs) can inhibit COX in peripheral tissues and therefore interfere with the mechanism of transduction of primary afferent nociceptors. The mechanism of analgesic activity of the extracts could be probably due to the blockade of endogenous

substances that excite pain nerve endings similar to that of NSAIDs. Thus, the statistically significant ($p^a < 0.001$) reduction of writhing indicates that the extracts might exert analgesic activity by inhibition of prostaglandin synthesis or by action on prostaglandin (Chandann *et al.*, 2012) (Table 1).

The hot plate method can evaluate centrally acting analgesic properties of a drugs or chemicals. Heat induced pain in this method is associated with the opioid receptors. So, the agent that gives positive effect in the hot plate test, acts through the opioid receptors and called centrally acting analgesics (Moniruzzaman *et al.*, 2014). In the present study, all the extracts were significantly ($P^b < 0.05$, $P^a < 0.001$) effective to prolong the latency period, and the effect was maximum at 90minute (Table 2). This finding suggests that the extracts have centrally acting analgesic effect.

Open field, hole cross and head deep tests (locomotor test) were applied to evaluate CNS-depressant potential of the *H. antidysenterica* extracts. The results of the study provided evidence that the extracts reduced locomotor activity confirming its CNS-depressant potential. Locomotor activity is considered as an index of alertness and a reduction of it is an indicative of sedative or CNS-depressant activity (Protapaditya *et al.*, 2011). Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (Kavita *et al.*, 2013), which is also involved in other physiological functions related to behavior and in various psychological and neurological disorders such as epilepsy, anxiety, depression, parkinson syndrome, and alzheimer's disease (Weinreb *et al.*, 2004). Diverse drugs such as anxiolytic, muscle relaxant and sedative-hypnotic exhibit their action via GABA. These drugs might modify the GABA system at the level of synthesis of it. Here they act by potentiating the GABA-mediated postsynaptic inhibition through an allosteric modification of GABA receptors, and by direct increase in chloride conductance or indirectly by potentiating GABA-induced chloride conductance with simultaneous depression of voltage activated

Ca⁺⁺ currents like barbiturates (Uma et al., 2011). Therefore, it is predictable that the extracts 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 (Kavita et al., 2013). It may also be due to enhanced affinity for GABA or an increase in the duration of the GABA-gated channel opening (Doha et al., 2013). In addition, the study on locomotor activity, as measured by hole cross, open field and head deep tests, showed that all doses of the extracts significantly ($p^b < 0.01$, $p^a < 0.001$) reduced the frequency and the amplitude of movements in dose dependant manner from the second observation (30 min) and continued up to the fifth observation (120 min) period (Table 3, Table 4 and Table 5). Since locomotor activity is a measure of excitability of the CNS. So decrease in spontaneous motor activity could be attributed to the depressant effect of the plant extracts with the presence of compound(s) having CNS-depressant potential (Kumaresan et al., 2011).

It is well established that alkaloids, flavonoids and tannins are potent analgesic compounds (Olubunmi et al., 2013). Flavanoids exert their effect through inhibition of prostaglandin synthetase (Islam et al., 2012). Various flavonoid derivatives including quercetin have been reported to inhibit the activity of arachidonic acid metabolizing enzymes (phospholipase A₂, cyclooxygenase and lipoxygenase). Various phytochemicals like flavonoids, saponins and tannins etc have CNS-depressant effect. Many flavonoids and neuroactive steroids are ligands for GABA_A receptors in the central nervous system which suggests that they can act as benzodiazepine-like agents (Protapaditya et al., 2013).

Literature review revealed the presence of polyphenols, flavonoids, tannins, alkaloids etc. bioactive compounds in the *H. antidysenterica* stem which are believed to be responsible for analgesic and CNS-depressant effects of the plant. Among the extracts, chloroform fraction has shown potent

analgesic and CNS- depressant effects. These effects might be due to presence of high portion of steroidal alkaloids (connessine) in it (Borris and Schaeffer, 1989).

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