



Research Journal of **Botany**

ISSN 1816-4919



Academic
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Investigations of Analgesic Activity of the Methanol Extract of *Haldina cordifolia* (Roxb.) Bark by using *in vivo* Animal Model Studies

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ABSTRACT

The present study was to investigate the analgesic activity of the methanol extract of *H. cordifolia* bark by using *in vivo* animal model studies. The effect was studied using acetic acid-induced abdominal constriction test and formalin induced hind paw licking test in mice model. The results of the study showed that the extract exhibited significant analgesic effect. In acetic acid-induced writhing test, significant percentage of inhibition (78.48%) shows that at the dose of 400 mg kg⁻¹ and at the same dose in formalin induced hand paw licking model shows significant activity in early phase (22.03% of inhibition) where in late phase (19.88% of inhibition) comparable to standard drug diclofenac sodium. These findings suggested that *H. cordifolia* bark has got the potential as a candidate for future analgesic agent.

Key words: *Haldina cordifolia*, analgesic activity, animal model, hind paw licking, writhing

INTRODUCTION

Pain is still one of the main health problems among the world's populations (Alemy *et al.*, 2012). It is an unpleasant sensation that is a consequence of complex neurochemical processes in the central and peripheral nervous systems. There are two major classes of drugs such as Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are used in management of mild to moderate pains and opioid analgesics are used in severe pains. These drugs have serious limitations due to their side effects. Opioid has several side effects such as respiratory depression, euphoria, tolerance and dependence; on the other hand Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) produce gastrointestinal irritation and renal damage. Therefore, it is a need to intensify research with the aim of developing efficacious agents with low toxicity profile (Musa *et al.*, 2009). Medicinal plants are an important therapeutic aid for various ailments (Fakruddin *et al.*, 2012). The World Health Organization (WHO) estimates that 80% of the population of some Asian and African countries presently uses herbal medicines for some aspect of primary health care because of better cultural acceptability, affordability, better compatibility with the human body and fewer side effects (Dash *et al.*, 2014; Parekh *et al.*, 2005). It has been also reported that developing countries people used medicinal plants for the management of pain and inflammatory conditions (Musa *et al.*, 2009). World Health Organization also encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of the great potential they hold in combating various diseases. It is estimated that about 30% of the pharmaceuticals are prepared

from plants derivatives (Rahman *et al.*, 2015) and many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis and quinine (Elumalai and Eswariah, 2012; Dash *et al.*, 2014).

It was realized from the various reports that *Haldina cordifolia* (Roxb.), Syn. *Adina cordifolia* (Roxb.) belongs to the family Rubiaceae, have displayed plethoras of potential biological activities (Dash *et al.*, 2014; Iqbal *et al.*, 2009; Sharma *et al.*, 2012). Geographically, it is found throughout central and South India to Srilanka. It has several biological activities such as anti-inflammatory (Kaushik *et al.*, 2009), anticancer (Sangameswaran and Saluja, 2012), antiulcer (Kasinadhuni *et al.*, 1999), hepatoprotective (Agarwal *et al.*, 2006), antifertility (Sabir and Razdan, 1970), antidiabetic (Chaudhary *et al.*, 2012), antiamebic (Iqbal *et al.*, 2009), antinociceptive (Jain *et al.*, 2007) etc. from its various parts. Traditionally, this plant has also used in curing various ailments such as rheumatism, stomachache, headache, cold/cough, toothache, fever, pain and swelling, bacterial infection, urinary problems, conjunctivitis, miscarriage etc (Dash *et al.*, 2014).

In this study, attempted to investigate the analgesic activity of the methanol extract of *H. cordifolia* bark by using *in vivo* animal model studies.

MATERIALS AND METHODS

Plant materials: The bark of *H. cordifolia* were collected from Vatiari area of Chittagong district and it is authenticated by Dr. Shaikh Bokhtear Uddin, Taxonomist and Associate Professor, Department of Botany, University of Chittagong, Chittagong, 4331, Bangladesh.

Extraction of plant materials: The fresh barks of *H. cordifolia* were cut, washed and air dried at room temperature ($24 \pm 2^\circ\text{C}$) for about 10 days. Dried leaves were macerated into coarse powder. Dried powder 250 g was then emerged using methanol. Then, methanol extract was shaken by rotary shaking apparatus for 7 days. The extract was collected using Buckner funnel. The Methanol was evaporated at a temperature below 45°C and concentrated extract was weighed 25 g stored at 4°C temperature.

Animals: Swiss albino mice weighing 25-30 g of both sexes were collected from the renowned animal laboratory of Jahangirnagar University (JU), Savar, Bangladesh. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature $23.0 \pm 2.0^\circ\text{C}$ and 12 h light: dark cycle) and acclimatized for 7 days. The animals were fed with standard diet and water. The study protocol was approved by the pharmacy and drug committee (institutional ethics committee), Department of Pharmacy, International Islamic University Chittagong, Bangladesh.

Acetic-acid writhing test: This test was based on the method described by Bukhari (2013) with slight modification where 1% tween-80 and 0.6% acetic acid used instead of normal saline and 0.7% acetic acid. Swiss albino mice of either sex were selected and divided into four groups and each group contains six animals. Before the 30 min of intraperitoneal injection of 0.6% acetic acid (10 mL kg^{-1}), the doses of extract i.e., 200 and 400 mg kg^{-1} administered orally, vehicle (1% tween 80 in water, p.o) and diclofenac sodium (10 mg kg^{-1} , i.p) were administered to the respective group. Immediately after administering acetic acid, mice were observed and the number of writhing or stretches was counted for 15 min. Reduction in the number of writhes compared to the control groups was considered as evidence of analgesic effect. The percent inhibition (% analgesic activity) was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

where, A represent average number of writhing of the control group and B represent average number of writhing of the test groups.

Formalin hind paw licking test: This test was based on the method described by Bukhari (2013) with slight modification where 1% tween and 2.5% formalin used instead of normal saline and 1% formalin. A 20 μL of 2.5% formalin was injected subcutaneously into the right hind paw of mice. The time (sec) spent in licking the paw and the biting responses of the injected paw were taken as an indicator of pain response. The mice were observed for 30 min after the injection of formalin and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. Extract (200 and 400 mg kg^{-1} , orally), diclofenac sodium (10 mg kg^{-1} , i.p) and 1% Tween-80 (0.5 mL mice $^{-1}$, p.o) were administered 30 min prior to formalin injection. The percentage inhibition of licking was calculated by the equation:

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

where, A represents the vehicle treated control group value for each phase and B represents the treated groups value for each phase.

Statistical analysis: Experimental values are expressed as Mean $\pm$ SEM. Independent sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p-value<0.05 in all cases.

RESULTS AND DISCUSSION

The results of the study showed that the extract exhibited significant analgesic effect. In acetic acid-induced writhing test, significant percentage of inhibition (78.48%) shows that at the dose of 400 mg kg^{-1} (Table 1) and at the same dose in formalin induced hind paw licking model shows significant activity in early phase (22.03% inhibition) where in late phase (19.88% inhibition) comparable to standard drug diclofenac sodium (Table 2). In all cases, significant p-value was noted less than 0.05.

Table 1: Analgesic activity of methanol extract of bark of *Holdina cordifolia* by using acetic acid induced writhing method in mice

Groups	Treatments	Dose (route)	No. of writhing	Inhibition (%)
Control	1% tween 80 in water	0.5 mL mice $^{-1}$ (p.o)	79.0 $\pm$ 1.63	-
Standard	Diclofenac sodium	10 mg kg^{-1} (i.p)	18.0 $\pm$ 0.82*	77.21
Test 1	MEHC	400 mg kg^{-1} (p.o)	17.0 $\pm$ 3.69*	78.48
Test 2	MEHC	200 mg kg^{-1} (p.o)	40.5 $\pm$ 10.89*	48.73

All values are expressed as Mean $\pm$ SEM (n = 6), *p<0.05, significant compared to control

Table 2: Analgesic activity of methanol extract of bark of *Holdina cordifolia* by using formalin induced hind paw licking test in mice

Groups	Treatments	Dose (route)	Early phase (sec)	Inhibition (%)	Late phase (sec)	Inhibition (%)
Control	1% tween 80 in water	0.5 mL mice $^{-1}$ (p.o)	57.31 $\pm$ 1.06	-	41.74 $\pm$ 0.60	-
Standard	Diclofenac sodium	10 mg kg^{-1} (i.p)	14.95 $\pm$ 0.60*	73.91	13.26 $\pm$ 0.94*	68.23
Test 1	MEHC	400 mg kg^{-1} (p.o)	44.65 $\pm$ 1.51*	22.03	33.44 $\pm$ 1.16*	19.88
Test 2	MEHC	200 mg kg^{-1} (p.o)	54.11 $\pm$ 0.67*	5.58	33.44 $\pm$ 1.16*	8.19

All values are expressed as Mean $\pm$ SEM (n = 6), *p<0.05, significant compared to control

The results obtained from the present study illustrate the analgesic activity of methanol extract of *H. cordifolia* bark. Acetic acid-induced abdominal constriction test is used for the evaluation of peripheral analgesic activity. The abdominal constriction response is thought to involve in part local peritoneal receptors (Musa *et al.*, 2009). Acetic acid indirectly releases endogenous mediators such as prostaglandin, kinin, histamine, etc which, stimulate neurons that are sensitive to other drugs such as narcotic and other centrally acting drugs (Bukhari, 2013; Kumbhare and Sivakumar, 2011). It is also believed that acetic acid increases the levels of prostaglandins E_2 and F_2 in peritoneal fluid as well as lipooxygenases (Musa *et al.*, 2009; Al-Sobarry *et al.*, 2011), so the mechanism of activity of the extract may be linked to cyclooxygenases and/or lipooxygenases. The extract may be possessed peripheral mediated analgesic activity and may also be interfered with these peritoneal receptors to bring about analgesia.

Formalin induced hind paw licking pain model is widely known method to elucidate the mechanism of pain and analgesia (Tjolsen *et al.*, 1992). Formalin induced pain involves two distinct phases, the first phase (neurogenic phase) in which pain is produced due to direct stimulation of the sensory nerve fiber by formalin and the second or late phase (inflammatory phase) in which the pain occurs due to release of inflammatory mediators such as histamine, serotonin, prostaglandin and bradykinin (Hunskar and Hole, 1987; Murray *et al.*, 1988). It is well established that centrally acting drugs such as narcotics inhibit both phases equally while peripherally acting drugs such as diclofenac inhibit the late phase (Shibata *et al.*, 1989; Santos *et al.*, 1994). In the present study, methanol extract of *H. cordifolia* bark produced marked analgesia in both phases of the test similar to standard drug diclofenac sodium, suggesting that central mechanism is involved in analgesic effect this extract. Study revealed that the presence of several active chemical constituents in plant such as flavonoids, terpenoids, saponin and tannins mainly responsible for analgesic activity (Kumbhare and Sivakumar, 2011; Yaqeen *et al.*, 2013; Ozturk *et al.*, 2002; Dongmo *et al.*, 2006). It is also well known that flavonoids may interact directly with the prostaglandin system and reduced availability of prostaglandins mediated effects of pain (Ahmad *et al.*, 2011; Zaman *et al.*, 2015). Saponin and terpenoid have also been reported to inhibit histamine release *in vitro* (Rao and Gurfinkel, 2000; Doshi *et al.*, 2014). The greatest possibility is presence of such kinds of bioactive compounds in this extract responsible for analgesic activity. Further investigation is needed to isolate the active compound in this extract are responsible for analgesic activity and it will be discovered through more quantitative and qualitative laboratory investigation.

In conclusion, *H. cordifolia* bark has got the potential as a candidate for future analgesic agent. In near future, it may be used as herbal or allopathic medicines to management of problems that cause chronic pain like headache, low back strain, cancer, arthritis and nerve damages etc.

ACKNOWLEDGMENTS

The authors greatly acknowledge to the respective authority of Department of Pharmacy, International Islamic University Chittagong for valuable laboratory facilities during the period of laboratory work. Authors also acknowledge to all the faculty members of Department of Pharmacy, International Islamic University Chittagong (IIUC).

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