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Research Article

ANALGESIC EFFECT OF ETHANOL EXTRACT OF *HEDYOTIS CORYMBOSA* L. WHOLE PLANT

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ABSTRACT

This study was aimed to evaluate the possible analgesic properties of the ethanol extract of *Hedyotis corymbosa* L. (Rubiaceae) a locally available plant used in traditional medicine for the management of pain and other conditions. The dried powder of the whole plant was extracted with 95 % ethanol and was subjected first to various chemical tests to ascertain the main constituents contained in the extract. The result revealed the presence of alkaloids, flavonoids, tannins, glycosides in significant amounts. Then the extract was investigated for analgesic effect by using hot plate test, acetic acid induced writhing test and formalin test. The results showed that the ethanol extract of the plant at two different doses (250 and 500 mg/kg body weight) showed significant ($p<0.05$), dose-dependent analgesic effect which is mediated by both central and peripheral mechanism. The analgesic activity was compared with a standard drug, ketorolac used at a dose of 10 mg/kg body weight.

Keywords: *Hedyotis corymbosa*, Analgesic, Pain, Ethanol extract.

INTRODUCTION

Pain is an unpleasant sensation no doubt, but it is a protective mechanism for the body that occurs whenever tissues are actually or potentially damaged and it causes the individual to react to remove the pain stimulus[1]. With many pathological conditions, tissue injury is the immediate cause of the pain, and this result in the local release of a variety of chemical agents, which are assumed to act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulation[2]. Pharmacologic management of pain requires the use of analgesic drugs. These drugs, though effective, can cause serious side effects. The search for potent analgesic agents with minimal side effects remains the goal of many scientific studies[3]. Medicines from indigenous plants form the basis of primary health care for a majority of people living in urban and rural or remote areas of the third world countries. The reason for this dependence is the perceived low cost, easy access and ancestral experience as well as the belief that these medicines are devoid of adverse effects[4]. *Hedyotis corymbosa* L. (syn: *Oldenlandia corymbosa* L., Family: Rubiaceae) is a flaccid, usually diffuse (sometimes erect) herb found in tropical regions of America, Africa, Asia and in the islands of the Western Pacific[5]. The plant is distributed all over Bangladesh in fallow lands. The plant has some folkloric uses as febrifuge, stomachic and anthelmintic. It is also used in gastric irritation, jaundice and liver complaints. Besides, leaf extract is used by Chakma in Chittagong Hill Tracts of Bangladesh to alleviate abdominal pain[6]. The biological studies performed so far on this plant reveal that the methanolic extract possesses the antioxidant, anti-inflammatory, cytotoxic, hepatoprotective and antibacterial activities[7]. The decoction of whole plant is used in traditional Thai medicine for antipyretic purpose to decrease body temperature[8]. The plant is used in traditional medicine of India and China to treat various hepatic disorders[9]. Chemical investigations on this plant have been shown to possess alkaloids, terpenoids, oleanolic acid and steroidal saponins[10]. Of these, oleanolic acid is the predominant compound in the whole plant. Oleanolic acid is a component of several botanical drugs and has been examined for its antitumor activity[11]. In the hepatoprotective study, plant extracts are reported to significantly reduce the acute elevation of serum glutamate oxalate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) concentration, and alleviates the degree of liver damage 24 hours after the intra peritoneal administration of hepatotoxins[12]. Although numerous studies have shown the medicinal values of this plant but its analgesic property is not yet reported. So far, this is the first attempt to investigate the analgesic activity of *Hedyotis corymbosa* and we report the results of our study using three established animal models in order to establish the scientific basis of the traditional uses of this plant in painful condition.

MATERIALS AND METHODS

Collection of the Plant Material

Hedyotis corymbosa was collected from Nagorpur, Tangail, Bangladesh in August, 2011 when the plant is fully flowered. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka. The accession no is DACB 32574. The plant is very well known and widely distributed in the north-western parts in Bangladesh.

Extraction of the plant

The whole plant was first sun dried for a week, crushed by hand and dried again. Then the crushed parts of the plant were ground into coarse powder with the help of a mechanical grinder. The whole powder (About 500 g) was extracted by cold extraction with 95 % ethanol and kept for a period of 3 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material followed by a second filtration through whatman filter paper. The filtrate (ethanol extract) obtained was evaporated by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and at 68ºc temperature. It rendered a gummy concentrate of

green black color. The gummy concentrate was designated as crude extract. Then the crude extract was dried by freeze drier and preserved at 4ºC[13].

Preliminary Phytochemical Screening

The freshly prepared crude extract was qualitatively tested for the identification of chemical constituents, such as, alkaloids, flavonoids, steroids, glycosides, saponins, terpenoids, gums and tannins. The tests were carried out by the method described by Harborne and Sazada *et al*[14,15]. In each test 10 % (w/v) solution of the extract was taken unless otherwise mentioned in individual test. Phytochemical screening of the extract was performed using the following reagents and chemicals: alkaloids with Dragendorff's reagent, flavonoids with the use of concentrated hydrochloric acid, tannins with 5 % ferric chloride, saponins with ability to produce suds, gum with Molisch reagents and concentrated sulfuric acid, reducing sugars with Fehling's solution and terpenoids with chloroform and concentrated sulfuric acid.

Assessment of Analgesic Effect

Animals

The experimental animals, cross breed Swiss -albino mice, of either sex (weighing 25-35 g) were purchased from the Animal Research Branch of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). The mice were acclimatized for a month in temperature controlled animal house in the Department of Pharmacy, North South University with a 12 h light/dark cycle and fed with standard laboratory food and tap water. The mice had no access to food during the whole day of experiment. The influence of circadian rhythms was avoided by starting all experiments at 8.30 a.m.

Hot Plate Test

The hot plate test method was employed to assess the analgesic activity in accordance with the method described previously with minor modification[16,17]. The experimental animals were divided into control, positive control and test groups with six mice in each group. The animals of test groups received test samples at the doses of 250 and 500 mg/kg body weight, positive control group was administered ketorolac at the dose of 10 mg/kg body weight and vehicle control group was treated with 1 % Tween 80 solution in distilled water at the dose of 10 ml/kg body weight orally. In this test, the animals were positioned on Eddy's hot plate kept at a temperature of 55 ± 0.5ºC. The test samples and the standard drug were administered 30 minutes before the beginning of the experiment. Mice were observed before and at 30, 60, 120, 180 and 240 minutes after administration. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60, 120,180 and 240 minutes after oral administration of the samples. A cut-off period of 20 seconds was observed to avoid the damage of the paw. The antinociceptive response latency was recorded from the time between placement and licking of fore or hind paws or jumping movements of the animals.

Acetic Acid Induced Writhing Test

To evaluate the analgesic effects of the plant extract, the method described by Dharmasiri JR *et al*, was used with slight modifications[18]. There were four groups for these tests each group containing six mice. Group-I was received normal saline orally as control (10 ml/Kg), Group-II was fed ketorolac (10 mg/kg), similarly Group-III and IV was given plant extract (250 and 500 mg/Kg). After 30 minutes, 0.6 % acetic acid solution was injected to all the animals in the four groups intraperitoneally. Writhing number was accounted between 5 to 15 minutes after the injection of acetic acid. To observe analgesic effect, rebate of writhes was counted in comparison of control group. The percentage inhibition of writhing was calculated using the following formula:

$$\text{Percentage Inhibition} = (1 - W_t / W_c) \times 100$$

Where, W_c and W_t represent the average number of writhing produced by the control and the test group, respectively.

Formalin Test

The formalin test was carried out as described by Hunskaar S and co- workers[19]. Four groups of mice were treated orally with the ethanol extract of *Hedyotis corymbosa* (250 and 500 mg/kg), Ketorolac (10 mg/kg) and normal saline (10 ml/kg). Formalin solution (0.5 % v/v) was injected subcutaneously into the right hind paw of mice, 30 minutes after administration of the extract, ketorolac and vehicle. The time (in seconds) spent in licking, biting and scratching responses of injected paw was considered as an indicator of pain response. Responses of the first 5 minutes were considered as early phase (neurogenic phase) and the period of 15-30 minutes as the late phase (inflammatory phase). The percentage inhibition was calculated by the following formula:

$$\text{Percent Inhibition} = (1 - T_t / T_c) \times 100$$

Where, T_c and T_t represent the average time (in seconds) spent for licking, biting and scratching by the control and the test group, respectively.

Statistical Method

SPSS software for windows version 17 was used to analyze the experimental data. Data are expressed as Mean ± SD. We used one way ANOVA to perform the analysis of the results following Dunnet's test where p values of 0.05 or less were thought statistically significant.

RESULTS

Phytochemical Studies

Preliminary phytochemical screening of the extract of *Hedyotis corymbosa* revealed the presence of various bioactive components of which flavonoids, alkaloids, terpenoids, tannins, gums and carbohydrates were the most prominent and the result of phytochemical test has been summarized in the Table 1.

Hot-plate Reaction Time

Results of hot plate test are presented in Table 2. The EtOH extract in both doses (250 and 500 mg/kg body weight) was found to exhibit a significant prolongation of latency time. The effect begun early at 30 minutes after administration of the extract and persist until the following fourth hour that was comparable to the standard drug ketorolac. The results were found to be statistically significant ($p < 0.001$).

Acetic Acid Induced Writhing Response of Mice

In the acetic acid-induced writhing assay the extract induced a significant decrease in the number of writhes and produced 31.33 % ($P < 0.05$) and 57.83 % ($P < 0.001$) writhing inhibition at the doses of 250 and 500 mg/kg body weight respectively, which was comparable to the standard drug ketorolac where the inhibition was 55.42 % at the dose of 10 mg/kg body weight (Table 3). The result at the dose 500 mg/kg was more significant than 250 mg/kg body weight.

Table 1: Qualitative analysis of the phytochemicals of the ethanol extract of *Hedyotis corymbosa*

Chemical constituent	Observation
Tannins	+
Saponins	+
Flavonoids	+
Gums and Carbohydrates	-
Alkaloids	+
Reducing Sugars	-
Terpenoids	+

Symbol (+) indicates presence and (-) indicates the absence of phytochemicals

Table 2: Effect of ethanol extract of *Hedyotis corymbosa* on hot-plate test in mice

Treatment group with dose	Latency time (s)[a]					
	0 minute	30 minutes	60 minutes	120 minutes	180 minutes	240 minutes
Control (vehicle, 10 ml/kg; p.o.)	10.7 ± 1.89	9.66 ± 2.09	8.00 ± 1.82	6.58 ± 1.43	5.52 ± 1.22	5.00 ± 0.98
Ketorolac (10 mg/kg; p.o.)	9.14 ± 1.17	11.02 ± 2.23	12.60 ± 2.11***	14.16 ± 2.40***	17.96 ± 1.51***	12.48 ± 1.56***
EtOH extract (250 mg/kg; p.o.)	8.46 ± 0.51	10.12 ± 0.36	11.38 ± 0.45**	12.62 ± 0.32***	14.44 ± 0.57***	10.82 ± 0.97***
EtOH extract (500 mg/kg; p.o.)	9.56 ± 0.39	10.66 ± 1.69	14.32 ± 1.07***	15.76 ± 1.16***	17.44 ± 0.92***	13.90 ± 0.90***

[a]Values are expressed as mean ± SEM; (Number of animals, n = 6); vehicle = 1 % tween-80 in distilled water p.o. = per oral; One way Analysis of Variance (ANOVA) followed by Dunnett's test was performed as the test of significance. The minimum value of $p < 0.05$ was considered significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ vs. control

Table 3: Effect of EtOH extract of *Hedyotis corymbosa* on acetic acid-induced writhing in mice

Treatment group with dose	No. of writhings[a]	Inhibition (%)
Control (vehicle; 10 ml/kg; p.o.)	16.60 ± 1.16	
Ketorolac (10 mg/kg; p.o.)	7.40 ± 0.92***	55.42
EtOHextract (250 mg/kg; p.o.)	11.40 ± 1.07*	31.33
EtOH extract (500 mg/kg; p.o.)	7.00 ± 1.09***	57.83

[a] Values are expressed as mean ± SEM (Number of animals, n = 6); vehicle = 1 % Tween-80 solution in distilled

Table 4: Effect of the EtOH extract of *Hedyotis corymbosa* on formalin-induced pain in mice

Treatment group with dose	Total time spent in licking (s)[a]			
	0-5 min (Early phase)	Inhibition (%)	15-30 min (Late phase)	Inhibition (%)
Control (vehicle; 10 ml/kg; p.o.)	30.6 ± 5.30		20.2 ± 4.86	
Ketorolac (10 mg/kg; p.o.)	9.80 ± 0.58***	67.1	5.00 ± 0.70**	75
EtOH extract (250 mg/kg; p.o.)	11.2 ± 1.35***	63.1	8.20 ± 1.98*	59
EtOH extract (500 mg/kg; p.o.)	9.80 ± 1.15***	67.0	5.80 ± 1.59**	71

[a] Values are expressed as mean ± SEM (Number of animals, n = 6); vehicle = 1 % Tween-80 solution in distilled water p.o. = per oral; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control

Formalin-Induced Pain

In this model, the extract demonstrated significant and dose-dependent inhibition in both early (63.1 % and 67 %) and late (59 % and 71 %) phases of the formalin induced pain as manifested by the licking responses at the doses of 250 and 500 mg/kg respectively (Table 4). In contrast, the treatment of animals with ketorolac showed significant inhibition of the late phase (75 %) but not in the early phase.

DISCUSSION

The antinociceptive activity of ethanol extract of *Hedyotis corymbosa* was tested by using three models (hot plate, acetic acid-induced and formalin) so that both the centrally and peripherally mediated effects could be investigated. The acetic acid-induced pain involves the peripheral mechanism whereas the hot plate test involves the central mechanism. The formalin test is believed to show the involvement of both peripheral and central mechanisms[20]. The hot plate test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the well-validated models used for studying central antinociceptive activity[21,22]. The extracts of the plants and ketorolac (10 mg/kg) also presented a longer latency time than the control group in the hot plate test in a dose related manner. Nociceptive pain inhibition was noticed higher at 180 minutes after administration of the extracts and the response was increased from 8.4 seconds to 17.4 seconds that was comparable to standard drug ketorolac where the response time was 17.9 seconds at 180 minutes of study. As the hot plate method is considered to be selective for the drugs acting centrally analgesics, the effect of the extracts on this pain model indicates that it must have a centrally acting antinociceptive activity. The acetic acid induced writhing test is normally used to evaluate the peripheral analgesic effect of drugs and chemicals. The possible mechanism is thought to be mediated by inhibition of lipooxygenase and/or cyclooxygenase in peripheral tissues induced by acetic acid, thereby reducing PGE2 synthesis and interfering with the mechanism of transduction in primary afferent nociceptor[23,24]. The EtOH extract significantly inhibited acetic acid-induced writhing in mice at both doses (250 and 500 mg/kg). But more significant response was found with 500 mg/kg dose level and the response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway[23,24]. Therefore, it may be inferred that the analgesic effect of the extract could be due to the inhibition of prostaglandin pathway which is peripherally mediated. The current studies showed that the early phase of formalin induced pain which starts immediately after injection seems to be caused predominantly by activation of C-fibers, which is responsible for the sensation of a sharp first pain and a combined process generated by peripheral inflammatory tissue. Then, there is a period (about 10 minutes) of reduced nociceptive activity. The late phase of moderate pain, which starts about 20 minutes after formalin injection, appears to be caused by tissue and functional changes in the dorsal horn of the spinal

cord[25,26]. The drugs that show their activity through central mechanism, such as narcotics, inhibited both phases almost equally, while peripherally acting drugs only inhibited the second phase. Administration of the extract demonstrated significant inhibition in both phases indicate that the extract contain active analgesic principles acting both centrally and peripherally. Preliminary qualitative phytochemical screening exhibits the presence of alkaloids, flavonoids, saponins and terpenoids in *Hedyotis corymbosa*. So, the observed analgesic activity may be attributed to these compounds. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins[27,28]. There are also reports on the role of tannins in anti-nociceptive activity[29]; besides alkaloids are well known for their ability to inhibit pain perception[30]. Flavonoids and other phenolics compounds of plant origin have been reported as antioxidants and as scavengers of free radicals which can also exert anti-inflammatory effects[31]. Therefore the results of phytochemical analysis strongly support the observed antinociceptive activity of the extract.

CONCLUSION

The results obtained in this study indicate that the ethanol extract of *Hedyotis corymbosa* possesses considerable antinociceptive activity at the investigated doses on the experimental laboratory animal and the activity is mediated via central and peripheral mechanisms. This could form the basis for its traditional uses in the management of pain and suggests further investigation and isolation of biologically active constituents responsible for its activity.

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