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EVALUATION OF ANALGESIC AND NEUROPHARMACOLOGICAL ACTIVITIES OF METHANOLIC RHIZOME EXTRACT OF *HEDYCHIUM CORONARIUM*

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ABSTRACT

This study was aimed to investigate analgesic and neuropharmacological actions of the methanolic extract of rhizomes of *Hedychium coronarium*. The analgesic activity was evaluated for its central and peripheral pharmacological actions using tail immersion method and acetic acid-induced writhing test in mice respectively. The extract, at the doses of 100, 200 and 400 mg/kg body weight, produced a significant increase in pain threshold in tail immersion methods in a dose dependent manner. In acetic acid-induced writhing test, the extract at 400 mg/kg dose showed a maximum of 73.12% writhing inhibition (p <0.001) compared to the control and this activity was comparable to 75.78% inhibition of writhing by standard drug Diclofenac-Na (25 mg/kg). The extract was also investigated for its neuropharmacological action using hole-cross and open field test in mice. The extract displayed dose dependent suppression of motor activity and exploratory behavior in mice in the tested models. The results of the study indicate that the plant possesses strong analgesic and central nervous system depressant activity.

INTRODUCTION: Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain acts as a warning signal against disturbances of the body and has a proactive function. However, it is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause [1]. Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect, new compounds with improved pain management capacity and fewer side effects are being searched every nook and corner of the world.

Hedychium coronarium Koen (Bengali name: Dolon Champa) is an erect herb belonging to the family Zingiberaceae. The plant is widely available in tropical and subtropical regions, such as Japan, India, Brazil, South China, Southeast Asian countries including Bangladesh. The rhizome of the plant is used in the treatment of diabetes [2]. It is also used as antirheumatic, excitant, febrifuge and tonic [3]. Previous phytochemical investigations showed that the plant contains the diterpenes- coronarin- A, coronarin- B, coronarin- C, coronarin- D and isocoronarin- D [4]. Though the plant is traditionally used in many parts of Bangladesh, no scientific report is available to validate the folkloric use. Again, Plants have been a promising source of drug molecules for ages. Bangladesh is blessed with rich floristic resources. Still the untapped wealth of plant kingdom is a major target for the search of new lead compounds in drug discovery. In Bangladesh, huge number of plants still remains unexplored. So well designed, systematic and objective research in this area might benefit our people who have been deluged with superfluity of disease, and who lack technological and economic resources.

In present study, we investigated the analgesic and central nervous system depressant activities of methanolic extracts of *H. coronarium.*

MATERIALS AND METHODS:

Chemicals and drugs: Diclofenac-Na and Diazepam injection were purchased from local market manufactured by Square Pharmaceuticals Ltd., Bangladesh.

Plant material: The rhizome of *Hedychium coronarium* was collected from the local area of Mirpur, Dhaka, during the first week of January, 2010 and was identified (Accession No. 34,484) by Bangladesh National Herbarium, Mirpur, Dhaka. Collected plants, after cutting into small pieces, were dried and pulverized into a coarse powder and stored into an air-tight container

Extraction and sample preparation: The pulverized coarse powder of the rhizome of *Hedychium coronarium* (150 gm) was extracted with methanol by successive cold extraction. The extracts obtained, were filtered off and evaporated to dryness in an oven at low temperature. The extracts rendered concentrate of reddish color.

Animal: For the experiment both male and female Swiss albino mice, 3-4 weeks of age, weighing between 20-25 gm, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temperature: (24.0±1.0^{o}C), relative humidity: 55-65% and 12 hrs light/12 hrs dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experimentation.

Analgesic activity:

Tail immersion test: The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice. The animals were treated as discussed above 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The

reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 12s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined at 0, 30, 60 and 90 min after the administration of drugs [5]. Percentage of elongation was calculated using the following formula

$$\textbf{Elongation (\%)} = \frac{\text{Latency (Test) - Latency (Control) x 100}}{\text{Latency (Test)}}$$

Acetic Acid-Induced Writhing Test: The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and Control were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac- Na was intra- peritoneally administered 15 min before injection of acetic acid and Diclofenac- Na (25mg/kg) was used as standard drug. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min [6-7]. Percentage inhibition of writhing was calculated using the following formula

$$\textbf{Writhing inhibition (\%)} = \frac{\text{Mean No. of writhing (control) - Mean No. of writhing (test) x 100}}{\text{Mean No. of writhing (control)}}$$

Neuropharmacological activity:

Hole cross test: A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage [8]. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after the oral treatment with *H. coronarium* methanolic extracts at the doses of 100, 200 and 400 mg/kg body weight (**table 3**). Percentage inhibition of movements was calculated using the following formula

$$\textbf{Movements inhibition (\%)} = \frac{\text{Mean no. of movements (control)-Mean no. of movements (test)x100}}{\text{Mean No. of movements (control)}}$$

Open field test: The animals were divided into control, standard, and test groups containing five mice each. The test group received *Hedychium coronarium* extract at the doses of 100, 200 and 400 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water) and standard group received Diazepam (3mg/kg body weight).The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90, and 120min after oral administration of the test drugs [9].

Percentage inhibition of movements was calculated using the following formula;

$$\textbf{Movements inhibition (\%)} = \frac{\text{Mean no. of movements (control)-Mean no. of movements (test)X100}}{\text{Mean No. of movements (control)}}$$

Statistical Analysis: Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the vehicle control group. p values < 0.05, 0.001 were considered to be statistically significant.

RESULTS: The analgesic activity of the methanolic extract of *Hedychium coronarium* was evaluated in tail immersion and acetic acid-induced writhing methods. The tail withdrawal reflex time following administration of the extract of *Hedychium coronarium* was found to increase with increasing dose of the sample. In this test maximum effect was observed after 60 and 90 min of drug administration.

The result was statistically significant (p<0.05-0.001) and was comparable to the control. The result was statistically significant (p<0.05-0.001) and was comparable to the control **(Table 1).** The doses of the extract significantly (p < 0.001) inhibited writhing response induced by acetic acid in a dose dependent manner as compared to control. At 100 mg/kg body weight the extract showed 26.15% inhibition, at 200 mg/kg body weight the extract showed 47.94% inhibition and at 400 mg/kg body weight showed 73.12% inhibition of writhing compared to the standard drug Diclofenac-Na which showed 75.78% inhibition of writhing at 25 mg/kg body weight dose (**Table 2**).

The extract at doses level of 100mg/kg, 200mg/kg and 400mg/kg body weight showed significant (p < 0.001) decrease of movement from its initial value during the period of hole cross experiment as compared to control (**Table 3**). The maximum decrease in movement was observed at 90 and 120 min after drug administration. In the open field test at dose level of 100mg/kg, 200mg/kg and 400mg/kg body weight the number of squares traveled by the mice was suppressed significantly from its initial score by both doses of the extract which is comparable to the standard drug Diazepam (**Table 4**). The maximum suppression was exhibited at 90 and 120 min after drug administration.

TABLE 1: EFFECTS OF THE METHANOLIC EXTRACT OF *H. CORONARIUM* ON TAIL WITHDRAWAL REFLEX OF MICE INDUCED BY TAIL IMMERSION METHOD

Groups	Dose (mg/kg)	Mean reaction time (s) before and after drug administration (% of tail flick elongation)			
		0 min	30 min	60 min	90 min
Control		1.73±0.125	1.60±0.125 (-)	1.47±0.17 (-)	1.33±0.105 (-)
Standard	25	2.53±0.29	5.33±0.235** (69.98%)	7.39±0.07** (80.10%)	8.8±0.17** (84.88%)
Group-I	100	1.82±0.02	4.45±0.385** (64.04%)	6.09±0.405** (75.86) %	7.06±0.50** (81.16%)
Group-II	200	1.86±0.035	5.53±0.335** (71.06%)	6.28±0.495** (76.59%)	7.44±0.305** (82.12%)
Group-III	400	1.82±0.005	6.50±0.24** (75.68%)	7.38±0.325** (80.08%)	9.88±0.495** (86.53%)

Control: animals received (1% Tween 80 in water), Standard group received Diclofenac-Na (25mg/Kg body weight i.p.), Group-I, Group-II and Group III were treated with 100, 200 and 400 mg/kg body weight of extract per oral. Values are mean ±SEM, (n = 5); ** $p < 0.001$, Dunnett's test as compared to control

TABLE 2: EFFECT OF THE *HEDYCHIUM CORONARIUM* METHANOLIC EXTRACT ON ACETIC ACID INDUCED WRITHING IN MICE

Group	Treatment and Dose	Writhings (Mean ± SEM)	% of writhing	% of writhing inhibition
Control	0.7% acetic acid (10 ml/kg, i.p.)	41.3±1.32	100.00	0
Standard	Diclofenac sodium (25mg/kg i.p.)	10.0±0.42**	24.21	75.78
Group-I	Extract (100mg/kg per oral)	30.5±1.035**	73.85	26.15
Group-II	Extract (200mg/kg per oral)	21.5±0.995**	52.06	47.94
Group-III	Extract (400mg/kg per oral)	11.1±2.88**	26.87	73.12

Diclofenac sodium was administered 15 min before 0.7% acetic acid administration. Writhing was counted for 15 min, starting after 5 min of acetic acid administration; **P<0.001 vs. control, values are mean ±SEM; (n=5)

TABLE 3: EFFECT OF *H. CORONARIUM* METHANOL EXTRACT ON HOLE CROSS TEST IN MICE

Group	Dose (mg/kg)	Number of movements (% of Number of movements inhibition)				
		0 min	30 min	60 min	90 min	120 min
Control		22.4±1.63	11.8±0.66 (-)	11.4±0.74 (-)	7.8±0.86 (-)	10.2±0.37 (-)
Standard	3	15.2±1.11	6.6±1.66* (44.07%)	4.0±1.09** (64.91%)	2.4±1.25* (69.23%)	1.6±0.87** (84.31%)
Group-I	100	14±2.74	9.4±1.63 (20.3%)	7.6±2.065 (33.33%)	4.6±1.53 (41.03)%	1.4±0.51** (86.24%)
Group-II	200	15±0.83	6.6±0.60* (44.07%)	5.2±0.20* (54.38%)	3.2±0.91* (58.97%)	2.0±0.54** (80.39%)
Group-III	400	14.2±4.3	7.2±2.22 (38.98%)	2.4±0.98** (78.95%)	1.4±0.74** (82.02%)	0.6±0.24** (94.12%)

Control: animals received (1% Tween 80 in water), Standard received Diazepam 3 mg/kg body weight i.p., Group-I, Group-II and Group III were treated with 100, 200 and 400 mg/kg body weight of the crude extract of *H. coronarium* per oral. Values are mean ±SEM, (n = 5); * $p < 0.05$, ** $p < 0.001$, Dunnett's test as compared to control

TABLE 4: EFFECT OF *H. CORONARIUM* METHANOLIC EXTRACT ON OPEN FIELD TEST IN MICE

Group	Dose mg/kg	Number of movements (% of Number of movements inhibition)				
		0 min	30 min	60 min	90 min	120 min
Control		113 ±3.22	106.6±1.69 (-)	91.2 ±1.53 (-)	87.4 ±1.63 (-)	98±2.43 (-)
Standard	3	83.2±14.21	39.4±8.14** (63.03%)	32.6±6.22** (64.25%)	24.2±6.9** (72.31%)	11±3.115** (88.77%)
Group-I	100	52±6.26	30.2±3.12** (71.67%)	25.2±2.22** (72.37%)	22.6±2.27** (74.14%	10±2.51** (89.79%)
Group-II	200	66.6±5.78	45.2±9.78** (57.60%)	27±7.98** (70.39%)	4.4±2.99** (94.96%)	2.2±1.02** (97.75%)
Group-III	400	51.4±14.46	30.6±3.4** (71.29%)	19.2±0.735** (78.94%)	10.2±1.67** (88.32%)	3.4±0.745** (96.53%)

Control: animals received (1% Tween 80 in water), Standard received Diazepam 3 mg/kg body weight i.p., Group-I, Group-II and Group III were treated with 100, 200 and 400 mg/kg body weight of the crude extract of *H. coronarium* per oral respectively. Values are mean ±SEM, (n = 5); ** $p < 0.001$, Dunnett's test as compared to control

DISCUSSION: Acetic acid induced writhing test is suitable for detecting both central and peripheral analgesia, whereas tail flick tests are most sensitive to centrally acting analgesics. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic mediators like PGE_2 and $PGF_{2\alpha}$ and their levels increase in the peritoneal fluid of the acetic acid induced mice [10]. The abdominal constrictions produced after administration of acetic acid is related to sensitization of nociceptive receptors to prostaglandins.

It is therefore possible that the extract exerts its analgesic effect by inhibiting the synthesis or action of prostaglandins which may be due to phytochemicals present in the extract. Thermally induced nociception indicates narcotic involvement [11]. The centrally acting analgesics generally elevate the pain threshold of mice towards heat. The extract significantly delayed the response time to thermal pain sensation in tail flick method indicating narcotic involvements.

Moreover, since the extract inhibited both peripheral and central mechanisms of pain, it is possible that the extract acted on opioid receptor [12-13]. While evaluating neuropharmacological activities of *Hedychium coronarium*, it was found that the plant extract possesses central nervous system depressant activity as indicated by decreased exploratory behavior in mice [14].

Results of the present investigation suggest that the extract of *Hedychium coronarium* possesses strong analgesic and CNS depressant activity and provide a scientific basis for the use of the plant in traditional system of medicine in the treatment of inflammatory disorders.

CONCLUSION: In light of the results of the present study, it can be concluded that the plant extract possesses remarkable analgesic and CNS depressant activity which may be mediated through the depression of central mechanism of pain; thereby lends support to the traditional use of the plant in pain and inflammatory disorders. However, further studies are needed to be conducted to understand the exact mechanisms of CNS depressant and analgesic activity isolating the compound (s) responsible for such actions.

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