

Evaluation of Anthelmintic, Analgesic and Neuropharmacological Activity of the Plant *Abutilon Indicum*

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ABSTRACT

The present investigation was conducted to determine the anthelmintic, analgesic and neuropharmacological activity of the methanolic extract from the barks of *Abutilon indicum*. Anthelmintic activity was investigated using earthworm (*Pheretima posthuma*) and fresh leaf juice of the plant. In anthelmintic activity test, the time of paralysis for leaves juice was range from 25min 37 sec to 65min 12 sec whereas the time ranges for standard drug albendazole was 31 min 57 sec to 75min 27 sec. On the other hand the time of death start from 31 min 43 sec and end to 72 min 09 sec for leaves juice and for albendazole the time of death start from 39 min 22 sec and end 84 min 19 sec respectively at different dose (5mg/ml, 10mg/ml, 20mg/ml, 50mg/ml, 100mg/ml) which indicate highly significant anthelmintic activity. Analgesic activity was investigated using two thermal (hot plate and tail immersion) and one chemical (acetic acid-induced writhing test) methods at the dose of 200 and 400 mg /kg in Swiss albino mice. In analgesic activity test, the latency time of thermal threshold was prolonged in hot plate and tail immersion tests, whereas it was inhibited by acetic acid-induced writhing test. Thus the extract of the *Abutilon indicum* barks showed moderate analgesic activity. Neuropharmacological activity of the plant was observed by open field, hole cross, and dark/light box tests at the doses of 200mg/kg and 400 mg /kg in Swiss albino mice. The extract significantly decrease the locomotor activity as shown by the open field, hole cross test which indicate highly significant antidepressant activity. The results also showed similar activity in dark/light box test. Thus the obtained results in the study provide a support for the use of this plant for medicinal purposes and encourage further investigations for more fruitful results.

Keywords: *Abutilon indicum*, anthelmintic, antidepressant, analgesic activity, medicinal plant

INTRODUCTION

Abutilon indicum is a medicinal plant that belongs to the family Malvaceae commonly known as Indian mallow. It is native to Africa, Asia and Australia and also found in India, Sri Lanka, tropical regions of America and Malaysia. It is also found in sub-Himalayan tracts, hills up to 1200m and in hotter parts of India. It is an erect, woody and shrubby plant with circular-ovate or heart shaped leaves with coarsely crenate-serrate margins. The plant can reach up to 1-2 m and yellow-orange color flower, fruits is quite interesting-it is circular in shape consisting of 11-20 radiating hairy carpels and seed are kidney-shaped. The bark, root, leaves, flowers and seeds are all used for medicinal purposes. It has been extensively used as a traditional medicine as a laxative, emollient, analgesic, anti-diabetic, anti-inflammatory, and blood tonic agent and also in the treatment of leprosy, urinary disease, jaundice, piles, relieving thirst, ulcers vaginal infection, diarrhea, blood dysentery some nervous and ear problems. The bark is used as astringent and diuretic. The flowers are traditionally used to increase semen in men. Various studies on the plant extract have been performed to confirm the anti-oxidant, anti-bacterial, analgesic, anthelmintic, anti-inflammatory, anti-cancer, hepato-protective and anti-diabetic activities of the plant [1].

MATERIALS AND METHODS

Plant Materials and Extraction

The bark of *Abutilon indicum* were collected from Noagoan, Bangladesh. The plant was identified by the scientist of Bangladesh National Herbarium, Mirpur, Dhaka where a voucher specimen has been deposited for future reference. The powdered material (150gm) was taken in separate clean, round bottomed flask and soaked in 1500ml of methanol. The container with its content was sealed by cotton plug and aluminium foil and kept

for a period of 15 days accompanying occasional shaking. The whole mixture is then filtered through cotton followed by Whatman no.1 filter paper and then obtained filtrate was concentrated by using a rotary evaporator at low temperature (40–50)°C. The concentrated extract is then air dried and the weight of the obtained methanolic extract was 25 gm respectively.

Chemicals

Drugs and chemicals used in the study include acetic acid (Merck, Germany), methanol (Merck, Germany), diclofenac-Na, albendazole and diazepam (Square Pharmaceuticals Ltd, Bangladesh).

Animals

For the experiment, adult earthworms (*Pheretima posthuma*) were collected from moist soil of a personal area of Bangladesh and 2 to 3 weeks old male Swiss albino mice weighing between 25-30g were collected from the Jahangirnagar University Bangladesh. Soft wood shavings were used as bedding cages. Animals were maintained under standard environmental conditions: temperature (24.0±1.0°C), relative humidity (55-65%) and 12h light /12h dark cycle. Husk and excreta were removed from the cages every day [2].

Phytochemical Testing

The methanol extract of *Abutilon indicum* was subjected to preliminary qualitative tests to identify the phytoconstituents present in the bark. It was observed that it contained alkaloid, glycosides, flavonoids, tannins, Saponin, carbohydrate and steroids [1].

Anthelmintic Activity

Collected leaves were weighted (75g) and blended into liquefactions in 250ml of water. Then the mixture was centrifuged at 100 rpm. The supernatant was filtered through sterile filter paper into a conical

flask as the study was expected that 1ml of filtrated was contain 0.3g, which is say 300mg/ml [3].

Adult earthworms (*Pheretima posthuma*) were washed with normal saline water to remove all faecal matter, for anthelmintic activity study. The earthworms of 3-8 cm in length and 0.1-0.2 cm width were used for experimental protocol [3].

Fresh leaves juice of *Abutilon indicum* were dissolved in saline water and volume was adjusted up to 10ml. All drug and juice were prepared before starting the experiment. In each case, 5 earthworms released into 10ml desired formulations as follows: Vehicle (normal saline water), Albendazole and fresh juice (5mg/ml, 10mg/ml, 20mg/ml, 50mg/ml, and 100mg/ml). Observation was made for the time to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in saline solution [3].

Analgesic Activity

The study of analgesic activity of the *Abutilon indicum* was conducted in animal models for both central and peripheral mechanism of pain. The peripheral and central analgesic activity was assessed by the acetic acid-induced writhing test (chemical method) [4], tail immersion test (thermal method) [5] and hot plate test (thermal method) [5] separately. Swiss albino mice were weighed and divided into control, positive control and test groups with five mice in each group. The animals of test groups received test sample at the dose of 200mg and 400mg/kg body weight respectively. Positive control group received standard drug diclofenac-Na (25mg/kg body weight) and vehicle control group was treated in distilled water (0.1ml/kg body weight).

Acetic Acid-Induced Writhing Test

Test samples and vehicle were administered orally 30 min before

intraperitoneal administration of 0.7% acetic acid, while diclofenac-Na was administered 15 min before injection of acetic acid. After an interval of 5min, the mice were observed for specific contraction of body referred to as “writhing” for next 10 min [6].

Tail Immersion Test

The test was conducted as per the method modified by D’Amour and Smith (1941). The screening cut-off time was set as 5 sec, while the test cut-off time was set as 10 sec. The extract was administered orally and diclofenac-Na was administered intraperitoneally. The tail of the mice was immersed to a constant level (3cm) in a water bath maintained at $55 \pm 0.5^\circ\text{C}$. The time to flick the tail from water (reaction time) was recorded. A maximum immersion time of 10 seconds was maintained to prevent thermal injury to the animals. A significant increase in reaction time compared to control animals was considered a positive analgesic response [7].

Hot Plate Test

Hot plate test was used to measure the response latencies based on the procedure described by Eddy and Leimbach (1953). In this experiment hot plate was maintained at $50 \pm 0.05^\circ\text{C}$. The reaction time was recorded for animals pre-treated with distilled water (10ml/kg body weight, 30 min before orally) and methanol extract (200mg and 400mg/kg body weight, 30 min before). Here diclofenac-Na (25 mg/ml body weight, 15 min before intraperitoneally). Animals were placed into the hot plate chamber and the time of latency was determined. The latent period of response was taken as the index of antinociception and was determined at the pre-treatment and after 0 min, 30 min, 60 min, 90 min and 120 min administration of the test sample and standard in the order to minimize the damage on the animal paws. The cut-off time was taken as 20 seconds [8].

Neuropharmacological Activity

The neuropharmacological activity was carried out by hole cross, open field and Light/dark box tests. In each test, the animals were divided into negative control, positive control and test groups containing five mice each. The test groups received bark extract of *Abutilon indicum* at 200mg and 400mg/kg body weight respectively and the negative control group received vehicle (0.1ml/mice distilled water) orally where as the positive control received the standard drug diazepam (2mg/kg body weight) intraperitoneally.

Hole Cross Test

A wood 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 centre of the cage. The number of passage of a mice through the hole from one chamber to other was counted for a period of 5 min and the reading was recorded at 0, 30, 60, 90 and 120 min after the administration of the test sample and the standard drug [9].

Open Field Test

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 animal was counted for 5 min and the reading was recorded at 0, 30, 60, 90 and 120 min after the administration of the test sample and the standard drug [9].

Light/Dark Box Test

The test was performed as per the method modified by Hascoet and Bourin (1998). An open-topped rectangular box (46×27×30 cm high) was divided into a small (18×27 cm) area and a large (27×27 cm) area with an opening door (7.5×7.5) located in the centre of the partition at floor level. The small compartment was painted black and illuminated by a dim red

light (60W; 4 1x), where as the large compartment was painted white and brightly illuminated with a 60W; (400 1x) light source. The compartments were equipped with infrared beam sensors (four in the White area, three in the black one). Each mouse was tested by placing it in the center of the white area, facing away from the dark one, and was allowed to explore the novel environment for 5 min and thereby enabling the detection of locomotion in each zone, time spent in each zone, latency of the first crossing from one compartment to the other, and shuttle crossing between both compartments [10].

LITERATURE REVIEW

By the literature review of *Abutilon indicum*, it was found that various studies on the plant extract have been performed to confirm the anti-oxidant [11], antibacterial [12], analgesic [13], anti-inflammatory [14], anti-ulcer [15], anthelmintic, and anti-arthritis [17] activities of the plant.

RESULTS AND DISCUSSION

Earthworms were released into the fresh leaves juice (5mg/ml, 10mg/ml, 20mg/ml, 50mg/ml, and 100mg/ml) to determine the anthelmintic activity. The leaves juice indicate significant anthelmintic activity of the plant which was comparable to the reference drug albendazole (Table 1). Preliminary phytochemical screening of methanol extract of *Abutilon indicum* bark showed the presence of steroids, flavanoids and tannins. It is an evident from experimental data that the extract of *Abutilon indicum* showed potent anthelmintic activity [3].

Dose dependent reduction of the paralysis and death time was observed in animals treated with different concentration of the leave juice of *Abutilon indicum* (Table 1).

Table 1: Anthelmintic activity of *Abutilon indicum*.

Test Samples Death (mins)	Conc.(mg/ml)	Time Taken for Paralysis (mins)	Time Taken for
Fresh juice of leaves	5	65 min 12 sec	72 min 09 sec
	10	53 min 17 sec	60 min 17 sec
	20	55 min 25 sec	62 min 21 sec
	50	35 min 13 sec	40 min 32 sec
	100	25 min 37 sec	31 min 43 sec
Albendazole	5	75 min 27 sec	84 min 19 sec
	10	63 min 15 sec	72 min 20 sec
	20	58 min 04 sec	70 min 48 sec
	50	40 min 39 sec	48 min 12 sec
	100	31 min 57 sec	39 min 22 sec

Methanol extract of the bark of *Abutilon indicum* was administered to the mice at 200mg and 400mg/kg body weight to determine the effects on acetic acid-induced writhing in mice. The extract significantly inhibited writhing response induced by acetic acid in a dose dependent manner which was comparable to the reference drug diclofenac-Na (Table 2).

Dose dependent reduction in the number of abdominal constriction was observed in animals treated with different concentration of the methanol extract of *Abutilon indicum* at 200 mg/kg and 400 mg/kg body weight and the inhibition of

writhing response was observed as 42.6% and 52.8% respectively, as compared to the inhibition of writhing response by diclofenac-Na which was 71%.

The tests for analgesic activity were carried out in the laboratory on four groups of mice by tail-flick and hot plate method. Time interval for the test was 30 minutes and the results are given in Table 3 and 4.

The tail withdrawal reflex time following administration of the bark extract of *Abutilon indicum* was found to increase with found to increase with increasing dose of the extract.

Table 2: Effect of bark extract of *Abutilon indicum* in acetic acid-induced writhing on mice.

Test Group	Dose	Mean $\pm$ SEM	% of Writhing	% of Inhibition
Control	0.1ml/kg	53 $\pm$ 8.804	100	0.00
Positive control	25mg/kg	15.4 $\pm$ 3.246	29	71
Group-I	200mg/kg	30.4 $\pm$ 0.781	57.4	42.6
Group-II	400mg/kg	25 $\pm$ 8.975	47.2	52.8

Values are expressed as Mean $\pm$ SEM (n=5).

Table 3: Results of tail immersion test for plant extract of *Abutilon indicum*.

Test Group	Dose	Response Time (seconds)				
		0min	30min	60min	90min	120min
Control	0.1ml/kg	1.32 $\pm$ 0.85	1.03 $\pm$ 0.21	2.35 $\pm$ 0.12	3.15 $\pm$ 0.98	2.59 $\pm$ 0.19
Positive control	25mg/kg	1.84 $\pm$ 0.95	2.11 $\pm$ 0.33	2.45 $\pm$ 0.41	3.45 $\pm$ 0.29	1.71 $\pm$ 0.171
Group-I	200mg/kg	2.12 $\pm$ 0.63	2.04 $\pm$ 0.19	2.35 $\pm$ 0.23	1.99 $\pm$ 0.36	2.52 $\pm$ 0.159
Group-II	400mg/kg	1.65 $\pm$ 0.19	2.85 $\pm$ 0.21	2.77 $\pm$ 0.19	1.77 $\pm$ 0.17	2.58 $\pm$ 0.325

Values are expressed as Mean $\pm$ SEM (n=5).

Table 4: Results of hot plate test for plant extract of *Abutilon indicum*.

Test Group	Dose	Response Time(seconds)				
		0min	30min	60min	90min	120min
Control	0.1ml/kg	3.19 $\pm$ 0.69	4.19 $\pm$ 0.98	5.19 $\pm$ 2.25	6.28 $\pm$ 1.01	6.32 $\pm$ 0.367
Positive control	25mg/kg	1.23 $\pm$ 0.07	4.54 $\pm$ 1.25	8.21 $\pm$ 2.92	9.44 $\pm$ 1.193	5.49 $\pm$ 0.605
Group-I	200mg/kg	2.12 $\pm$ 0.46	7.15 $\pm$ 1.12	6.23 $\pm$ 0.36	7.32 $\pm$ 1.494	5.13 $\pm$ 0.82
Group-II	400mg/kg	3.81 $\pm$ 0.31	4.03 $\pm$ 1.12	4.23 $\pm$ 0.45	5.11 $\pm$ 0.650	3.89 $\pm$ 0.95

Values are expressed are Mean $\pm$ SEM (n=5).

The data obtained clearly indicated that the bark extract of *Abutilon indicum* showed dose dependent analgesic effect in central analgesic effect in tail immersion (Table 3) and hot plate (Table 4) tests.

The most important step in evaluating drug action on CNS is to observe its effect on locomotors activity of the animals. The activity is a measure of the level of excitability of the CNS and any decrease in CNS activity may be closely related to sedation resulting from depression of the central nervous system. The CNS potential of the extract was evaluated using hole cross (Table 5), open field (Table 6) and Light/dark box test (Table 7) to understand

the CNS activity in Swiss *albino* mice at 200 mg/kg and 400 mg/kg body weight. The results of these tests revealed potential CNS depressant activity of the extract which was comparable to the standard drug, diazepam (2mg/kg body weight).

Both hole cross and open field tests showed that the depressing activity of the bark extract was evident from the 2nd observation period in the test animals at the doses of both 200 mg/kg and 400 mg/kg body weight. Maximum depressant effect was observed from 2nd (30 min) to 4th (90 min) observation period at the dose of both 200 mg/kg and 400 mg/kg b.w. The result was also dose dependent.

Table 5: Results of hole cross test for plant extract of *Abutilon indicum*.

Test Group	Dose (mg/kg b.w)	Number of Movements				
		0min	30min	60min	90min	120min
Control	0.1ml/mice	15.6±1.53	12.2±0.65	15.6±0.91	14.8±0.65	11±0.36
Diazepam	2	7.4±0.76	0.2±0.23	0.2±0.23	1.8±0.35	1.4±0.27
Group-I	200	6.8±0.65	6.2±0.42	2.2±0.65	1.6±0.65	0.6±0.45
Group-II	400	4±0.79	3.2±0.42	1.2±0.65	0.4±0.275	0.2±0.23

Value expressed as Mean ± SEM (n=5)

Table 6: Results of open field test for plant extract of *Abutilon indicum*.

Test Group	Dose (mg/kg b.w)	Number of Movements				
		0min	30min	60min	90min	120min
Control	0.1ml/mice	75.6±4.73	77.2±4.06	58.4±3.90	56.6±1.75	41.2±3.6
Diazepam	2	71.4±6.65	1±0.5	3.2±1.43	14.4±1.75	13.2±2.45
Group-I	200	54.4±1.48	34±1.97	26.6±8.7	14.4±3.77	35.2±4.56
Group-II	400	47.8±2.82	41.2±2.88	29±4.69	15.4±3.4	20.2±3.87

Value expressed as Mean ± SEM (n=5).

Table 7: Result of light/dark box test for plant extract of *Abutilon indicum*.

Test group	Dose (mg/kg b.w)	No of entry in light area	No of entry in dark area	Time spent in light area (seconds)	Time spent in dark area (seconds)
Control	0.1ml/mice	6.6±1.82	7.6±1.81	33.2±14.2	266.8±14.2
Diazepam	2	0.6±0.89	1.2 ±0.89	157.8±131.1	142.2±131.1
Group-I	200	3.6±2.19	4.6± 2.19	96.8±88.73	203.2±88.73
Group-II	400	4±3.46	5±3.46	57.2±28.01	242.8±28.01

Value expressed as Mean ± SEM (n=5).

CONCLUSION

Based on the results of the present studies, it can be concluded that the crude extract of the bark of *Abutilon indicum* possesses anthelmintic, analgesic and neuropharmacological activity. Dose dependent significant anthelmintic activity

was observed. At higher dose, moderate analgesic activity was observed from hot plate, tail immersion and acetic acid-induced writhing tests. Significant antidepressant activity can also be suggested from the results of neuropharmacological test. Dose

dependent activity was observed in all the performed pharmacological investigations. Hence, further studies are suggested to pinpoint the bioactive compounds from the extract of *Abutilon indicum* and to better understand the mechanism of such action scientifically.

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