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ANALGESIC AND CNS DEPRESSANT ACTIVITY OF METHANOLIC EXTRACT OF *ALSTONIA SCHOLARIS* LEAVES.

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Abstract:

The present study was aimed to investigate the possible analgesic action & CNS (Central Nervous System) depressant effect of methanolic extracts of Alstonia scholaris (Family: Apocynaceae) leaves on animal model. The analgesic activity of the extracts of the plant was investigated for its peripheral pharmacological actions using acetic acid-induced writhing test in rats. The methanolic extracts of leaves of Alstonia scholaris at dose 200 mg/kg body weight, displayed 72.85% pain inhibition which was significant ($p<0.001$) compared to reference drug Indomethacin. CNS depressant activity was evaluated by observing the effects of the plant extracts on exploratory activity in rats using hole cross and open field method. The methanolic extracts of the plant was administered orally at dose 500 mg/kg- body weight to each rat and the movement of the rats through the hole from one chamber to another was observed. Here Diazepam was used as standard drug. The result was found statistically significant ($p<0.01$) compared to control group for the leaf extract of the plant that decreased the exploratory behavior of rats significantly. From the pharmacological point of view, A. scholaris appears to be a valuable plant which can be treated as analgesic and CNS depressant.

Key Words: *CNS-depressant, analgesic action, hole cross tests, writhing.*

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INTRODUCTION:

A. scholaris, commonly known as chatim and devils tree which belongs to the family of apocynaceae, is an evergreen and tropical tree native to the Indian subcontinent and parts of Indonesia, Malaysia as well as Australia. It grows up to 40 m tall and has rough, grey bark and large leaves that grow in clusters of seven. The flowers are small and greenish-yellow in color and it blooms in the month of October. The fruits are thin pods that can grow up to 20 inches long. The sap of the tree is sticky and bitter.

A. scholaris has a promising place in various ayurvedic preparations to treat whooping cough, malaria, jaundice, gastric complaint, headache, asthma, stomachache, wound, fever [1]. *A. scholaris* is used in Nigeria to treat mental illness by traditional psychiatrists [2]. This traditional use of *A. scholaris* is reported to be remarkably compatible with its profile in experimental animals [3].

The plant is a rich source of alkaloids, flavonoids, saponins, steroids, reducing sugar and phenolic compounds which witness the ample of medicinal potential of the herb [4]. Eight flavonoids were isolated and identified as kaempferol, quercetin, isorhamnetin, kaempferol-3-0-beta-D-galactopyranoside, quercetin-3-0-beta-D-galactopyranosi- de, isorhamnetin-3-0-beta-D-galactopyranoside, kaempferol-3-0-beta-D-xylopyranosyl-(2-1)-0-beta-D-galactopyranoside,quercetin-3-0-beta-D-xylopyranosyl-(2-1)-0-beta-D-galactopyranosi- de [5]. Four picrinine-type monoterpenoidindole alkaloids, 5-methoxyaspidophylline, picrinine, picralinal and 5-methoxystrictamine were obtained from the leaves of *A. scholaris*. Ten alkaloids in *Alstoniascholaris* leaves were identified by HPLC as Scholaricine; 19-Epi-scholaricine; Sarpagine; N4-Demthylechitamine; Echitamidine; Strictamine; Akuammidine; Vallesamine; Picraline; Picralinal. Seven triterpenes in *A. scholaris* leaves were identified by HPLC as Cylicodiscic acid; Betulin; Betulinic acid; Oleanolic acid; Ursolic acid; Cycloeucalenol; α-amyrin acetate [6].

Several plants have been reported to have CNS depressant and anxiolytic activity due to the presence of flavonoids [7]. Therefore, our present work carried out on leaves of *A. scholaris*, to explore the analgesic and CNS depressant activities.

MATERIALS AND METHODS:

Plant materials:

The leaves of plant *A. scholaris* were collected from Gazipur in the month of March, 2011. The plant was taxonomically identified and Voucher Specimen No - 34479 has been maintained for *A. scholaris* in the Bangladesh National Herbarium Dhaka, Bangladesh.

Preparation of Methanol Extract of *A. scholaris*

The leaves were first washed with water to remove adhering dirt and then dried at 45°C for 36 hrs in an electric oven, then powdered with a mechanical grinder, passing through sieve #40 and stored in a tight container. The dried powdered material (147.74 g) was taken in a clean, flat bottomed glass container and soaked in methanol. The container with its contents was sealed & kept for a period of 7 days accompanying occasional shaking & stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. The total filtrate was concentrated to dryness, *in vacuo* at 40^0C to render the methanol extract (390 g) of brownish red color.

Drugs & Chemicals

All chemicals and drugs were obtained commercially and were of analytical grade. The active drugs Indomethacin and Diazepam were the generous gift samples from Square Pharmaceuticals Ltd., Bangladesh. Acetic acid was obtained from Merck, Germany. Tween-80 was obtained from BDH Chemicals, UK. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh.

Animals

Long-Evan rats of either sex weighing about 120-170 gm were used for the experiment. The rats were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were maintained under standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed ICDDR, B formulated rodent food and water ad libitum. The set of rules followed for animal experiment were approved by the institutional animal ethical committee [8] .

Acute toxicity study

The median lethal dose (LD_{50}) of the extract in mice was estimated by the up and down method [9]. Doses were adjusted up or down by a constant multiplicative factor (1.5) depending on the previous outcome.

Analgesic activity:

Acetic acid induced writhing in rats

For evaluation of analgesic response, the animals were divided into 4 groups of 6 animals each. The writhing test was carried out as described in literature [10]. Each rat was given an intra peritoneal injection of 0.7% v/v acetic acid at 0.1 ml/10 g bodyweight. 30 minutes before the administration of acetic acid, each

set of animals were orally injected with 10 ml/kg bodyweight of 1% tween 80 solution (Group I, control group) and samples extract of *A. scholaris* (Group III, experimental group). But 10 mg/kg body weight of standard drug, Indomethacin (Group II, standard group) Indomethacin (standard drug) was administered orally 15 minutes prior to acetic acid injection. Then the animals were placed on an observation table. Each rat of all groups was observed individually for counting the number of writhing. The number of writhing was recorded between 5 min and 20 min after acetic acid injection. The writhing was considered as contraction of the abdominal muscles followed by the stretching of hind limbs. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated groups was compared to that of a control group. Samples having analgesic activity will reduce number of writhes of treated rats.

The percent inhibition (% analgesic activity) was calculated by

% inhibition = {(A-B)/A} X 100

Where, A= Average number of writhing of control group; B= Average number of writhing of experimental group.

CNS Depressant Activity

Hole Cross Test

The method described by Takagi *et al.*, 1971 [11] was implemented for this study. Again the 24 rats were equally divided into 4 groups. The control group received 1% Tween 80 in water (10ml/kg body weight), the standard group received Diazepam (1mg/kg body weight) and the experimental groups received crude extract of 500 mg/kg body weight. 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 partition. The number of passages of rats through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 120 and 240 minutes after the oral treatment with extract. The reduction of the passage of rats through the hole will indicate the presence of CNS depressant activity of methanolic extract of leaves of *A. scholaris.*

Statistical analysis

All the values in the test are expressed as mean ± standard deviation (SD). The data were statistically analyzed by ANOVA (Analysis of variance) and post-hoc Dunnett's tests with the Statistical Package for Social Sciences (SPSS16.0, USA) program. Dissimilarity between the means of the various groups were measured significant at $p< 0.05$ and $p< 0.01$.

RESULTS:

Acute toxicity

Oral administration of graded doses of the methanolic extract of *A. scholaris* leaves (500 – 5000 mg/kg, body weight) did not cause any death in the different dose groups. The LD_{50} value for oral administration of the plant extract was found to be greater than 5000 mg/kg.

***In vivo* analgesic activity**

The result of analgesic activity of *A. scholaris* leaves is shown at the Table- 1. The methanolic extract of *A. scholaris* leaves at the dose 200 mg/kg body weight showed highly significant ($p<0.001$) analgesic effects compared to control on acetic acid induced rats.

Table 1: Analgesic effect of methanolic extract of *A. scholaris* leaves in acetic acid induced writhing test.

Group	Dose (mg/kg b.w.)	No of writhing	% of inhibition
Control	-	26.25±0.986	-
Standard	10	9.25±1.28***	64.76%
Extract	200	7.125±2.742***	72.86%

Statistical analysis was conducted through one way ANOVA post hoc dunnett's test & the data of standard & test drug was found highly significant ($p<0.001$) compared to control.

CNS Depressant Activity

Table 2: CNS depressant effect of methanolic extract of *A. scholaris* leaves on Hole cross test.

Treatment	Doses	No. of movement				
		0 min	30 min	60 min	90 min	120 min
1% Tween 80 in water (Control)	10 ml/kg	13.5±1.37	14±1.49	14.25±.99	14±1.27	13.5±0.33
Diazepam (Standard drug)	1mg/kg	10.75±0.55	5.5±0.75**	4.5±1.00**	3.0±0.82**	1.75±0.55**
Leaves extract of *A. scholaris*	500mg/kg	7.25±2.33**	4.5±1.11**	5.5±0.75**	6.25±1.44**	5.5±1.53**

Statistical analysis was conducted through one way ANOVA post hoc dunnett's test. All data were highly significant ($p<0.01$) compared to control. Only exception was at o minute standard drug is insignificant compared to control.

Hole cross test

In this test, the extracts showed a decrease in locomotion in the test animals. The number of crossing hole from one chamber to another by rat of the control group was remain almost normal from 0 minutes to 120 minutes (Table 2). But the methanolic extract of *A. scholaris* leaves showed a noticeable decrease on exploratory behavior in the test animals at dose 500mg/kg-body weight from its initial value 0 to 120 which was found highly significant ($p<0.01$) compared to control. Only exception is at 0 minute standard drug was insignificant compared to control.

DISCUSSION:

Present study was conducted to elucidate analgesic and CNS depressant activity of the methanolic extract of *A. scholaris* leaves. The relatively high oral median lethal dose (LD_{50}) in rats suggests that the extract is relatively non toxic when taken orally[12].

Reduced number of writhing may confirm the peripheral analgesic activity of the test plant *A. scholaris* by inhibiting prostaglandin synthesis, a peripheral mechanism of pain inhibition [13]. It has been already reported that *A. scholaris* contains alkaloids [4] that may produce the anti-inflammatory and analgesic effect peripherally.

Anxiety and hypnosedation are principally mediated in the CNS by the $GABA_A$ receptor complex, 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 [14] . Diverse drugs that are used in various psychological and neurological disorders might modify the GABA system at the level of the synthesis of GABA, induce anxiolysis or hypnosis in animals by potentiating the GABA-mediated postsynaptic inhibition through an allosteric modification of GABA receptors, [15] and thirdly by direct increase in chloride conductance or indirectly by potentiating GABA-induced chloride conductance with simultaneous depression of voltage activated Ca^{++} currents like barbiturates [16]. In this study, CNS depressant activity of methanolic extract of *A. scholaris* was evaluated by hole cross test to observe its effect on locomotor activity of the animal. The activity is a measure of the level of excitability of the CNS, and decreased activity results from CNS depression [17].

Our extract had decreased locomotor activity indicating its CNS depressant activity. Therefore it is possible that extract may acts either by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extract [18]. Many research showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders and many flavonoids and neuroactive steroids can act as benzodiazepine like molecules [19] and may exert their action through $GABA_A$ receptors. So the presence of flavonoids, steroids, phenolic compounds & many other chemical constituents in the methanolic extract of *A. scholaris* might be produce CNS depressant activity.

CONCLUSION:

Based on the results of the present study, we can confirm that the methanolic extract of *A. scholaris* leaves possesses remarkable analgesic and CNS depressant activities. However, further studies are indispensable to examine underlying mechanisms and to isolate the active compounds responsible for these pharmacological activities. In future experiments, studies with purified fractions of the extract can be conducted for further pharmacological and

toxicological characterization, such as the research of the mechanisms involved in the central and peripheral analgesic effect as well as CNS depressant activity.

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