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Research Article

ANTIDIARRHEAL AND CYTOTOXIC ACTIVITIES OF *ALSTONIA SCHOLARIS* BARK

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ABSTRACT
Alstonia scholaris (Apocynaceae) is an evergreen and tropical tree native to the Indian subcontinent and South-east Asia. It is a well known medicinal plant and commonly known as 'Chhatim' in Bangladesh. Traditionally the bark is used to treat anemia, menstrual disorders, colic, diarrhea, dysentery and acute arthritis. In the present study, the ethanolic extract of bark was investigated for phytochemical properties as well as antidiarrheal and cytotoxic activities. In antidiarrheal activity test (castor oil induced diarrhea), the extract was taken as a dose of 250mg/kg body weight orally in Swiss albino mice and the standard drug loperamide, at a dose of 50mg/kg. The cytotoxic test was performed on brine shrimp (*Artemia salina*). The ethanolic extract contains alkaloid, reducing sugar, saponin and tannin. It significantly increased mean latent period (1.09 hr) and decreased the frequency of defecation, which were comparable to the standard drug (P<0.01). In case of cytotoxic test, the LC_{50} & LC_{90} were found as 22.25 & 95.28µg/ml, respectively. The findings suggest that *Alstonia scholaris* possesses antidiarrheal and cytotoxic activities.
Keywords: *Alstonia scholaris;* Apocynaceae; Antidiarrheal activity; Cytotoxic activity; Castor oil induced diarrhea; Brine shrimp lethality bioassay.

INTRODUCTION

Alstonia (A.) scholaris belongs to the Apocynaceae family and is indigenous to the South and South-east Asia. The tree is commonly known as 'Chhatim' in Bangladesh and has been used for centuries in Ayurvedic medicine for treatment of various disorders. The bark yields a tonic and antiseptic medicine which is used to treat anemia, menstrual disorders, malarial fever, colic, diarrhea, dysentery and acute arthritis. It contains alkaloids like ditamine, echitenine and echitamine, and is used to serve as an alternative to quinine. Decoction of the bark is used to treat diarrhea and malaria, and as a tonic, febrifuge, anti-periodic, astringent and anthelmintic drug. Bark is also used in snakebite and produces protective effect on hepatotoxin induced acute liver damage. Sap, gum and roots are used in tumor and cancer[1]. The alkaloid fraction of *A. scholaris* exhibited antitussive and antiasthmatic activities *in vivo*[2]. Extract of the bark was found to have antibacterial[3], immunostimulant[4], antimalarial[5] and anticancer[6] activities. Although the plant has widespread applications in traditional medicine, very few scientific studies have been performed to ascertain its medicinal potential. Therefore, the objective of the present investigation was to evaluate the antidiarrheal and cytotoxic effects of ethanolic extract of stem bark of *A. scholaris.*

MATERIALS AND METHODS

Collection and Identification

The bark of *A. scholaris* was collected from Gosaibari, Bogra, Bangladesh in January 2010 on the daytime from the fresh tree. The plant was taxonomically identified by the Bangladesh National Herbarium, Mirpur, Dhaka (Accession No.: DACB 32100) and a voucher specimen was deposited at Pharmacy Discipline, Khulna University, Khulna.

Drying and Grinding

The collected plant part (stem bark) was separated from undesirable material, plant or plant parts, and sun dried for one week. The dried bark was ground into a coarse powder with a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until the analysis was commenced.

Extraction

About 500 gm of powered material was taken in a clean and flat-bottomed glass container and soaked in 1400 ml of 80% ethanol. The container was sealed and kept for a period of seven days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean and white cotton. Then it was filtered through Whatman filter paper. The filtrate (Ethanolic extract) was evaporated under ceiling fan until it was dried. It rendered a gummy concentrate of yellowish color. The gummy concentrate was designated as crude ethanolic extract.

Experimental Animals

Young Swiss albino mice of either sex with average weight of 19-25 gm were employed in the experiment taking five in a group. The mice were purchased from the Animal Research Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B). The mice were kept in a room well ventilated for air and light. Foods formulated by ICDDR, B and fresh water were given to the mice regularly. The mice were kept for seven days in the laboratory to get them adapted with the environment before being employed in the experiment.

Phytochemical Screening

Small amount of dried extract was appropriately treated to prepare sample solution and then subjected to the specific phytochemical tests. Benedict's test, Fehling's test and ring test were performed to investigate the presence of reducing sugar. Ferric chloride test and potassium dichromate test were performed to identify tannin. Frothing test was performed to identify the presence of saponin. Libermann-Burchard test was performed to identify steroid. Mayer's reagent, Wagner's reagent and Dragendorff's reagent were used to identify alkaloid. For Gum, flavonoid and glycoside, other identifying tests were performed[1,7].

Antidiarrheal Activity Test

All the animals were screened initially by giving 0.3 ml of castor oil and only those showing diarrhea were selected for the final experiment. The animals were divided into control, positive control and test groups containing five mice in each group. Control group received vehicle (1% Tween 80 in distilled water) at a dose of 10 ml/kg body weight orally. The positive control group received loperamide at the dose of 50 mg/kg and test group received the ethanolic extract at the dose of 250 mg/kg body weight orally. Each animal was placed in an individual cage. The floor was lined with blotting paper which was changed every hour. Diarrhea was induced by oral administration of 0.3 ml castor oil to each mouse, 40 minutes after the above treatments. During an observation period of 4 hours, the total number of fecal outputs excreted by the animals was counted. The latent period of each mouse was also recorded[8,9].

Brine shrimp Lethality Bioassay

Cytotoxic activity test was performed using brine shrimp lethality bioassay [9,10]. 500 mg of dried ethanolic extract of *A. scholaris* bark was dissolved in DMSO and made the volume 10 ml. The concentration of this solution was 50µg/µl. 38g Sea salt (pure NaCl 20g and table salt 18g) was weighed accurately, dissolved in distilled water to make one liter and then filtered off to get a clear solution. Sea water was taken in the small tank and shrimp eggs were added to the one side of the divided tank and the side was covered. The shrimps were allowed for two days to hatch and mature as nauplii (larvae). The hatched shrimps were attracted to the lamp through the perforations in the dam and they were taken for bioassay. 20 clean test tubes were taken, 10 of which were for the samples in five concentrations (two test tubes for each concentration) and 10 for control test. Then 4ml of seawater was given to each of the test tubes. Then with the help of the micropipette, specific volumes (2, 4, 8, 16 and 32µl) of extract solution were transferred from the stock to the test tubes to get final sample concentrations of 10, 20, 40, 80 and 160µg/ml respectively. Sea water was added to adjust the volume of each test tube to10 ml. For the control, same volumes of DMSO (as in the sample test tubes) were taken in the rest of the 10 test tubes. Finally with the help of a Pasteur pipette 10 living shrimps were transferred to each of the test tubes. These were then incubated at 29^{o}C for 24 h in a water bath, after which each tube was examined and the surviving brine shrimps were counted and recorded. The percentage of mortality was calculated at each concentration. The LC_{50} and LC_{90} values were calculated with best fit line by using Microsoft Excel 2007.

Table 1: Phytochemical investigation of ethanolic extract of *A. scholaris* bark

Extract	Steroid	Alkaloid	Reducing sugar	Tannin	Gum	Flavonoid	Saponin
Ethanol extract of *A. scholaris* bark	-	+	+	+	-	-	+

+ = Presence - = Absence

Table 2: Effect of ethanolic extract of *A. scholaris* bark on castor oil induced diarrhea in mice

Group	Dose (/kg, p.o.)	Mean Latent Period in hr ± SEM	Mean Number of Stools in 4 hrs ± SEM
I Control	1% tween 80 solution in water, 10ml	0.686 ± 0.103	3.8 ± 0.732
II Positive control	Loperamide, 50mg	1.52 ± 0.355**	1.15 ± 0.427*
III Test group	Ethanolic extract of *A. scholaris* bark, 250mg	1.09 ± 0.312**	1.67 ± 0.506*

Values are expressed as mean ± SEM (n=5); **: P<0.001; *: P<0.01 vs control; p.o.: per oral.

Table 3: Result of Brine shrimp lethality bioassay of ethanolic extract of *A. scholaris* bark

Test sample	Conc. (µg/ml)	Log (Conc.)	No. of alive shrimp	% mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Ethanolic extract of *A. scholaris* bark	10	1.0	7	30	22.25	95.28
	20	1.3	6	40		
	40	1.6	3	70		
	80	1.9	1	90		
	160	2.2	0	100		

RESULTS

The ethanolic extract of *A. scholaris* was subjected to qualitative phytochemical tests for detection of different classes of biologically active chemical compounds and the results are summarized in the Table 1. Alkaloid, reducing sugar, tannin and saponin were present; however, steroid, gum and flavonoid were not found in the extract.

Ethanolic extract of *A. scholaris* caused an increase in mean latent period (1.09 hr) for diarrheal episode to ensue at 250mg/kg dose compared to the standard antidiarrheal drug loperamide (mean latent period 1.52 hr) and the results were statistically significant (Table 2). It also decreased the frequency of defecation (mean number of stools: 1.67) which was comparable to standard drug (1.15 hr).

In brine shrimp lethality bioassay, the crude extract of *A. scholaris* bark showed lethality indicating the biological activity of the extract. The percentage of mortality vs log concentration was plotted and a best fit line was obtained using Microsoft Excel 2007. From the equation of the line, the LC_{50} and LC_{90} of the test sample were found to be 22.25 and 95.28µg/ml, respectively (Table 3).

DISCUSSION

Antidiarrheal activity of the ethanolic extract of *A. scholaris* was tested using the castor oil induced diarrheal model in mice. Castor oil, which is used to induce diarrhea in mice, mixes with bile and pancreatic enzymes (e.g., lipase) and is degraded into ricinoleic acid and glycerin upon oral administration. Ricinoleic acid remains in the intestine and produces its anti-absorptive or secretory effect. It also forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The salts, formed as such behave like soap or surfactant within the gut and at the mucosal surface[9,11]. The most-agreed view is that ricinoleate salts stimulate the

intestinal epithelial cell's adenyl cyclase[12] or release prostaglandin and platelet activating factor, which result in inflammation of the intestinal mucosa and an increase in the net secretion of water and electrolytes in the small intestine[13,14]. The ethanolic extract of *A. scholaris* significantly delayed the onset of diarrhea and decreased the frequency of defecation in mice at the dose of 250 mg/kg of body weight. On the basis of the results of castor oil induced diarrhea, it can be concluded that the ethanolic extract of *A. scholaris* possesses a significant antidiarrheal activity.

The brine shrimp lethality bioassay can be recommended as a guide for the detection of antitumor and pesticidal compounds because of its simplicity and low cost. It indicates cytotoxicity as well as a wide range of pharmacological activities of plant extracts[15]. The test is based on whether the brine shrimps are dead or alive at the end of the test or on the ability to kill laboratory cultured *Artemia* nauplii[16]. From the results of cytotoxicity test of ethanolic extract of *A. scholaris,* an approximate linear correlation was found between logarithms of concentration versus percentage of mortality. The LC_{50} and LC_{90} calculated from the best-fit line equation were very promising. Thus, the results tend to suggest its possible cytotoxic activity.

In conclusion, it can be suggested that the crude ethanolic extract of *A. scholaris* bark possesses antidiarrheal and cytotoxic properties, which correlate well with the traditional uses of the plant. Therefore, further researches are essential to find out the active principle(s) responsible for those activities.

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