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THROMBOLYTIC, ANALGESIC AND ANTIDIARRHOEAL ACTIVITIES OF METHANOL EXTRACT OF *BAUHINIA ACUMINATA* LINN

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Abstract: *The present study was designed to evaluate the analgesic and antidiarrheal activities of a methanol extract and thrombolytic activity of methanol extract and its different partitionates obtained from the leaves of Bauhinia acuminata Linn. The analgesic activity was determined on mice model for its central and peripheral pharmacological actions using acetic acid-induced writhing and tail immersion tests, respectively and antidiarrheal effect was assessed by castor oil-induced diarrhea model at 200 and 400 mg/kg b.w. In in vitro thrombolytic study, the petroleum ether soluble materials of methanol extract revealed highest thrombolytic activity with clot lysis value of 46.66±0.67% as compared to 63.26±0.47% exhibited by the standard streptokinase. The extract significantly (P< 0.05) attenuated the acetic acid-induced writhing with the highest activity observed at 400 mg/kg b.w. (41.43%) comparable to that of the standard drug, diclofenac sodium (47.62%). A significant dose-dependent increase (P< 0.05) of latency period was also observed in the tail immersion method. During the castor oil-induced diarrheal assay, the extract showed significant (P<0.05) and dose dependant antidiarrheal effect. These findings indicate that sthe extract has potential thrombolytic, analgesic and antidiarrheal activity which support the folkloric claim and thus have great potential as a source of natural products.*

Keywords: *Bauhinia acuminata; Thrombolytic; Analgesic; Antidiarrheal.*

Introduction

Medicinal plants are important to combat diseases from the dawn of civilization[1]. Herbal medicine is widely used by 75 - 80% of the world population, mainly in the developing countries, for primary health care[2]. This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available[3]. According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times. *Bauhinia acuminata* Linn. (Common Name- White orchid; Family- Fabaceae) is a species of flowering shrub native to the tropical southeastern Asia. Different parts (dried leaf, flower bud and a decoction of the root and bark) of *B. acuminata* are used by the African doctors. The Indian Vaiydas recommends the bark and leaves of *B. acuminata*

for treating biliousness[4]. The leaves possess antidiabetic property[5]. While in India the leaves and bark of this plant is used for treating venereal diseases and asthma attack[4]. Moreover, all the part of the plant is recommended in combination with other drugs for the treatment of snake bite and scorpion-sting. Previous phytochemical screening of *B. acuminata* revealed the presence of kaempferol-7-O-rhamnoside, kaempferol-3-O-glucoside, quercetin-3-O-glucoside and quercetin-3-O-rutinoside[6]. In contribution of our ongoing efforts to study medicinal plants of Bangladesh[7,8,9], the present study has been undertaken and we, herein, report the analgesic, antidiarrheal and thrombolytic properties of the leaves of *B. acuminata* for the first time.

Materials and Methods

Plant material and extraction: The leaves of *B. acuminata* were collected from Khulna and a voucher specimen (DUSH-10775) for this plant sample has been deposited in the Department of Botany, University of Dhaka for future reference. The collected leaves were sun dried for several days and then oven dried for 24 hours at 40° C to facilitate grinding. The powdered leaves (600 gm) of *B. acuminata* was extracted with 1.5 L methanol for 7 days at room temperature and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated by using a rotary evaporator at reduced temperature (40-45° C) and pressure. The concetrated methanol extract was partitionated by the modified Kupchan method[10] and the resultant patitionates i.e., methanol extract (ME), pet ether (PE), carbon tetrachloride (CT), chloroform (CL) and aqueous (AQ) soluble materials were used for thrombolytic activity.

Animals: Swiss albino mice (either sex), weighting 23-25 g, bred in the Animal House of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh were used for the experiments. All the animals were acclimatized to laboratory condition one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 ± 2^0C, and 12 h light dark cycle). The animals were fed with standard diet (ICDDRB, B formulated) and had free access to tap water but were fasted 12 h prior to each experiment. Standard protocols for animal experiment were followed to minimize discomfort.

Drugs: The drugs and chemicals used in this study include Streptokinase, 0.6% acetic acid, diclofenac sodium, tramadol, 1% Tween 80, loperamide

Measurement of thrombolytic activity

The thrombolytic activity was assessed by evaluating their ability to break the clot of human blood following the method developed by Daginawala[11]. Here streptokinase (SK) was used as the standard substance and the percentage of weight loss of clot after the application of extract solution was taken as the functional indication of thrombolytic activity.

Streptokinase (SK): Commercially available lyophilized Alteplase (Streptokinase) vial of 15,00,000 IU was collected and 5 ml sterile distilled water was added and mixed

properly. This suspension was used as a stock from which 100 µl (30,000 IU) was used for *in vitro* thrombolysis.

Measurement of analgesic activity

Acetic acid-induced writhing method: The method developed by Koster[12] was employed for this test. Here four groups of 4 mice each were pretreated with the methanol extract (200 mg/kg, 400 mg/kg b.w.), diclofenac sodium (50 mg/kg b.w.) and normal saline (20 ml/kg b.w.), respectively. Forty five minutes later each mouse was injected with 0.7% acetic acid at a dose of (10 ml/kg) body weight. The number of writhing responses was recorded for each animal during a subsequent 5 min period after 15 min of intraperitoneal administration of acetic acid and the mean abdominal writhing for each group was obtained. The percentage inhibition was calculated using the formula: Inhibition (%) = [(Mean number of writhing of control) – (Mean number of writhing of test)] / (Mean number of writhing of control) x 100.

b) Tail immersion test: The tail immersion test was used to measure response latency according to the method[13]. The temperature of the warm water was maintained at (55 ± 2 °C). Mice were divided into 4 groups of 4 animals each. Group 1, the control group received normal saline, p.o. (10 ml/kg b.w.); group 2, the standard group received tramadol (10 mg/kg b.w.). Group 3 and 4 received crude extract (200 and 400 mg/kg b.w.). The lower 5 cm portion of the tail is marked. This part of the tail is immersed into the warm water (55 ± 2 °C). Within a few seconds the rat reacts by withdrawing the tail. The reaction time is recorded in 0.5 seconds units by a stopwatch. After each determination the tail is carefully dried. The reaction time is determined before and periodically such as 0, 30, 60 and 90 minutes after oral administration of the test and standard substances. The cut-off time for tail immersion latency was set at 15 seconds.

Measurement of Antidiarrhoeal activity

Antidiarrhoeal activity of extract of *B. acuminata* was tested by using castor oil-induced method in mice[14] with slight modification. Sixteen Swiss albino mice were randomly divided into four groups (n=4). Control group received 1% Tween 80 in water of 10 ml/kg of body weights, positive control group received loperamide at 50 mg/kg b.w. as standard drug and test groups received the extracts at the doses of 200 mg and 400 mg/kg body weight. Mice were housed in separate cages having paper placed at the bottom for collection of fecal matters. Diarrhea was induced in the mice by oral administration of castor oil (1 ml/mice). Extract and drugs were given orally 1 hr before the administration of castor oil. During an observation period of 5 hours, the total number of fecal output by the animals were recorded. Percent inhibition of defecation in mice was calculated by using the following equation: % inhibition = {(Mean defecation of control- Mean defecation of test sample)/ Mean defecation of control} × 100

Statistical analysis: Statistical analysis was conducted by using GraphPad InStat 3.05. All the values are presented as mean ± SEM. Student's *t*-test was used to determine a significant difference between the control group and experimental groups, where p values < 0.05 were considered to be statistically significant.

Result

The methanol extract of *B. acuminata* and its different partitionates were assessed for thrombolytic activity as a part of the discovery of cardio protective drugs from natural resources and the results are presented in Table 1. Addition of 100 µl SK, a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37° C, showed 63.26±0.47% lysis of clot. On the other hand, distilled water, treated as negative control, exhibited a negligible percentage of lysis of clot (2.32±0.41%). The mean difference of percentage in clot lysis between the positive and negative control was found to statistically significant ($p < 0.01$). In this study, the petroleum ether soluble fraction of *B. acuminata* exhibited highest thrombolytic activity (46.66±0.67%).

Table 1: Thrombolytic activity (in terms of % clot lysis) of *B. acuminate*

Sample	% of clot lysis
Blank	2.32±0.41
SK	63.26±0.47
ME	30.26±0.34
PE	46.66±0.67
CT	24.72±0.51
CL	10.93±0.25
AQ	11.64±0.19

SK = streptokinase, ME= Methanol extract, PE= Petroleum ether soluble fraction, CT= Carbon tetrachloride soluble fraction, CL= Chloroform soluble fraction, AQ= Aqueous soluble fraction.

Oral administration of methanol extract *B. acuminata* caused significant inhibition of writhing effect in mice induced by the acetic acid. At both doses, the extract inhibited the acetic acid-induced writhing. At 200 mg/kg b.w. the extract inhibited 37.77 % writhing while at 400 mg/kg b.w. it was 41.43%. The standard analgesic drug, diclofenac sodium caused 47.62% inhibition (Table 2).

Table 2: Analgesic activity of *B. acuminata* in acetic acid induced writhing test

Groups	Dose (mg/kg)	Number of writhing (mean ± SEM)	Inhibition (%)
Control (Vehicle)	-	18.27±1.68	-
Diclofenac Sodium	50	9.57± 1.44	47.62 **
Methanol extract of *B. acuminata*	200	11.37±1.22	37.77 *
	400	10.70±0.41	41.43 **

All values are expressed as mean ± SEM; n=4, * P<0.05, **P<0.01, significant compared to control.

The extract of *B. acuminata* when administered orally to the mice showed significant analgesic activity in tail immersion method as supported by increase in latency time. The increase in latency was found to be dose dependant (Table 3).

Table 3: Analgesic activity of *B. acuminata* in tail immersion test

Groups	Dose (mg/kg)	Reaction time in seconds at time			
		0 min	30 min	60 min	90 min
Control (Vehicle)	-	4.22±1.2	4.61±0.63	3.10±0.78	2.67±0.07
Tramadol	10	8.42±1.77	8.83±0.17 **	5.77±0.15 *	3.27±0.12 **
Methanol extract of *B. acuminata*	200	4.33±1.24	4.15±0.42	5.38±0.28 *	2.71±0.33
	400	4.76±0.24	5.34±0.1.40	5.47±0.64 *	3.11±0.30

All values are expressed as mean ± SEM; n=4, * P<0.05, **P<0.01, significant compared to control.

In the castor oil-induced diarrheoa, the extract of *B. acuminata* produced a marked antidiarrheal effect in the rats, as shown in Table 4. The antidiarrheal activity was found to be dose dependant.

Table 4: Antidiarrheal activity (in terms of % inhibition) of *B. acuminata*

Groups	Dose (mg/kg)	Number of feces	Inhibition of diarrhoea (%)
Control (Vehicle)	-	8.27±1.78	-
Loperamide	50	2.45± 0.41	70.37 **
Methanol extract of *B. acuminata*	200	3.0±1.22	48.72 *
	400	2.67±0.41	57.71 *

All values are expressed as mean ± SEM; n=6, * P<0.05, ** P<0.01, significant compared to control.

Discussion

The results demonstrated significant thrombolytic, analgesic and antidiarrheal activities of the methanol extract of *B. acuminata*. These indicated that this plant could be a potential source for discovery of new thrombolytic, analgesic and antidiarrheal "leads" for drug development. These findings support its traditional claims and provides a scientific basis for thrombolytic, analgesic and antidiarrheal effect of *B. acuminata*. However, further detailed studies are necessary to identify the active principle(s) and elucidate the mechanism(s) of extract behind these pharmacological properties.

Conclusion

In in vitro thrombolytic study, the petroleum ether soluble materials of methanol extract revealed highest thrombolytic activity with clot lysis value of 46.66±0.67% as compared to 63.26±0.47% exhibited by the standard streptokinase. The extract significantly (P< 0.05) attenuated the acetic acid-induced writhing with the highest activity observed at 400 mg/kg b.w. (41.43%) comparable to that of the standard drug, diclofenac sodium (47.62%). A significant dose-dependent increase (P< 0.05) of latency period was also observed in the tail immersion method. During the castor oil-induced diarrheal assay, the extract showed significant (P<0.05) and dose dependant antidiarrheal effect.

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