

ANTI-DIARRHEAL ACTIVITY AND ACUTE TOXICITY OF METHANOLIC BARK EXTRACT OF *Adenanthera pavonina* LINN (FABACEAE) AND ITS ELEMENTAL COMPOSITION

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Abstract

In folk and traditional medicine in Bangladesh, Adenanthera pavonina L. has been used for the treatment of asthma, diarrhea, gout, inflammations, rheumatism, tumour, ulcers and as a tonic. In castor oil induced diarrhea model on rat, the methanol extract of A. pavonina bark has significantly reduced the cumulative wet fecal mass in the dose dependant protection. At the doses of 500 mg/kg body weight found 17.91% reduction and at 1000 mg/kg body weight found 34.32% reduction in comparison to the control. In acute toxicity study on mice, the LD₅₀ value of the methanolic extract was found to be 1453.44 mg/kg. The elements N, P, K, S, Ca and Mg were found to be the highest amount of the powdered bark sample. The observed activities as well as mineral composition of the bark powder possesses pharmacological potential to develop natural compounds based pharmaceutical products and also justifies its use in traditional ayurvedic form of medicine.

Key words: Fabaceae, Adenanthera pavonina, Anti-diarrheal activity, Castor oil, Acute toxicity, Elemental compositions.

Adenanthera pavonina Linn'nın (Fabaceae) Metanollü Kabuk Ekstresinin Antidiyareyik aktivitesi, Akut Toksisitesi ve Bitkinin Elementel Kompozisyonu

Adenanthera pavonina L. Bangladeş'te halk arasında ve geleneksel tedavide astım, diyare, gut, inflamasyonlar, romatizma, tumor, ülserlerin tedavisinde ve tonik olarak kullanılmaktadır. Sıçanlarda hint yağı ile oluşturulan diyare modelinde, A. pavonina kabuğunun metanollü ekstresi kümülatif yaş feçes kütlesini doza bağlı olarak belirgin olarak azaltmıştır. Kontrol grubu ile karşılaştırıldığında, 500 mg/kg vücut ağırlığında azalma % 17.91 ve 1000 mg/kg vücut ağırlığında % 34.32 azalma bulunmuştur. Fareler üzerinde yapılan akut toksisite çalışmasında, metanollü ekstrenin LD₅₀ değeri 1453.44 mg/kg olarak bulunmuştur. Toz edilmiş kabuk örneğinde N, P, K, S, Ca ve Mg elementlerinin yüksek miktarlarda olduğu bulunmuştur. Gözlenen aktiviteler ve bunun yanında kabuğun mineral kompozisyonu doğal bileşiklere dayalı farmasötik ürün geliştirmek için farmakolojik potansiyeli olduğunu göstermiş ve aynı zamanda ayurvedik tıpta geleneksel kullanımını desteklemiştir.

Anahtar kelimeler: Fabaceae, Adenanthera pavonina, Antidiyareyik aktivite, Hint yağı, Akut toksisite, Elemental kompozisyon.

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INTRODUCTION

Plants and plant extracts have been used for centuries throughout the world in traditional cures and herbal remedies and as homeopathic medicines. Different medicinal plant parts are used for extract as raw drugs and they possess varied medicinal properties. The phytochemical research based on ethno-pharmacological information is generally considered to be an effective approach in the discovery of new bioactive principles (1). A need is therefore felt to search newer drug, which are highly effective at non toxic doses, cheaper economically and do not have severe side effect.

Adenanthera pavonina L. (Fabaceae) commonly known in Bangladesh as “Rakta Kombol”, is an important medicinal plant native to tropical Asia, Western and Eastern Africa as well as in most islands of both the Pacific and Caribbean regions. Bark of this plant is dark brown to greyish in colour. Various parts of this plant have also been used in traditional medicine for the treatment of asthma, boil, diarrhea, gout, inflammations, rheumatism, tumor, ulcers and as a tonic (2-6). The literature revealed various secondary metabolites mainly flavonoids, steroids, saponins triterpenoids and glycosides are present in the plant. (7-14). The analgesic, anti-inflammatory, antibacterial, antifungal, antioxidant, cytotoxic and blood pressure reducing activities of the leaf and seed extracts have been reported (14-21).

Diarrhoea and the associated fecal urgency results from an imbalance between the absorptive and secretory mechanism in the intestinal tract accompanied by hypermotility. This results in excess loss of fluid and electrolytes in feces (22). Diarrhoea may be acute and severe. It can be very severe in infants and elderly people because of the risk of some potentially fetal dehydration. Diarrhoea occurs worldwide and results in significant morbidity and mortality (23). Since long time our local herbalists (local name: Cabiraj and Hakim) have depended on medicinal plants as a reliable means of treating diarrhoea. Hence the use of medicinal plants that possess anti-diarrhoeal activities has been explored as a measure that could be of benefit in combating widespread diarrhoea infections especially in third world countries (24) including Bangladesh. On the basis of scientific validation, a number of plants have been chemically and biologically evaluated for their anti-diarrhoeal properties. However, the effectiveness of many anti-diarrheal plant used in traditional medicines has not been validated scientifically. The search of a new anti-diarrheal medicine in this respect, the present work was undertaken to evaluate anti-diarrhea activity, to determine the LD₅₀ value of the methanolic bark extract.

EXPERIMENTAL

Plant material and extraction

The bark of *Adenanthera pavonina* L. were collected from the Jahangirnagar University campus, Savar, Dhaka-1342, Bangladesh, at the mature stage. A voucher specimen (accession No.-DACB 34196) was deposited in the National Herbarium of Bangladesh. Collected plant parts, after cutting into small pieces and then dried in shade at temperature between 21-30° C for 15-20 days. The cutting pieces were pulverized by a mechanical grinder and sieved through 60 mesh sieve to obtain fine powder. One kilogram of this bark powder was extracted successively with petroleum ether, dichloromethane, ethyl acetate and methanol (ME) by cold extraction process. All the extracts were filtered off, evaporated to dryness (40° C) under reduced pressure by rotary evaporator and refrigerated at 4° C temperature. The methanol extract of *A. pavonona* bark extract (yield 3.0% w/w) was used in this study.

Drugs and reagents

The solvents petroleum ether, dichloromethane, ethyl acetate, methanol, acetic acid (the BDH laboratory grade), loperamide, tween-80 (Sigma Chemical Co. St. Louis, MO, USA), Castor oil (Qualikems fine chemicals Pvt, Ltd. New Delhi, India) and saline (0.9% NaCl

solution) were used. Rest of the chemicals & drugs were used from BDH and E-Merck of analytical grade.

Animals

Male *albino* rats (150-200 g) and *Swiss albino* mice (weighing 30±2 g.) of either sex were collected from BCSIR laboratories, Chittagong, Bangladesh. The animals were acclimatized to room temperature (28±5°C) with a relative humidity of 55±5% in a standard wire meshed plastic cages for one week prior to performing the experiment. During the entire period of study, the animals were supplied with standard pellet diet and water *ad libitum*. In this study, the entire experimental animals were carried out according to the guidelines of Ethical Review Committee, Faculty of Pharmacy, University of Dhaka, Bangladesh.

Castor oil-induced diarrhea

Castor oil-induced diarrhea model was carried out using the method described by Shoba and Thomas (25). Twenty rats were randomly divided into four equal groups with five in each of either sex. The control group received only distilled water (10 ml/kg), positive control group received loperamide 2 mg/kg and two graded dose groups received methanolic bark extract at the doses of 500 and 1000 mg/kg body weight. The animals were housed in separate cages with papers placed underneath, which was changed every hour. Diarrhea was induced in rats by oral administration of castor oil (3.0 ml/rat). Extract and drugs were given orally 1 hour before the administration of standard dose 3.0 ml of castor oil. The numbers of both hard and soft pellets were counted at every hour over 6 hour period for each rat. Diarrhea was defined as the presence in the stool with fluid material that stained the paper placed beneath the cages. Percent inhibition (PI) was calculated as follows:

$$PI = \frac{\text{Mean defecation (control group - treated group)}}{\text{Mean defecation of control group}} \times 100$$

Acute toxicity study

In the acute toxicity study model (26), the mice were administered orally with test samples (ME extract) in five different doses of 900, 1125, 1350, 1575 and 1800 mg/kg body weight. Mortality rates in the treated mice were recorded 24 h. after the treatment and any behavioral changes, locomotion, convulsions and mortality were observed for next 14 days. The number of animals dying during the period was noted. The toxicological effects were observed in terms of mortality and expressed as LD₅₀. The probit analyses were calculated by the Finney method (27) for determining the LD₅₀ value.

Digestion and instrumentation for elemental analysis

A. pavonina bark powder (0.5 g) was dissolve in 5 ml of acid mixture (HNO₃:HClO₄=5:1) in a Kjeletec Autoplus II mineralization set till to complete mineralization (about 4-5 hours) and volume was made up to 15 ml with distilled water, then filter in a dry flask. The instrument Varian Cary 50 Conc UV-Visible spectrophotometer was used for the analysis of P, S and B, Chemito AA 203 atomic absorption spectrophotometer for K, Ca, Mg, Cu, Fe, Mn and Zn and Varian Spectra AA 55B atomic absorption spectrophotometer for Pb, Cd, Ni and Cr were used. N₂O flow gas was used with oxy-acetylene flame and the specific Hollow Cathode Lamp for each element was used in the atomic absorption spectrophotometer. The minimum detection limits of the elements were determined at ppm level. N was estimated by Kjeldhal method (28).

Data analysis

The results were analyzed for anti-diarrheal and acute toxicity activity by using SPSS version 13 and Biostat 2007 software respectively with statistical significance (p<0.05) using

one-way analysis of variance (ANOVA) followed by student 't' test. The results were expressed as mean \pm SEM.

RESULTS

Effect of ME extract on diarrhea

The percentage yield of ME extract of *A. pavonina* was 3.0%. In the castor oil-induced diarrhea experiment, the ME extract of *A. pavonina* produced a marked anti-diarrheal effect in the rats, as shown in Table 1. At doses of 500 and 1000 mg/kg body weight a significant ($p < 0.05$) reduction of diarrhea was observed representing 17.91% and 34.32% inhibition respectively compared to control. Loperamide (2 mg/kg) inhibited the castor oil induced diarrhea by 56.71%. The highest dose (1000 mg/kg body weight) produced inhibition of defecation that compared favorably with loperamide. However, both the doses were shown to reduce the total number of feces in a dose dependant manner in comparison to control.

Table 1. Effect of methanolic bark extract (ME) of *Adenanthera pavonina* on castor oil-induced diarrhea in mice.

Treatment	Dose <i>p.o.</i> (mg/kg body weight)	Total Number of feces	Diarrhoeal Episode (SF+DF)	Inhibition Defecation (SF+DF)
Control	2 ml/rat	24.25 \pm 0.94	16.75 \pm 0.94	Nil
Loperamide	2mg/kg	11.75 \pm 1.10	7.25 \pm 1.25	56.71
ME extract	500mg/kg	20.75 \pm 1.10	13.75 \pm 1.31	17.91
ME extract	1000 mg/kg	20.50 \pm 1.25	11.0 \pm 0.70	34.32
		F Value=22.966 df=15	F Value =13.951 df=15	

Values are expressed as mean \pm SEM (n=5), $p < 0.05$ when compared to control, df= degree of freedom, SF=Soft pellet with minimum Fluid, DF=Deformed pellet with excess fluid.

Acute toxicity

Acute toxicity is involved in estimation of LD₅₀ which is usually an initial screening step in the assessment and evaluation of the toxic characteristics of a substance. Oral consumption of ME extract produce 80%, 60%, 40% and 20% mortality at the doses of 1800 mg/kg, 1575 mg/kg, 1350 mg/kg and 1125 mg/kg body weight respectively (Table 2). Treated animals those were alive, showed some symptoms associated with toxicity like slow movement and drowsiness at these doses level. The LD₅₀ value of the test substances was found 1453.44 mg/kg body weight in mice (Fig. 1).

Table 2. Mortality percentage and LD₅₀ value of ME extract of *Adenanthera pavonina*

Doses (mg/kg body weight)	Log dose	Total Mice	Dead	Alive	Mortality Percentage	Probit	LD ₅₀
1800	3.255	5	4	1	80	5.84	1453.44 mg/kg
1575	3.197	5	3	2	60	5.25	
1350	3.130	5	2	3	40	4.74	
1125	3.051	5	1	4	20	4.17	
900	2.954	5	0	5	0	0	

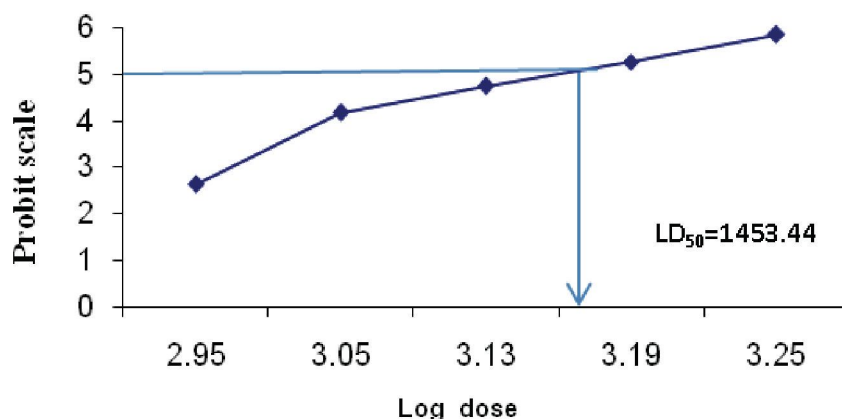


Figure 1. LD₅₀ value of the ME extract of *A. pavonina* bark in mice.

Elemental compositions

The concentrations of 16 elements in *Adenanthera pavonina* expressed in dry weight basis are listed in Table 3. Each result is the mean value of three replicates. In the experimental data the concentration of elements decreases as follow: N>Ca>P>K> Mg>S >Mn>Zn>B>Fe>Pb>Ni>Cr>Co>Cd. The elements like Pb, Cd, Ni, Cr and Co were found in trace amount whereas N, Ca, Mg and Fe were found in the highest amount.

DISCUSSION

Since ancient times, diarrhoea has been treated orally with several medicinal plants or their extracts based on folkloric medicine. Vast majority of the people in developing countries including Bangladesh still rely on herbal drugs for the management of diarrhoea despite the availability of allopathic medicine. In the present study the ME extract inhibited significantly (p<0.05) castor oil-induced diarrhea in rats in dose depended manner which is comparable to those of the standard drug loperamide. The pharmacological effect of loperamide is due to its

anti-motility and anti-secretory properties (29). This drug is widely employed against diarrhoea disorders which effectively protect diarrhoea induced by castor oil, prostaglandin and cholera toxin (30). Several mechanisms had been previously proposed to explain the diarrheal effect of castor oil. The ethanolic leaf extract of *A. pavonina* was studied on rat by castor oil induced diarrhea (20). Most recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil (31). The diarrhea lasts for at least 8 h (32) and is a consequence of the action of ricinoleic acid liberated from castor oil by lipase enzymes (22). The freed ricinoleic acid irritates the intestinal mucosa causing inflammation and release of prostaglandins and nitric oxide, which stimulate gastrointestinal secretion, motility, epithelial permeability and edema of the intestinal mucosa (33-34) thereby preventing the re-absorption of sodium, chloride and water.

Table 3. Elemental composition of *Adenanthera pavonina*.

Sl No.	Elements	Concentration (ppm)
1	N	11900±0.02
2	P	2300± 0.01
3	K	1800±0.00
4	S	1400± 0.02
5	Ca	5500±0.02
6	Mg	1500±0.01
7	B	60.0 ±1.0
8	Cu	3.60±0.06
9	Fe	199±1.0
10	Mn	25.9±0.13
11	Zn	25.8± 0.03
12	Pb	12.0±0.10
13	Cd	0.60±0.03
14	Ni	4.80±0.09
15	Co	1.14±0.0
16	Cr	4.0±0.04

Each value is the mean ± SD of three replicates. P<0.01

The anti-dysentric and anti-diarrheal properties of medicinal plants have been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, sterols and reducing sugars (35). Flavonoids, present in the powder inhibit to release of autacoids and prostaglandins, thereby may inhibit motility and secretion induced by castor oil (36). Therefore, the anti-diarrhoeal activity in this study may be attributed to the presence of these compounds (13). The LD₅₀ value in the acute toxicity test in mice suggests that the bark extract may be generally regarded as safe with remote risk of acute intoxication. The elemental compositions indicating its nutritional value in the bark powder make it more valuable and may find possibility to use in the aurvedic preparation.

CONCLUSION

On the basis of the results of the present study, it can be concluded that the extract contains pharmacologically active compounds which possess anti-diarrheal properties. The observed activities of methanol extract on castor oil-induced diarrhoea and acute toxicity in mice as well as mineral composition of the bark powder justifies its use in traditional ayurvedic medicine. Further research is to be carried out to fractionate and purify the extract, in order to find out the compounds responsible for the activities.

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