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Diuretic and laxative activities of stem bark extracts of *Alstonia boonei* in rats

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ABSTRACT: **Background:** *Alstonia boonei* is a plant of medicinal importance especially in traditional medicine. It has been used for treating malaria, inflammatory disorders, rhematic pain, toothache, ulcer and diabetes in Nigeria. In the present study, we have reported the diuretic and laxative properties of stem bark extracts of the plant on male Sprague-Dawley rats. **Materials and Methods**: The experimental animals (rats) were grouped into 10 groups of 6 each comprising control group (administered normal saline), test groups (administered various concentrations of the extract- 250 and 500 mg/kg methanol extracts, 125 and 250 mg/kg of flavonoid, saponin and neutral alkaloid fractions) as well as a group administered standard drugs (25 mg/kg furosemide/sodium picosulfate). The mode of administration was oral and normal saline was used as the vehicle. **Results:** Only the 500 mg/kg methanol extracts and the saponin fraction significantly improved ($p<0.05$) the excretion of urine volume and electrolytes (Na^+, K^+ and Cl^-), the rest of the extracts did not exhibit diuretic properties. Even more, the ME fraction showed good natriutic index. All the fractions of the extract did not affect fecal production when compared to the control, however, compared to the standard drug the fractions have potential of controlling fecal incontinence. **Conclusions:** This study has shown that some fractions of *A. boonei* possess diuretic properties and can be used for the development of therapeutic interventions against kidney disorders, hypertension or congestive heart failure. Studies to determine its laxative property shows that based on the low fecal output, fractions of *A. boonei* can be used to tackle fecal incontinence.

Keywords: *Alstonia boonei*; Diuretic; Laxative; Fecal incontinence; Traditional medicine.

Introduction

Medicinal plants have been used for ages as the source of chemical compounds with potent therapeutic effects. A common example is the discovery of artemisinin from *Artemisia annua* for the treatment of

malaria (1). The discovery of chemical compounds with potent therapeutic effect from plants begins with crude extraction, either with polar or non-polar solvents, followed by assays to determine the activity of the crude extract on a particular disease-causing organism or a medical condition. A potent chemical compound with good activity on a disease-causing organism or a medical condition is then isolated and can be chemically synthesized. Despite the avalanche of compounds discovered from medicinal plants thus far, a lot more is yet to be discovered. Hence, there is need for more research in this area.

In clinical conditions like nephrotic syndrome, hypertension, cirrhosis, renal and heart failure, diuretics which are medicines taken in order to enhance the rate of flow of urine and electrolyte excretion, are needed to control the amount and/or composition of fluids in the body. The electrolytes targeted by diuretics involve both cations (Mg^{2+}, H^+, Na^+, K^+, and Ca^{2+}) and negatively charged ions ($H_2PO_4^-$, Cl^- and HCO_3^-), as well as uric acid. Common examples of diuretics in clinical practice includes; furosemide, hydrochlorothiazide, mannitol, bumetanide, chlorothiazide and amiloride (2, 3).

Laxatives stimulate evacuation of feces and can be used to manage constipation especially during aging. Constipation is a mild or chronic gastrointestinal disorder associated with infrequent bowel movements and might lead to restlessness, abdominal distension and gut obstruction (4, 5). The current drugs used in the management of constipation are insufficient leaving patients with unresolved symptoms of constipation and dissatisfaction.

Alstonia boonei (De Wild) belongs to the dogbane family Apocynaceae (6). It is a tree of 30-40m high by over 3m in girth, bole cylindrical and long to 27 m, with high narrow buttresses commonly called; Alstonia, cheesewood, timber trade — pattern wood, stoolwood in English and emien in French. In Nigeria, it is common in the southern part and commonly called égbū *or egbun* by the Igbos, ahùn by the Yorubas, Ukhu by the Edos while the Urhobo people call it Ukpukunu (7).

There are several reports that *A. boonei* expresses biological activity against various medical conditions, justifying its use in traditional medicine. For example, it has been used traditionally for treating malaria, inflammatory disorders, rhematic pain, toothache and ulcer in Nigeria. Stem bark extracts of the plant have been shown to be anti-diabetic by reducing blood glucose levels (8) while echitamine, a phytochemical from the stem bark of the plant has been reported as anti-hypertensive (9). Polar extracts from the stem and root bark of *A. boonei* are also known to be anti-inflammatory (10, 11). There are also several reports on the anti-malarial properties of this plant (12-14). Recently, the anti-oxidant and anti-bacterial properties of the plant has been reported (15)

However, despite several reports on the pharmacological properties of *A. boonei,* none has considered the diuretic and laxative properties of this plant. In this report, we have shown the diuretic and laxative properties of various extracts of the stem bark of *A. boonei*. This can form the basis for the development of drugs used in the management of kidney disorders, congestive heart failure and hypertension as well as constipation.

Materials and Methods

Plant material

The stem bark of *A. boonei* was harvested from an area in Umuekwune, Ngor-okpala, Imo State, Nigeria. Authentication of the plant was done by Dr. J. O. Ihuma, Department of Biological Sciences, Bingham University, Nigeria. The plant was allocated the voucher number, GA133-27522 and specimen deposited in the Department of Biological Sciences, Bingham University, Nigeria.

Methanol extract preparation

After harvesting the stem bark of *A. boonei*, it was chopped into tiny pieces, shade-dried at 22-23^{o}C, followed by milling into bristly powder. The non-polar solvent, n-hexane was first used to extract 1 kg of the powder for 12 hrs after which methanol extraction was conducted by maceration for 72 hrs. The process was repeated three times. Muslin cloth and Whattmann filter paper were used to get the filtrate of which

methanol was effectively removed under reduced pressure at 40ºC with a rotary evaporator, giving a total percentage yield of 14.8%.

Partition of methanol extracts into fractions

Saponin fraction preparation

Preparation of saponin fraction was done as described previously (16, 17). Briefly, water was used to extract 75g of concentrated defatted methanol extract followed by partition with n-butanol (3x500ml). After n-butanol partition, separating funnel was used to separate the aqueous partition followed by addition of diethyl ether to precipitate crude saponin mixture. Thereafter, decantation and centrifugation were used to collect the saponin fraction at a yield of 20.25%.

Preparation of flavonoid fraction

A mixture of methanol and water in the ratio 4:1 was used to extract powdered plant material in three replicates. After concentration of the filtrate to 10% of the initial quantity, 2 M H_2SO_4 was added to help in the extraction of flavonoids. It was then partitioned into layers with chloroform three times following a previous method (18). The chloroform layer was pooled together and concentrated under reduced pressure to give a total flavonoid yield of 9.2%.

Neutral alkaloid preparation

To obtain the neutral alkaloid fraction, we slightly modified a previous method described by (17, 19) according to the peculiarity of our laboratory procedures. Briefly, 1N H_2SO_4 was used to extract 50g of concentrated defatted methanol extract in triplicates. This was followed by basification with ammonia on an ice cold bath until a pH of 7.0 is obtained. Ethyl acetate was then used to partition the extract and the aqueous partition was separated using separating funnel. The ethyl acetate portion was then concentrated with the help of rotary evaporator to give a yield of 7.9% neutral alkaloid fraction.

Standard drugs

The reference drug used for the diuretic study was Lasix (furosemide) which is produced by Sanofi-aventis Pakistan Limited while Laxoberon (sodium picosulfate) produced by Merck (Private) Limited, Pakistan was used as the laxative reference drug.

Animals used for the research study

Ethical approval was obtained from the Research Ethics Committee of Pharmacy Department, CIIT, Abbottabad (PHM-0024/EC/M-4-5.15) and the experimental procedures concerning the experimental animals were according to the European Community guidelines (EEC Directive of 1986; 86/609/EEC) for animal use. Sixty male Sprague–Dawley rats (180 ± 10g) which were procured from the National Institute of Health (NIH), Islamabad, Pakistan and were made to acclimatize in cages for 2 weeks at the Animal Care unit of Pharmacy Department, COMSATS University Islamabad, Abbottabad Campus, Pakistan at regulated temperatures (22 ± 2°C) and light/dark cycle (12:12 h) with food and water *ad libitum.*

Mode of drug/extracts/fractions' administration

Normal saline was used as the vehicle for the administration of the extracts/fractions with the help of oral gavage. Administration was done based on the group of the rats as shown in Table 1.

Table 1: Experimental design for diuretic and laxative studies of plants

Group (n=6)	Dose (mg/kg b.wt)	Description	Treatment
1	25 ml/kg	Normal control	Normal saline
2	25	Standard drug	Furosemide/Laxoberon
3	250	*A. boonei*	Methanol extract
4	500	*A. boonei*	Methanol extract
5	125	*A. boonei*	Saponin fraction
6	250	*A. boonei*	Saponin fraction
7	125	*A. boonei*	Flavonoid fraction
8	250	*A. boonei*	Flavonoid fraction
9	125	*A. boonei*	Neutral Alkaloid fraction
10	250	*A. boonei*	Neutral Alkaloid fraction

Determination of diuretic activity

Diuretic activity was determined as described previously (3, 20). The animals were group into 10 with each group made up of 6 rats. The animals were not allowed to take food and water 18 hrs prior to the experiment. Table 1 shows the identity of the various groups. To determine the diuretic activity of the fractions, two rats per cage were placed in metabolic cages after administering the extracts. The metabolic cages are made in such a way that they can collect urine separately from feces. The volume of urine was determined 5 hrs after administration during which period the experimental animals were starved of water and food. Estimation of urinary electrolytes concentration, Na^+, K^+ and Cl^- was also carried out.

Urinary electrolytes determination

Conventional digestion method was used to prepare the urine sample according to (21). In a 50ml flask, 0.5 ml of urine was added followed by 5ml of concentrated HNO_3-H_2O_2 (2:1, v/v). The set up was placed on an electric hot plate and heated for 3 hrs at 80^0C until we obtained a clear supernatant digest. Then, the solution was made up to 10 ml with 2M HNO_3 and portions were placed in transparent glass vials for the analysis of Na^+ and K^+ using flame by the Atomic Absorption Spectrophotometer (AAnalyst 700 Perkin Elmer). Calibration graphs on the computer screen connected to the AAS were then used to determine the concentration of Na^+ and K^+.

To determine Cl^- concentration, Mohr's method was used with slight modifications as described by (22). The urine sample was diluted 20-fold with distilled water in flask and an indicator (1 ml of 5% potassium chromate) was included. The sample was then titrated with 0.02N silver nitrate. Red-brown color shows the end point of the titration.

Chloride ion concentration (mg/l) was calculated using the formula, $(V_s\text{-}V_b) \times N \times 35.5 \times 1000/$ ml sample,

where;

V_s = volume of $AgNO_3$ used for sample titration;
V_b = volume of $AgNO_3$ used for blank titration and
N = Normality of $AgNO_3$

Measurement of laxative activity

At least two weeks wash out period for the rats from the previous experiment was observed before laxative activity was determined as described by (23). The grouping of the animals as well as the mode of administration was as described earlier and in Table 1. Basically, the experiment commenced after the rats were fasted for 12 hrs however water was available *ad libitum*. Feces of the experimental animals were then collected and weighed at the 8th hr.

Data analysis

Experimental data were expressed as Mean ± S.E.M. GraphPad Prism was used for statistical analysis involving one-way ANOVA and Dunnett's multiple comparison tests at 95% confidence level.

Results

***A. boonei* extracts exhibit varying diuretic properties**

Apart from the group that received 500 mg/kg b.wt of *A. boonei* and the saponin fractions (SF), all other treated groups showed no significant difference ($p > 0.05$) in the urine volume excreted by the rats when compared to the control group after 5 hours (Fig. 1). However, compared to the standard diuretic drug, furosemide, the urine volume excreted by all the rats in the treated groups was significantly reduced at 95% level of confidence.

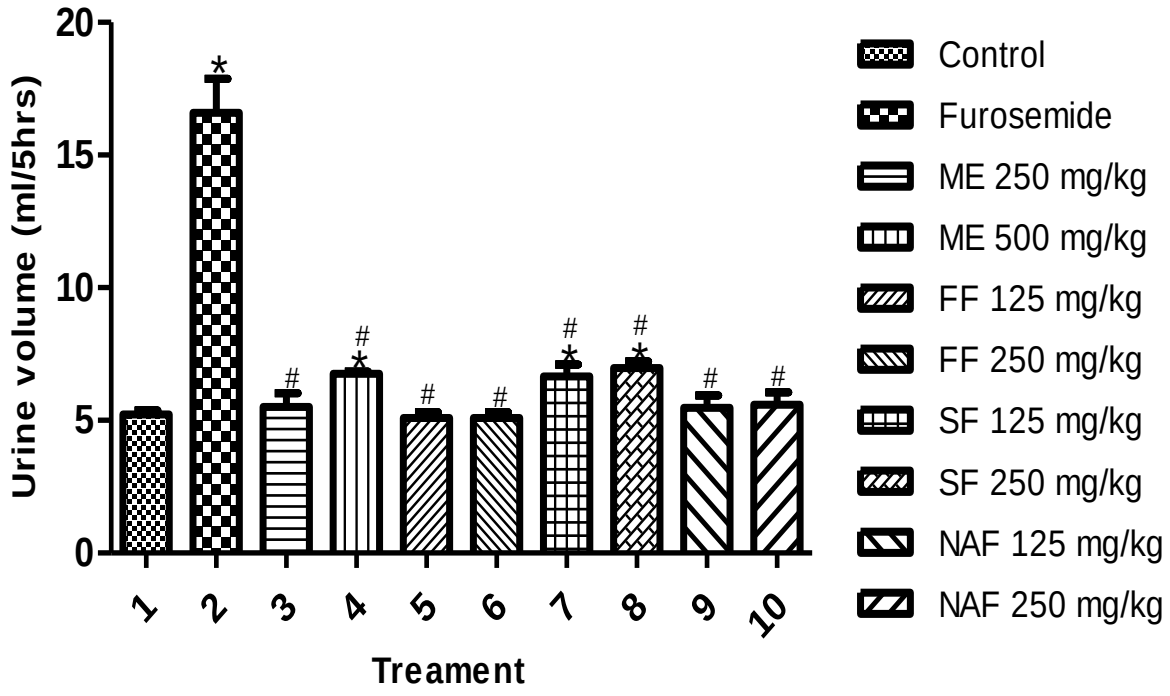

Fig. 1: Bar chart showing the volume of urine from experimental animals' 5 h post *A. boonei* extract/drug administration

The chart is based on data expressed in the form; mean ± SEM, * indicates difference from control group ($p < 0.05$), # shows difference from standard drug, furosemide ($p < 0.05$).

Effect of *A. boonei* extracts on urine electrolytes

Urine electrolyte levels of rats treated with the various extracts of *A. boonei* was measured after 5hrs of oral administration as shown on Table 2. There was significant increase in Na^+ and K^+ levels in groups treated with 500mg/kg b.wt of ME and 250mg/kg b.wt of SF ($p < 0.05$), while other treatments showed no significant difference ($p > 0.05$) when compared to the control group. All treated groups had significant increases ($p < 0.05$) in the levels of Cl^- when compared to the control group. However, furosemide treatment had significantly increased levels of urinary Na^+, K^+ and Cl^- ($p < 0.05$) when compared to the normal control and all the treatments.

***A. boonei* fractions do not affect fecal output**

The fecal output of experimental animals treated with the various fractions of *A. boonei* was not significantly different compared to the control after 8hrs of administration of extracts/drug ($p > 0.05$). On the corollary, treatment with the standard drug, sodium picosulfate revealed significantly increased fecal output compared to the control and treatment groups ($p < 0.05$, Fig.2).

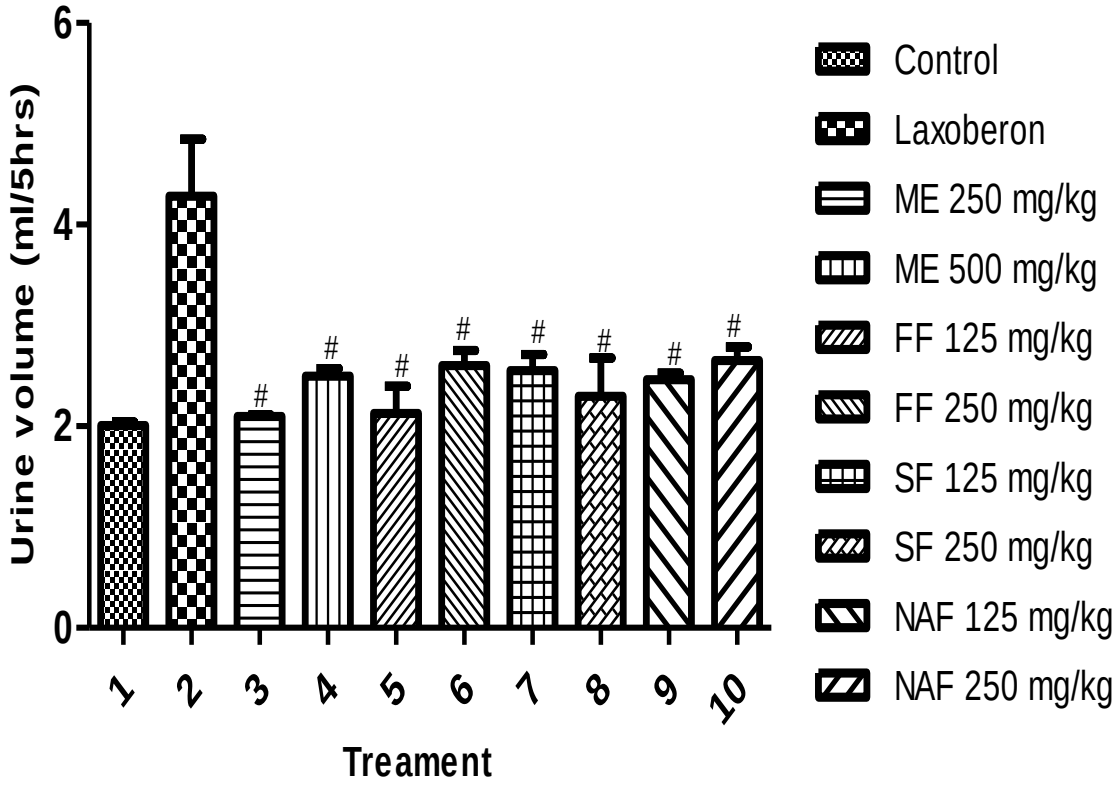

Fig. 2: Graphical representation of fecal output from the experimental animals 8 h after the administration of *A. boonei* extract/drug

The graph is based on data expressed in the form; mean ± SEM, * indicates difference from control group ($p < 0.05$), # shows difference from standard drug, Laxoberon (sodium picosulfate) ($p < 0.05$).

Table 2: Urinary electrolyte excretion of *A. boonei* extracts/fractions in rats after 5 hrs of oral administration

Group	Dose (mg/kg)	Diuretic index	Urine Electrolyte Concentration (mEq/L/5hrs)			Saluretic index			Na^+/ ratio	K^+
			Na^+	K^+	Cl^-	Na^+	K^+	Cl^-		
Normal Control		1.00	55.26 ± 0.14^{c}	25.11 ± 0.21^{de}	232.00 ± 4.04^{e}	-	-	-	2.20	
Furosemide	25	3.17	159.36 ± 14.16^{a}	80.41 ± 6.50^{a}	761.84 ± 44.84^{a}	2.88	3.20	3.28	1.98	
Methanol Extract	250	1.05	58.32 ± 0.91^{bc}	26.61 ± 0.16^{cde}	429.52 ± 2.38^{cd}	1.06	1.06	1.85	2.19	
	500	1.29	71.21 ± 1.14^{b}	32.58 ± 0.55^{bc}	515.90 ± 10.29^{b}	1.29	1.30	2.22	2.19	
Flavonoid Fraction	125	0.98	51.40 ± 2.12^{c}	24.52 ± 0.93^{e}	381.74 ± 21.04^{d}	0.93	0.98	1.65	2.10	
	250	0.98	53.34 ± 2.20^{c}	24.98 ± 0.95^{de}	385.35 ± 21.24^{d}	0.97	0.99	1.66	2.14	
Saponin Fraction	125	1.27	66.97 ± 1.96^{bc}	32.28 ± 1.10^{bcd}	482.57 ± 17.96^{bc}	1.21	1.29	2.08	2.07	
	250	1.33	72.63 ± 2.63^{b}	34.05 ± 1.40^{b}	523.36 ± 23.11^{b}	1.31	1.36	2.26	2.13	
N. Alkaloid Fraction	125	1.04	54.79 ± 2.72^{c}	26.11 ± 1.38^{cde}	413.79 ± 21.74^{d}	0.99	1.04	1.78	2.10	
	250	1.07	58.61 ± 2.02^{bc}	27.11 ± 1.01^{bcde}	434.47 ± 15.50^{cd}	1.06	1.08	1.87	2.16	

Data are expressed as mean ± SEM, Values not sharing the same letter as superscript are significantly different from control or standard drug ($P<0.05$). Saluretic index = mEq test group / mEq control group. Diuretic index = volume problem group/volume control group.

Discussion

Alstonia boonei is a plant of choice in African medicine for controlling various pathological conditions like intestinal colitis through its anti-oxidant potential (24). Leaf extracts of the plant has also been shown to be hepatoprotective against oxidative stress related diseases like tuberculosis (25).

Kidney disorders, hypertension and congestive heart failure are medical conditions that can be treated with drugs with diuretic properties (3). In this report, we have examined the diuretic and laxative properties of *A. boonei*. Result from the diuretic study showed that, rats in the group that received 500 mg/kg ME of *A. boonei* and the saponin fraction (SF) increased the volume of urine and electrolytes (Na, K and Cl) excreted by the rats. Thus, these fractions of *A. boonei* possesses diuretic activity. This result is in agreement with an earlier report on the diuretic activity of this plant (26). A wide range of phytochemicals are responsible for diuretic activity of plants which include alkaloids, glycosides, tannins, phenolics coumarins and triterpenoids (27). However, our study has revealed that the saponin fraction of *A. boonei* stem bark might be responsible for its diuretic activity.

Therefore, the diuretic potential of *A. boonei* validates its traditional use in the management of swellings, oedema and hypertension. The increased excretion of urine by the extracts suggests a positive diuretic effect in the treatment of hypertension. By increasing sodium excretion, extracellular fluid volume expansion (a common feature in hypertensive state) is reversed, thereby lowering blood pressure. Diuretics are useful in the management of persons with chronic kidney disease and cardiovascular disease (28, 29). The combination of diuretics and antihypertensive agents (ACE inhibitor or beta-blockers) leads to improved outcomes in the treatment of hypertension (29, 30).

The ratio of Na^+ to K^+ was not different in the group administered standard drug compared to that administered fractions of the plant extract. The fact that the Na^+/ K^+ ratio is slightly greater than 2 for the group treated with ME suggests that aside the diuretic potential of this extract, it also exhibits a good natriuretic index (31).

Despite some promising diuretic properties and good natriuretic index of some fractions of *A. boonei,* there was no significant impact of the fractions on fecal output compared to the control group. Compared to the standard drug used, the fecal output upon administration of the fractions was significantly reduced. This suggests that the fractions have the potential of controlling fecal incontinence.

Overall, this study has shown that some fractions of *A. boonei* possess diuretic properties and can be harnessed for the development of therapeutic interventions against kidney disorders, hypertension or congestive heart failure. Studies to determine its laxative property shows that based on the low fecal output, fractions of *A. boonei* can be used to tackle fecal incontinence. Further studies to isolate the actual bioactive compounds responsible for the above activities are required.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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