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Available also online at www.sciencedirect.com.

• Research Article

Evaluation of diuretic and laxative activity of hydro-alcoholic extract of *Desmostachya bipinnata* (L.) Stapf in rats

Upendarrao Golla[1,2], Praveen Kumar Gajam[2], Solomon Sunder Bhimathati[2]
1. Department of Biological Sciences, Indian Institute of Science Education and Research (IISER), Bhopal-462023, Madhya Pradesh, India
2. Department of Pharmacology, Faculty of Pharmacy, Bharat Institute of Technology, Hyderabad-501510, Andhra Pradesh, India

OBJECTIVE: In continuation to the growing evidence for therapeutical potential of *Desmostachya bipinnata* (Linn) Stapf, the current pharmacological study was carried out to evaluate the diuretic and laxative activity of its hydro-alcoholic extract in rats.
METHODS: The hydro-alcoholic extract of *D. bipinnata* whole plant was prepared by using Soxhlet extractor and subjected to analysis by standard preliminary phytochemical tests. Evaluation of both diuretic and laxative activity was carried out using standard methods as reported earlier. Frusemide (20 mg/kg) was served as positive control for diuretic activity and sennosides (10 mg/kg) served as negative control for laxative activity.
RESULTS: The hydro-alcoholic extract showed significant diuretic activity and was found to be the most potent in increasing the urinary output at 500 mg/kg when the effect was compared with that of the standard frusemide (P<0.01). Moreover, this extract was found to be most effective in increasing urinary electrolyte concentration (Na^+, K^+, and Cl^-) at both doses tested. Whereas the results for laxative activity showed minimal increase of feces output at the dose of 500 mg/kg and the increase was negligible when compared with that of the standard drug sennosides.
CONCLUSION: Altogether, the above significant findings validate and support its folkloric diuretic use and lend pharmacological credence to the ethno-medical use of this plant in traditional system of medicine, which demands further studies to investigate its active constituents, as well as its use and safety.
KEYWORDS: plant extracts; bioassay; diuretic; laxative; frusemide; sennosides; ethnomedicine; rats

http://dx.doi.org/10.1016/S2095-4964(14)60029-7
Golla U, Gajam PK, Bhimathati SS. Evaluation of diuretic and laxative activity of hydro-alcoholic extract of *Desmostachya bipinnata* (L.) Stapf in rats. *J Integr Med*. 2014; 12(4): 372–378.
Received January 21, 2014; accepted March 7, 2014.
Correspondence: Upendarrao Golla; Tel: +91-9584520895; E-mail: golla.upendarrao@gmail.com

1 Introduction

Medicinal plants can be significant sources of undiscovered chemical substances with potential therapeutic effects. In fact, the World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic healthcare needs[1]. Diuretics are drugs that increase the rate of urine flow and sodium excretion and are used to adjust the volume and/or composition of body fluids in a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis. Diuretics not only alter the excretion of Na^+ but also may modify renal handling of other cations (*e.g.,* K^+, H^+, Ca^{2+}, and Mg^{2+}), anions (*e.g.*, Cl^-, HCO_3^-, and $H_2PO_4^-$), and uric acid[2].

Constipation is a common, often chronic, gastrointestinal disorder with a well-recognized tendency to cause discomfort and to affect quality of life[3–5]. Constipation increases during aging and can be a chronic condition requiring the use of laxatives over the long term. Constipation causes not only discomfort but also abdominal distension, vomiting, restlessness, gut obstruction, and perforation; in extreme cases it may be associated with aspiration or fatal pulmonary embolism[6]. The treatment with classic drugs is often insufficient, leaving patients with inadequate relief of bloating and other symptoms, and with the lack of efficacy in relieving constipation. So far, half of patients receiving treatment with laxatives state that they are not satisfied with improvements to their quality of life[7,8].

Desmostachya bipinnata (Linn) Stapf (DBPS), an official drug of Ayurvedic pharmacopoeia, belonging to family Poaceae, is commonly known as "sacrificial grass", for its use in Yagnas and religious rites[9]. It is distributed throughout India, also found in Syria, Pakistan, Persia, Middle East to Indo-China and North, Northeast and tropical Africa. The various parts of this plant are used extensively in traditional medicine to cure various human ailments. Its uses include: astringent, aphrodisiac, galactogogue, analgesic, antipyretic, wounds, anti-inflammatory, anti-asthma, diuretic, prevention of miscarriage, as well as treatment of dysentery, diarrhea, jaundice, vomiting, dysuria, diabetes, menorrhagia, skin eruptions, urinary calculi and other diseases of bladder and skin[10–12]. This plant extract is reported to maintain euglycemic status[13]. Further, this plant is one of the ingredients of trinapanchamoola, a composite herbal formulation found to have effective anti-urolithiatic activity[14]. Previous studies on this plant have resulted in the isolation of some known coumarins, amino acids, carbohydrates[15], flavonoids[16,17], sterols[18], terpenes[19] and triterpenoids. In addition, pharmacological studies have established its antioxidant[20], antiulcerogenic[16], analgesic, antipyretic[19,21], anti-inflammatory[19,21], antidiarrhoeal[22], antifungal[23,24] and anti-helicobacter activities[17].

Although quite a number of scientific investigations have been undertaken to validate the traditional uses of this plant, no pharmacological or clinical studies have been carried out to test its diuretic and laxative activities. Thus, the present investigation was aimed at experimentally verifying these claimed diuretic and laxative activities of DBPS hydro-alcoholic extract in rats. Furosemide was selected as the reference drug for diuretic activity; *Senna* glycosides were used as reference drug for laxative activity.

2 Materials and methods

2.1 Drugs and chemicals

Frusemide injection (Lasix injection, Aventis Pharma Ltd., India) and *Senna* glucosides (Senasof tablets, Wanbury Ltd., India) were obtained from a pharmacy. Potassium chromate solution, silver nitrate and other reagents used were of analytical grade.

2.2 Plant material

The plant DBPS was collected in and around Nalgonda city, Andhra Pradesh, India. The plant material was taxonomically authenticated by Dr. T. Shankara Chary, Government Degree College for Women, Nalgonda, India. The voucher specimen (No: DBP/GDCWN/54/2010) was deposited in the college herbarium for future reference.

2.3 Preparation of extract

The whole plant of DBPS was shade dried, powdered coarsely (sieve No. 40) and then extracted in a Soxhlet extractor using 70% of methanol as a solvent at 55 ℃ until the extract became colorless. The filtrate obtained by vacuum filtration was concentrated to dryness using a vacuum evaporator under controlled temperature (40–50 ℃). The dried concentrated extract was suspended in saline before administering to the animals[25].

2.4 Preliminary phytochemical screening

The crude hydro-alcoholic extract of DBPS was subjected to preliminary qualitative phytochemical screening for the identification of major functional groups and various phytochemical constituents such as carbohydrates, glycosides, alkaloids, flavonoids, saponins, tannins, phenolic compounds, terpenoids, steroids, proteins, gums and mucilage using standard tests[26,27].

2.5 Animals

Healthy Wistar albino rats (180–220 g) used in this study were maintained at institutional animal house, kept in standard polypropylene cages with 12 h light-dark cycle at (22±3) ℃. The animals were fed a standard rodent chow diet and accessed water *ad libitum*. After proper acclimatization, the animals were used for study. Approval for the usage of animals in the experiments was obtained as per the Indian Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines outlined by the Institutional Animal Ethical Committee (IAEC) of Bharat Institute of Technology and approval number for the study was 1015/c/06/CPCSEA[28].

2.6 Acute oral toxicity studies

Wistar albino rats (180–220 g) of both sexes were used to determine the toxicity of DBPS, following the acute oral toxic class method of Organization of Economic Co-operation and Development (OECD) according to 423 guidelines[29]. Animals were fasted for 3 h prior to the experiment and doses ranging from 0.1 to 2 g/kg were administered orally. The rats were observed for 48 h following dosing, and evaluated for neurological, behavioral and autonomic symptoms, as well as mortality for 14 d following administration of extract.

2.7 Evaluation of diuretic activity

Diuretic activity was carried out in accordance with slight

modification of earlier methods[30,31]. Twenty-four male rats (180–200 g) were randomly divided into four groups of six each. Each group was fasted and deprived of water for 18 h prior to the experiment. The first group of animals (I) serving as control, received normal saline (15 mL/kg, p.o.); the second group (II) received furosemide (20 mg/kg, i.p.) in saline[32,33]; the third (III) and fourth (IV) groups received the hydro-alcoholic extract at the doses of 250 and 500 mg/kg (p.o.) respectively, in normal saline. Animals grouped in groups III and IV were considered as test groups. Immediately after dosing, the animals were hydrated with saline (15 mL/kg) and placed in metabolic cages specially designed to separate urine and feaces (3 per cage; B.I.K Industries, Mumbai, India). Food and water was witheld for 5 h after the animals were placed in their cages, and the cages were maintained at (25.0±0.5) ℃ throughout the experiment. The volume of urine collected was measured at the end of 5-hour treatment and urine collected was subjected to analysis.

2.8 Urine analysis

The water excretion rate (urine volume), pH and conductivity of urine collected were estimated using a pH meter and a conductometer. Concentration of Na^+ and K^+ in urine was also measured using a flame photometer (Mediflame 127, Systronics, Ahmedabad, India). For sodium and potassium, the flame intensity corresponding to the concentration of Na^+ and K^+ calibration standards was noted by using appropriate filters. The results were plotted in a graph. The concentration of the Na^+ and K^+ was calculated from the graphs and expressed in terms of mEq/L[34]. The Cl^- concentration was estimated by titration with silver nitrate solution (N/50) using three drops of 5% potassium chromate solution as an indicator[35].

2.9 Evaluation of laxative activity

Evaluation of laxative activity was carried out according to the method described previously with few modifications[36]. Twenty-four rats of either sex (180–200 g) were randomly allocated to four groups of six each. All animals were fasted for 12 h prior to the experiment, but water was provided *ad libitum*. The four experimental groups in this study were as follows. The first group was given saline (5 mL/kg, p.o.) and served as a negative control. The second group received sennoside glucosides (10 mg/kg, p.o.) (Senasof tablets, Wanbury Limited, Mumbai), and served as the positive control. The third and fourth groups received 250 and 500 mg/kg (p.o.) of the hydro-alcoholic extract of DBPS in normal saline[37]. Immediately after dosing, the animals were placed in individual cages lined with clean filter paper, designed to collect feaces[38]. The faeces production (total number of normal as well as wet faeces) was monitored for up to 16 h. Production was quantified by measuring total faeces weight in each of the cages.

2.10 Statistical analysis

The results were expressed as mean ± standard error of mean (SEM), and data was statistically analyzed by one-way analysis of variance followed by Dunnett's multiple comparison test using GraphPad Prism Version 5.0 (GraphPad Software, San Diego, CA, USA). All the results obtained in this study were compared with the vehicle control group. The values of $P<0.05$ were considered statistically significant. The control group and treatment groups were analyzed separately for statistical significance.

3 Results

3.1 Preliminary phytochemical screening

The whole plant of DBPS was extracted using methanol (70%) as solvent in Soxhlet extractor, which has given reddish brown sticky mass. The percentage yield of hydro-alcoholic extract was found to be 5.4% (w/w).

The preliminary phytochemical screening of crude hydro-alcoholic extract revealed the presence of phytochemical constituents such as alkaloids, carbohydrates, proteins, tannins, phenolic compounds, flavonoids, triterpenoids and glycosides. However, steroids, saponins and gums and mucilage were absent. The results are shown in Table 1.

3.2 Acute oral toxicity studies

Table 1 Preliminary phytochemical screening of hydro-alcoholic extract of *Desmostachya bipinnata* (Linn) Stapf

Screening No	Phytochemical test	Inference
1	Alkaloids	+
2	Carbohydrates	+
3	Proteins	+
4	Tannins and phenolic compounds	+
5	Flavonoids	+
6	Steroids	−
7	Triterpenoids	+
8	Saponins	−
9	Glycosides	+
10	Gums and mucilage	−

'+' indicates the presence; '−' indicates the absence of that phytochemical constituent.

In the acute toxicity study, it was found that the DBPS extract induced diuresis and there were no significant behavioral or neurological changes and there was no mortality at any of the tested doses throughout the 14-day observation.

3.3 Diuretic activity

The hydro-alcoholic extract of DBPS was evaluated for

diuretic activity using the standard methods, measuring urinary output, pH and conductivity. We found that both the hydro-alcoholic extract of DBPS at the higher dose tested (500 mg/kg, p.o.), and standard frusemide (20 mg/kg, i.p.) significantly increased urinary output (Table 2). As there was an increase in urinary output in DBPS-treated rats at 500 mg/kg, we were interested in investigating the effect of DBPS extract on the concentration of electrolytes in the urine in order to identify the mechanism of its diuretic activity. We found that the DBPS extract produced a significant increase in excretion of sodium, potassium and chloride ions at both of the treatment doses (250 and 500 mg/kg p.o.; Table 3).

Changes in other parameters such as conductivity and pH were not significant when compared with that of the vehicle control group. The saluretic index and Na^+/K^+ ratio were also calculated and are shown in Table 3.

3.4 Laxative activity

In addition to diuretic activity, we were interested in investigating the effect of DBPS on relief of constipation and thus for its laxative effect. We used sennoside, a plant-based alkaloid, as a standard drug for comparing the laxative effect. Interestingly, we found that the DBPS extract showed a dose-dependant increase in fecal output of rats relative to the control group (Figure 1). The feces output following a single administration of sennosides significantly increased to (5.023 ± 0.130) g (P<0.01) relative to the vehicle control group ((1.527 ± 0.064) g). A single administration of DBPS at a dose of 500 mg/kg significantly increased the feces output to (1.984 ± 0.101) g (P<0.05) relative to the vehicle control. There was no significant difference between the extract at the dose of 250 mg/kg (p.o.) and the control group (Figure 1). DBPS at a dose of 500 mg/kg (p.o.) increased the fecal output of rats significantly relative to the control group (P<0.05). However, the effect of the extract at a dose of 500 mg/kg (p.o.) was very small compared with that of standard drug sennosides (10 mg/kg, p.o.; Figure 1). Thus, the extract failed to exhibit significant laxative activity in comparison to standard laxative drug, sennoside.

4 Discussion

The present study was carried out to evaluate the diuretic and laxative effects of DBPS hydro-alcoholic extract. This study served the secondary purpose of evaluating the use of this herb to treat constipation in the practice of traditional medicine. In support of earlier reports, our preliminary phytochemical analysis also revealed the presence of alkaloids, carbohydrates, proteins, tannins, phenolic compounds, flavonoids, terpenoids and glycosides in DBPS extract (Table 1). Earlier investigations and primary literature demonstrate that *D. bipinnata* is regularly consumed by the African population, that this herb contain adequate amounts of protein, and that it does not exhibit any toxic properties, even failing to show toxic effects on brine shrimp[25]. Further supporting these data, our acute oral toxicity analysis also showed that DBPS extract was safe for administration in rats at the highest dose tested.

Here we present the first experimental evidence supporting

Table 2 Effects of frusemide and hydro-alcoholic extract of DBPS on urinary volume, pH and conductivity in normal rats

Treatment	Dose	Urine volume (mL)	pH	Conductivity
Control	Saline (15 mL/kg), p.o.	3.46±0.13	7.12±0.23	15.65±0.44
Frusemide	20 mg/kg, i.p.	8.23±0.23*	7.22±0.18	18.21±1.24
DBPS	250 mg/kg, p.o.	3.72±0.37	7.24±0.31	17.76±0.63
DBPS	500 mg/kg, p.o.	6.43±0.13*	7.02±0.26	17.80±1.26

Values are expressed as the mean ± standard error of mean (n = 6), *P<0.05, *vs* vehicle control (one-way analysis of variance and Dunnett's multiple comparison test). DBPS: *Desmostachya bipinnata* (Linn) Stapf.

Table 3 Diuretic activity of hydro-alcoholic extract of DBPS in rats

Treatment	Dose	Concentration of ions (mEq/L)			Saluretic index			Na^+/K^+ ratio
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	
Control	Saline (15 mL/kg), p.o.	24.23±0.36	8.48±0.25	14.28±0.57	–	–	–	2.86
Frusemide	20 mg/kg, i.p.	53.25±0.26**	31.12±0.32**	33.52±0.33**	2.232	3.330	2.287	1.72
DBPS	250 mg/kg, p.o.	31.63±0.67*	14.63±0.34**	17.40±0.53**	1.243	1.450	1.291	2.16
DBPS	500 mg/kg, p.o.	43.58±0.34**	22.38±0.45**	24.8±0.568**	1.740	2.330	1.450	1.94

Values are shown as the mean ± standard error of mean (n = 6), *P<0.05, **P<0.01, *vs* control (one-way analysis of variance and Dunnett's t test); saluretic index = test $mEql^{-1}$/control $mEql^{-1}$. DBPS: *Desmostachya bipinnata* (Linn) Stapf.

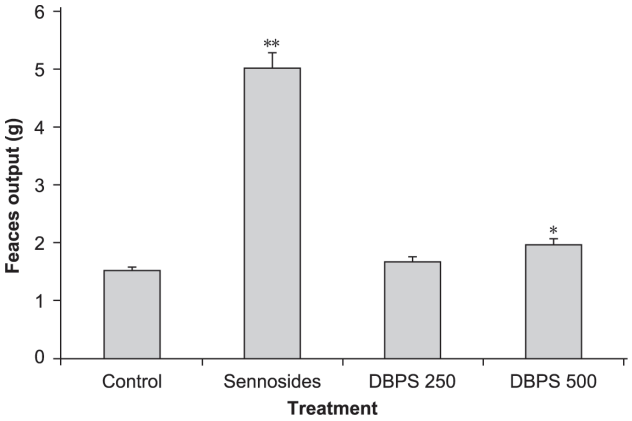

Figure 1 Effects of DBPS hydro-alcoholic extract on feaces output (laxative activity) in rats compared with that of standard sennosides
Data are shown as the mean ± standard error of mean, $n = 6$. $^{*}P<0.05$, $^{**}P<0.01$, *vs* control (one-way analysis of variance and Dunnett's *t* test). DBPS: *Desmostachya bipinnata* (Linn) Stapf.

the use of DBPS as a diuretic in traditional medicine. The hydro-alcoholic extract of DBPS was found to be the most potent in increasing the urinary output at 500 mg/kg; the effect was comparable to that of the standard drug, whereas, the extracts at low concentration (250 mg/kg) did not significantly increase urinary output. The control of plasma sodium is important in the regulation of blood volume and pressure. The control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles[39]. Thus, the regulation of sodium and potassium balance is also intimately related to renal control of acid-base balance. Evaluation of urinary electrolyte concentration revealed that the hydro-alcoholic extract increased urinary electrolyte concentration for all the three ions tested (Na^+, K^+, and Cl^-) at both the doses tested. These findings are also consistent with the traditional use of this plant for the treatment of diabetes and hypertension. The specific conductivity, which is an indirect measure of the ionic content of the urine, was increased in a dose-dependent manner in all the extract-treated groups irrespective of its diuretic activity. Thus the diuretic effect of the extract may not be attributed to the increase in both water excretion and excretion of sodium and potassium. The chemical components responsible for the diuretic effect of the DBPS extract have not yet been described but the preliminary phytochemical analysis of DBPS extracts revealed the presence of polar compounds such as flavonoids and terpenoids. Phytochemicals such as flavonoids and terpenoids are known to be responsible for diuretic activity[40]. So, the diuretic activity of the DBPS extract might be attributed to the presence of these compounds. The current study supports the traditional medical use of *D. bipinnata* as a diuretic agent. Further studies are required to isolate the active constituents responsible for *D. bipinnata's* diuretic activity and to determine the mechanism of their action.

Fecal consistency is correlated to the ratio of the water-holding capacity of the insoluble solids, such as those that derived from dietary fiber, and to the total water in the lumen[41]. Many conventional laxatives, especially the stimulant and saline laxatives affect water absorption and/or secretion in the gut. The presence of phyto-constituents like terpenoids, sterols, flavonoids, phenolic compounds, tannins and alkaloids can be responsible for the laxative effects of plant material[42]. Although the phytochemical screening of the DBPS extract revealed the presence of these constituents, the extract failed to produce significant laxative activity in comparison to standard sennosides at either of the doses tested.

The intestinal transit is controlled by both neural and myogenic mechanisms[43]. In general, an increase in the contractile activity of the smooth layers is responsible for acceleration of intestinal propulsion. Several mediators and neurotransmitters govern these motor patterns. Acetylcholine is the main excitatory neurotransmitter in the enteric nervous system[44]. Thus, the absence of cholinomimetic constituents in the plant extract could explain the weak laxative activity of DBPS. Our results also support the recent study showing the presence of calcium antagonist activity in DBPS[45], possibly underlying its ineffectiveness as a laxative.

In summary, preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, proteins, tannins, phenolic compounds, flavonoids, triterpenoids and glycosides in DBPS extract. In addition, DBPS hydro-alcoholic extract exhibited significant diuretic activity in rats, which may be attributed to the presence of several bioactive diuretic phytochemical constituents. Moreover, DBPS extract failed to exhibit significant laxative activity in accordance with earlier reports showing its calcium antagonist activity. Although a large number of potentially active compounds have been found in DBPS, further studies are needed for isolation, structural elucidation, and screening of any of the above mentioned active principles with regard to specific medicinal uses of the extract. It is thus apparent from above findings that hydro-alcoholic extracts of DBPS possess diuretic activity. This study provides experimental evidence supporting its use as a diuretic in traditional system of medicine. Nevertheless, further studies are required to elaborate its use, active constituents and safety.

5 Acknowledgements

The authors sincerely thank the Ministry of Human Resource and Development (MHRD) for providing financial support to 'UG and PKG' in the form of PG Scholarship Grant from All India Council for Technical Education (AICTE), New Delhi, India.

6 Conflict of interests

The authors declare no conflict of interests.

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