Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 2, 2015

Original Article

EVALUATION OF DIURETIC ACTIVITY OF BOSWELLIA SERRATA LEAF EXTRACTS IN ALBINO MICE

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Received: 22 Nov 2014 Revised and Accepted: 15 Dec 2014

ABSTRACT

Objective: The objective of the current study is to evaluate the diuretic activity of *Boswelliaserrata* leaf extracts in albino mice.

Methods: The leaf extracts were prepared using petroleum ether, chloroform, ethanol and distilled water. The effective dose of 250mg/kg, 200mg/kg, 250mg/kg and 500mg/kg were used respectively. A preliminary phytochemical screening was performed to know the constituents in each extract. All the extracts were then evaluated for their diuretic activity. The volume of urine and excretion of electrolytes in the urine was measured to assess the diuretic activity.

Results: Phytochemical screening showed the presence of alkaloids, tannins, flavonoids, terpenoids, anthraquinone and steroids. Petroleum ether extract and aqueous extract showed a higher volume of urine excreted when compared to other groups. In analyzing the discharge of electrolytes, petroleum ether extract showed excretion of Na+ and K+ while Chloroform extract showed excretion of Cl- ions. Ethanolic group portrayed low excretion of both electrolytes and urine volume. In the petroleum ether extract and chloroform extract, BUN levels were significantly more when compared to other groups. There was no significant effect on the levels of serum creatinine in all the extracts. Overall the petroleum ether extract substantiated a near to equal value in comparison with the standard drug [furosemide].

Conclusion: The data obtained in this study showed that the leaves of *Boswelliaserrata* showed diuretic activity with no toxic effects on the kidneys.

Keywords: Diuretic, *Boswelliaserrata*, Herbal drugs, Leaf extracts.

INTRODUCTION

Traditional medicines obtained from plants and animals have made an immense contribution to human health and wellbeing [1]. In these, ingredients sourced from plants and animals are increasingly valued as raw materials for the preparation of modern medicines [2]. Some developing countries have outdated medicine as the mainstay of their health care system [3]. Recently it is witnessed that many people from developed countries are also turning to herbal remedies due to the safety of plant based substances when compared to the contemporary medicines [3].

Herbal preparations use a wide variety of plant products for curing and prevention of various diseases. Among all the plant species, medicines obtained from the Burseraceae family are of great importance for their therapeutic potential [1]. It consists of nearly 17 genera and 600 species of plants in its familial classification[4]. *Boswelliaserrata* [Indian Frankincense] belonging to the Burseraceae family is among the primal and most valued herb in the Ayurveda [4]. It is a moderate to large size tree which grows in the dry hilly areas of India, Northern Africa and the Middle East [5, 6]. In India, its principal commercial sources are Andhra Pradesh [A. P], Gujarat, Madhya Pradesh, Jharkhand and Chhattisgarh. In these regions, *Boswelliaserrata* is also known by other locally used names such as Phirangi, Dhup, Kundur, Salai or SaliGuggul[4]. Studies suggest that various herbal supplements obtained from this plant are beneficial for numerous ailments. In a thorough review of literature it is seen that this plant has been screened for its anti-inflammatory [7], anti-oxidant [8], anti-ulcer [9], anti-arthritic [10], anti-asthmatic[11], anti-atherosclerotic[12], anti-cancer [13], anti-diarrheal[14], Hepatoprotective[15], anti-microbial [16], anti-hyperglycemic [17], wound healing [18] and analgesic [19] activities. The traditional Ayurveda and Unani text in India also showed that it is an effective remedy for diarrhoea, dysentery, ringworm, boils, fever, skin infections, blood disorders, cardiovascular diseases, bronchitis, asthma, cough, jaundice, hemorrhoids and syphilitic diseases [20]. Recently, pharmacological studies have been carried out to confirm its use as an antiarthritic, antiinflammatory, antihyperlipidemic, antiatherosclerotic, analgesic and hepatoprotective drug [21-25]. But studies which suggest its role as a diaphoretic, astringent and diuretic drug lack sufficient experimental validation [20].

In the light of above, an attempt is proposed to evaluate the diuretic activity of *Boswelliaserrata*. Diuretics are beneficial in many life threatening disease conditions such as congestive heart failure, pulmonary edema and also in the treatment of hypertension [26]. The allopathic medicines currently in use like the thiazides and the high ceiling loop diuretics have a number of adverse effects, such as; electrolyte imbalance, metabolic alterations, development of new-onset diabetes, activation of the rennin–angiotensin–neuroendocrine systems and impairment of sexual functions [26]. With regards to the long list of side effects it could be said that there is a need for experimental evaluation and confirmation of naturally occurring plant extracts to be used as diuretics[3]. Previous studies have analyzed that the flavonoids and tannins of various plant species such as *Tribulusterrestris, Urticadioica*, Oleaeuropaea; and the alkaloids of *Aervalanata*, *Foeniculumvulgare, Azimatetracantha; Allium sativum* for their involvement in the mechanism of diuretic action [27, 28]. Similarly, the extracts of the leaves of *Boswelliaserrata* are also rich in alkaloids, flavonoids and tannins. Hence the current study is experimentally designed to evaluate the diuretic activity of the leaves of *Boswelliaserrata* using albino mice.

MATERIALS AND METHODS

Plant collection and authentication

Fresh plant of *Boswelliaserrata* [Burseraceae] was collected from the forest of Narsapur, Medak district [A. P] and was authenticated at Central research institute of unani medicine, Hyderabad [Voucher number CRIUM – 114/2004].

Preparation of plant extracts

The leaves were first air dried at room temperature and then mechanically reduced to moderate coarse powder. The powdered

material was made to undergo successive extraction using different solvents in increasing order of their polarity [petroleum ether, chloroform, and then ethanol] using soxhlet apparatus [29, 30]. In order to obtain the aqueous extract the powdered leaf material was subjected to cold maceration with distilled water at room temperature for 48 hours and then filtered. All these extracts were concentrated with the help of vacuum evaporator and then stored in a dessicator [31].

Standard drug

Furosemide, a commonly used high ceiling diuretic served as the standard drug in this investigational study.

Procurement of animals

Male albino mice weighing between 22 - 30 gms were procured from Mahaveer enterprises [Reg. No. 149/1999/CPCSEA] Amberpet, Hyderabad, India. Evaluation of diuretic activity was approved by the ethical committee Reg. No. 1330/ ac/10/CPCSEA.

Housing of animals

The animals were housed at room temperature of 24 ± 1°C with 12:12 hours light and dark cycles respectively. The animals were given commercially available rat feed pellets as food and it was made sure that the water was maintained at adlibitum.

Chemicals and drugs used

Solvents [petroleum ether, chloroform and ethyl alcohol] were obtained from S. D fine chemicals, Mumbai. Standard Drug Furosemide [Lasix] was obtained from a local pharmacy.

Preliminary phytochemical screening

All the extracts were screened for various phytochemicals [Table 1] such as tannins, alkaloids, glycosides, terpenoids, flavonoids, anthraquinones and proteins using standard methodology [32].

Acute toxicity studies

Acute toxicity test is a demonstration in which a single dose of the drug is used in each animal on one occasion in order to analyze its gross behavior toxicity. Initially a preliminary pharmacological study was carried out to assess the acute pharmacological effects and safety effects of all the prepared extracts. The acute toxicity studies were conducted as per CPCSEA and OECD/OCDE guidelines. Four groups of three mice each weighing about 22 – 30 gms were selected. Each group was then administered with the prepared drugs respectively and kept 3-4 hours of fasting with free access to water. Later, these animals received a starting dose of 2000 mg/kg orally of the prepared drug and were observed for 24 hours. The lethal dose was calculated and 1/10th of the lethal dose was regarded as the effective dose. The effective dose of petroleum ether [PEE], chloroform [CFE], ethanol [ETE] and aqueous extract [AQE] was found to be 250 mg/kg, 200mg/kg, 250mg/kg and 500mg/kg of body weight respectively.

To conduct the experimental study, the animals were at first divided into 6 groups, with each group consisting of 5 animals. All of these animals were subjected to overnight fasting with unrestricted access to water. Metabolic cages were used for placing the animals. Care was ensured that the animals were placed in individual metabolic cages for 24 hours prior to the commencement of the experiment in order to achieve the adaptation.

Diuretic activity

Prior to receiving treatment, all the animals were given normal saline [5 ml/100 gms] to maintain uniform water and salt load. Later each group received its assigned drug to check the desired activity.

Group I [Control] - Received distilled water [10 ml/kg BW p. o]

Group II - Received petroleum ether extract [250mg/kg BW, p. o]

Group III - Received chloroform extract [200 mg/kg BW, p. o]

Group IV - Received ethanol extract [250 mg/kg BW, p. o]

Group V - Received aqueous extract [500mg/kg BW, p. o]

Group VI [Standard] - Received furosemide [10mg/kg BW, p. o]

After the drug administration, these animals were placed at room temperature [25+ 0.5^{o}C] in the metabolic cages which effectively helped in separating urine and fecal matter. The volume of urine and excretion of electrolytes in the urine was measured to assess the diuretic activity. Total urine volume was gathered from the control, standard and all the extract groups in measuring cylinders after 5 and 24 hours. During this period, no food and water was delivered to animals [33]. The bladder was emptied by pulling the base of the tail of each animal [34]. Evaluation of diuretic activity was carried out by monitoring the total urine volume and urine concentration of Na^{+}. K^{+}, Cl^{-}. Organ protection activity of the extracts was assessed by estimating the Blood urea nitrogen [BUN] and serum creatinine level. Urine volume was measured using a measuring cylinder and electrolytes were analyzed using easylyteanalyser. Blood urea nitrogen [BUN] and serum creatinine levels were tested using BUN analyzer and serum analyzer respectively [33, 35-37].

RESULTS

Preliminary phytochemical screening

A preliminary screening was carried out to check the presence of various phytoconstituents in the extracts [Table 1]. The results of phytochemical analysis showed the presence of alkaloids, tannins, flavonoids, terpenoids, anthraquinone and steroids [Table 1]. Cardiac glycosides and proteins were not present in any of the extracts. Except the ethanolic extract, all others showed a positive result for the presence of alkaloids. Most of the phytoconstituents such as the steroids, carbohydrates, flavonoids and proteins were not seen to be present in the petroleum ether extract.

Table 1: Phytochemical constituents of leaf extracts from *Boswelliaserrata*

S. No.	Phyto constituents	PEE	CFE	ETE	AQE
1.	Tannins	+	+	+	+
2.	Alkaloids	+	+	-	+
3.	Cardiac glycosides	-	-	-	-
4.	Steroids	-	+	+	+
5.	Carbohydrates	-	+	+	-
6.	Saponins	-	-	+	+
7.	Flavanoids	-	+	+	+
8.	Anthraquinones	+	+	+	+
9.	Proteins	-	-	-	-
10.	Terpenoids	+	+	+	+

[+] indicates the presence of phytoconstituent in the extract and [-] indicates the absence of phytoconstituent in the extract

Effect on urine volume

On checking the effect of the extracts on the total urine volume, it is observed that all the extracts i. e. petroleum ether, chloroform, ethanolic and aqueous extracts showed a significantly high level of diuresis when compared to the control group. The petroleum ether extract [16.1±0.49] and the aqueous extract [16.8±1.32] group showed a slightly higher value than others in terms of volume of urine excreted. The value is seen to be nearly equivalent to the standard drug, furosemide [Table 2]. The ethanolic extract group has portrayed a value [14.8+0.96] which is notably low than the value obtained by the other extracts.

Effect on the excretion of electrolytes

In the analysis of the excretion of electrolytes, the results reveal that all the plant extracts except the ethanolic extract showed a simultaneous increase in the excretion of electrolytes with an increase in the total urine volume [$p < 0.001$vs Control]. The extract obtained through petroleum ether is seen to remarkably induced the excretion of Na^{+} and K^{+} while the chloroform extract showed an increase in the excretion of Cl^{-}ions [Table 3].

Table 2: Urine volume of leaf extracts from *Boswelliaserrata*

Group	Control	Furosemide	PEE	CFE	ETE	AQE
Urine Volume	8.0+0.36	19.1+0.48*	16.1+0.49*	17.5+0.71	14.8+0.96	16.8+1.32*

Values are mean±SEM [n=5];*p<0.[00]1VsControl

Table 3: Excretion of electrolytes from leaf extracts from *Boswelliaserrata*

Group	Control	Furosemide	PEE	CFE	ETE	AQE
Na^+	126+0.89	252.2+0.97	225.5+1.34	201.8+1.23*	160+0.52*	213.7+0.85
K^+	104.6+0.80	222.2+0.59*	184.6+0.66*	135.2+0.43	142.3+0.64	156.5+0.44
Cl^-	144.8+0.46	380.0+0.63	321.7+0.42*	370.8+0.43	188.0+0.40	193.5+0.50

Values are mean±SEM [n=5];*p<0.[00]1VsControl

Table 4: BUN and serum creatinine levels of leaf extracts from *Boswelliaserrata*

Group	Control	Furosemide	PEE	CFE	ETE	AQE
BUN	32.8+1.05	38.6+0.89**	28.5+0.68**	40.6+1.12*	48.6+0.95	39.8+0.68
Serum creatinine	2.0+0.28	3.2+0.35	1.9+0.23	2.6 +0.27	1.6+0.20	2.8+0.20

Values are mean±S.D [n=5];*p<0.[00]1VsControl,**p<0.[01]Vs Control

Effect on BUN and Serum creatinine

The BUN and serum creatintine level were checked to know the toxic effect of the extracts on kidneys. Analysis revealed that the value obtained by the extract was remarkably close to the control group with a P- value of < 0.001. Petroleum ether and chloroform extract groups showed a more nearer value in comparison with the additional extracts [Table 4]. There was not any significant effect seen on the serum creatinine value when compared to the control animals, indicating that the serum creatinine level was non-significant vs control.

DISCUSSION

Diuretics are known to play an important role in hypertensive patients as they are effective in decreasing the blood pressure by reducing the syndrome of volume overload and in turn reducing the cardiac workload [38]. The results of the current study revealed that different leaf extracts of *BoswelliaSerrata* were successful in functioning as a diuretic drug, but the effectiveness varied individually when compared to the standard drug [furosemide]. The chloroform extract was observed to be the most effective in demonstrating an increase in urine volume when compared to all the other extracts [Table 2]. The values obtained were in accordance with the standard drug [furosemide] used in the study [Table 2]. Whereas the ethanolic extract was seen to be the least potent in terms of total urine excretion, with comparable value of 14.8+0.96.

In a broader aspect to check the diuretic activity of a drug it is necessary that apart from the increase in the total volume excreted, the drug should also demonstrate a net loss of solutes [i. e. electrolytes] when tested [39]. In the current study, all the extracts of the leaves of *Boswelliaserrata* showed a substantial increase in the excretion of sodium and potassium ions which can contribute in relieving pulmonary congestion and peripheral edema in patients with ischemic heart diseases[38]. The most effectual for the overall sodium and potassium ion release was petroleum ether extract [Table 3]. Whereas chloroform extract showed an increase in the chloride ions [Table 3]. The electrolyte concentration obtained by the ethanolic extract was significantly less in value when compared to the other extracts [Table 3].

With the analysis of both, the total urine volume excreted and the electrolyte release it is deduced that petroleum ether extract substantiated a near of equal value in comparison with the standard drug [furosemide]. If the pattern of excretion of electrolytes is taken into consideration, it is apparent that the active constituent present in this extract possesses furosemide like activity. In examining the foregoing researches it is seen that the plant extracts which contain alkaloids have potent diuretic and hypotensive action [40]. The presence of certain flavonoids had also seen to enhance the diuretic activity, mainly by binding with adenosine A1 receptor [41]. Concurrently the expressive level of diuretic activity seen with the *Boswelliaserrata* leaf extracts could be through one of these possible mechanisms. This is interpreted due to the high amount of alkaloids and flavonoids found in all the extracts [Table 1]. Further detail investigations are required to confirm as to which of the active constituents are responsible for showing the diuretic activity.

Finally, the toxic effects of the extracts on the kidneys were checked by measuring the levels of BUN and Serum creatinine [42]. Usually dehydration leads to a rise in BUN levels more than creatinine level resulting in increased BUN to creatinine ratio. Kidney disease is also seen to be associated with a rise in BUN and creatinine level to a similar degree [43]. On analyzing the prepared extracts, it was observed that the level of BUN was raised and the creatinine level was found to be within the normal limits. The rise in BUN value is indicated due to the dehydration caused by the diuretic effect of the plant extracts. Thus, confirming that none of the extracts showed any toxic effect on the kidneys. Additional studies have to be carried out in order confirm the lack of renal toxicity and also to rule out other organ toxicity before conducting advanced trials.

CONCLUSION

To conclude, the present study demonstrated the overall effective diuretic activity associated with the leaves of *Boswelliaserrata* plant. This observation supports the folklore consideration of this plant as a diuretic. The study could form the basis for exploring the use of this plant in the management of certain cardiovascular diseases.

CONFLICT OF INTERESTS

Declared None

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