Diuretic activity of *Boswellia serrata* Roxb. oleo gum extract in albino rats

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Abstract: The aim of the study was to evaluate the effect of crude aqueous extract of *Boswellia serrata* Roxb. oleo gum on urinary electrolytes, pH and diuretic activity in normal albino rats. Moreover, acute toxicity of the gum extract was assessed using mice. Albino rats were divided into five groups. Control group received normal saline (10 mg/kg), reference group received furosemide (10 mg/kg) and test groups were given different doses of crude extract (10, 30 and 50 mg/kg) by intra-peritoneal route, respectively. The Graph Pad Prism was used for the statistical analysis and *p* < 0.05 was considered statistically significant. Significant diuretic, kaliuretic and natriuretic effects were observed in the treated groups in a dose dependent manner. Diuretic index showed good diuretic activity of the crude extract. Lipschitz values indicated that the crude extract, at the dose of 50 mg/kg, showed 44 % diuretic activity compared to the reference drug. No lethal effects were observed among albino mice even at the higher dose of 3000 mg/kg. It is concluded that aqueous extract of *Boswellia serrata* oleo gum, at the dose of 50 mg/kg showed significant effects on urinary volume and concentration of urinary electrolytes with no signs of toxicity.

Keywords: *Boswellia serrata* Roxb, kaliuretic, natriuretic, lipschitz, diuretic index.

INTRODUCTION

Plants are important sources of unknown chemical substances with potential therapeutic uses. World Health Organization (WHO) has assessed that more than three quarter of the world’s population still depend on plant-derived medicines for their basic healthcare needs (Farinworth, 1985). Herbal products are used by chronically ill patients especially those suffering from cancer (12 %), liver diseases (21%), acquired immune deficiency syndrome (22 %), asthma (24 %) and rheumatological disorders (26 %) (Inamdar, 2008). Traditional medicines also give useful synthetic clues of modern drugs. Most of the drugs were originally discovered through study of herbal cures and folk knowledge of traditional people. Despite all the advancements in synthetic chemistry, some of the traditional remedies are still not replaced by the synthetic drugs (Gurib-Fakim, 2006).

According to the WHO report, cardiovascular diseases are among the leading causes of adult mortality throughout the world (World Health Organization, 2003). Conditions such as high blood pressure lead to several other types of complications, such as stroke, heart diseases and renal disorders. Most common therapeutic strategies to attain lower blood pressure include the use of angiotensin converting enzyme inhibitors, calcium-channel blockers, beta blockers and diuretics (Asif et al., 2013; Williams et al., 2004). All of these therapeutic agents work by reducing arterial resistance and/or decreasing cardiac output.

Diuretics normally cause an increase in urinary output and urinary excretion of sodium from the body (Asif et al., 2013; Gallagher et al., 2006). Thiazides and the high-ceiling loop diuretics are more commonly used in clinical practice, and are associated with several side effects (Gasparotto et al., 2009). Hence, there is a need for novel diuretics which are considered to be relatively safe with better or equivalent diuretic activity.

*Boswellia serrata* (Bs.) Roxb. of family Burseraceae is commonly known as Indian Olibanum and Indian Frankincense. The plant is commonly found throughout the greater parts of Pakistan and India (Kirtikar and Basu 1918). Phytochemical studies have shown that the oleo gum resin of Bs. contains 8–9 % essential oils, 20–30 % gum and about 50 % resin. The gum portion contains sugars such as pentose and hexose, and some oxidizing and digestive enzymes (Sharma et al., 2007). α-pinene (73.30 %) is the major constituent of the essential oil. Other constituents are β-pinene (2.05 %), cis-verbenol (1.97 %), trans-pinocarveol (1.80 %), borneol (1.78 %), myrcene (1.71 %), limonene (1.42 %), thuja-2,4(10)-diene (1.18 %) and p-cymene (1.0 %), while α-copaene (0.13 %) is the only sesquiterpene identified in the oil (Upaganlawar and Ghule, 2009). Boswellic acid, a pentacyclic triterpene, is the active moiety of the resin portion. The triterpenes include acetyl-β-boswellic acid, 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid (Sharma et al., 2007).

In folklore medicine, the oleo-gum resin of Bs. is commonly used for the treatment of many diseases including chronic ulcers, rheumatic disorders and urinary...
problems (Nadkarni, 1996). Studies have shown that Bs. is effective in the treatment of various gastrointestinal, urinary, hepatic, cardiovascular, skeletal, nervous and skin disorders (Kimmatar et al., 2003; Gupta et al., 2001; Madisch et al., 2007; Gayathri et al., 2007; Kimmatar et al., 2003; Singh et al., 2008; Weckesser et al., 2007; Shareef, 2011; Borrelli et al., 2006; Moussaieff and Mechoulam, 2009; Menon and Kar, 1971, Pungle et al., 2003; Al-Awadi et al., 2011; Borrelli et al., 2006). It has also been proved to have reno-protective (Pandey et al., 2005), hepatoprotective (Upaganlawar and Ghule, 2009), immunosuppressive (Sharma et al., 2007), anticarcinogenic and anti-tumor activities (Huang et al., 2000). Studies have further shown that it has been found to prevent hyperlipidemia and atherosclerosis (Tripathi et al., 2000; Al-Awadi et al., 2011; Borrelli et al., 2006). Despite the herbal use of Bs. as a diuretic agent, to date, no study in existing literature has evidenced its diuretic activity. Therefore, the primary objective of this study was to evaluate the diuretic effects of aqueous extract of this plant in a rat model. We also aimed to study the effect of aqueous extract on urinary excretion of electrolytes. Furthermore, acute toxicity of this plant was assessed in albino mice.

MATERIALS AND METHODS

Plant material and extraction
Dried oleo gum resin of Bs. was purchased from the local market of Bahawalpur, Punjab, Pakistan. After identification from the herbalist, the sample was submitted to the herbarium in the Pharmacology laboratory at the Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan, and was tagged as BS-GM-08-10-010 for future reference. After removing the foreign material, the gum was crushed into a coarse powder with an electric grinder (National, Model MJ-176NR, China). Approximately, 500 g of ground material was soaked in one liter of hot water at the room temperature (23–25°C) for 3 days. The soaked material was shaken occasionally. Material was then filtered and the residue was again soaked in hot water for 3 days and this procedure was repeated thrice. A thick, semi-solid pasty mass of dark brown color was obtained by evaporating the filtrate in a rotary evaporator (Heidolph Laborota 4000-efficient, Germany) under reduced pressure (~760mmHg). Crude extract of Boswellia serrata (Bs.Cr.) was completely solubilized both in normal saline and distilled water for use in in-vitro and in-vivo experiments (Patel et al., 2009).

Reference and control drugs
Furosemide (Lasix, Aventis Pharma, Pakistan), was used as a reference drug (positive control). Normal saline (Merck, Germany) was used as a control drug (Asif et al., 2013).

Animals and treatment
Adult albino rats of either sex, kept in the animal house of the Department of Pharmacy, IUB, Pakistan, were used to study the effect of Bs.Cr. on urinary pH, level of urinary electrolytes and diuretic activity. Animals were housed in polycarbonate cages (Techniplast, Italy), under the standard conditions of temperature, humidity and dark light cycle (12 h–12 h), and were given pelleted food (Master feed Ltd. Colombo, Sri Lanka) and drinking water ad libitum. The bedding of the animal cages was changed after every 48 hours.

Acute toxicity test of Bs.Cr. was performed on albino mice of 18–25 g body weight. Animals were kept in polycarbonate cages (Techniplast, Italy), and were given pelleted food (Master feed Ltd. Colombo, Sri Lanka) and drinking water ad libitum (Ratnasooriya et al., 2004). The study was approved by the Board of Advance Studies at The Islamia University of Bahawalpur as a part of M.Phil. research project.

In-vitro experimentation (phytochemical screening of the crude extracts)
Preliminary screening of Bs.Cr., for a variety of chemical constituents (alkaloids, flavonoids, tannins, saponins, coumarins and anthraquinones), was carried out using standard methods (Tona et al., 1999).

In-vivo experimentation
Adult albino rats of either sex weighing between 200 and 220 g were divided into five groups of six animals each. Prior to experimentation, animals were screened for any visible signs of disease and only the healthy animals were selected. The study was performed at room temperature, and through out the study duration, animals were kept at the same temperature. Before experimentation, the bladder of rats were emptied by gentle compression of the pelvic area and by the pull of their tails. Group I (control group) was given normal saline 10 ml/kg, group II (reference group) was given 10 mg/kg of furosemide and test groups (III, IV and V) were given graded doses of Bs.Cr., respectively. All the doses were made in same volume of normal saline to administer uniform volume in each group. Intra-peritoneal route (IP) was used for the administration of drugs because of its benefits over other routes. Immediately after administration, animals were kept in metabolic cages (Asif et al., 2013).

Assessment of diuretic activity
The urine was collected in graduated vials and total volume was measured at the end of 6 hours and expressed as ml/100 g of body weight per 6 hours (Asif et al., 2013; Ratnasooriya et al., 2004).

Determination of electrolytes level
Levels of sodium and potassium, in fresh urine samples, were estimated using calibrated Flame Photometer.
Before estimating urinary sodium and potassium levels, samples were filtered to remove debris and shedding. Concentration of electrolytes was expressed in parts per million (ppm) (Asif et al., 2013; Sathianarayanam et al., 2011).

**Determination of pH of urine**

A calibrated pH meter (Model: WTW-Series pH-720) was used to estimate the pH of the fresh urine samples (Abdala et al., 2012; Asif et al., 2013).

**Assessment of acute toxicity**

To perform the acute toxicity test of Bs.Cr., albino mice (18–25 g) were used. Animals were divided in different groups of five mice each. The control group received normal saline (10 ml/kg), while test groups were administered graded doses of Bs.Cr. (up to 3000 mg/kg). All the doses were administered by the oral gavage. Animals were observed carefully for 2 hours, then at 30 minute breaks for 6 hours, for any evident signs of toxicity (salivation, ptosis, lacrimation, convulsions, squinted eyes, tremors, writhing, loss of hair and yellowing of fur), stress (exophthalmia and erection of fur), behavioural abnormalities (cleaning of face, ataxia, impairment of spontaneous movement, climbing and other postural changes) aversive behaviour (licking of tail, paw and penis, biting and scratching behaviour, intense grooming behaviour and vocalization) and diarrhea (Asif et al., 2013; Ratnasooriya et al., 2004). Mortality of the animals was noted at 24 hours.

**Calculation of diuretic index, lipschitz value, saliuretic index and Na+/K+ ratio**

The following equations were used for the calculation of these parameters (Abdala et al., 2012; Asif et al., 2013; Danamma et al., 2011).

- **Diuretic Index** = Mean urine volume of the test group/ Mean urine volume of the control group.
- **Lipschitz value** = Mean urine volume of the test group/ Mean urine volume of the reference group.
- **Saliuretic Index** = Concentration of electrolyte in urine of the test group/ Concentration of electrolyte in urine of the control group.
- **Na+/K+ Ratio** = Concentration of Na+ in urine of a group/ Concentration of K+ in urine of the same group.

**RESULTS**

**Phytochemical Analysis**

The phytochemical analysis of Bs.Cr. was positive for saponins and flavonoids, while negative for alkaloids, coumarins and anthraquinones (Table 1).

**Table 1: Phytochemical Screening of Boswellia serrata crude extract**

<table>
<thead>
<tr>
<th>Constituent to be tested</th>
<th>Bs.Cr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
</tbody>
</table>

Negative (-) sign indicates the absence of phytochemical constituent, Positive (+) sign indicates the presence of phytochemical constituent.

**Effect of plant extract on urinary output in rats**

The IP administration of Bs.Cr. increased the urinary output in a dose-dependent manner (fig. 1). Compared with group I (control group, normal saline treated), approximately 1.3, 2.1 and 2.9 times increase in urine output was observed in the test groups (group III, IV and V), respectively.

**Fig. 1:** The effect of Bs.Cr. on urination in albino rats. Values shown are mean ± S.E.M of six observations and the values are compared with the control group and considered significant as *p < 0.001.
Table 2: Effect of Boswellia serrata on urinary volume and electrolyte concentration

<table>
<thead>
<tr>
<th>Group</th>
<th>Extract &amp; dose (mg/kg)</th>
<th>Volume of urine (ml/6 hrs)</th>
<th>Urine Sodium (ppm)</th>
<th>Urine Potassium (ppm)</th>
<th>pH</th>
<th>Diuretic Index</th>
<th>Lipschitz value</th>
<th>Saliuretic Index</th>
<th>Saliuretic Index</th>
<th>Na⁺ / K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal saline 10 (ml/kg)</td>
<td>1.0±0.02</td>
<td>479.2±0.8</td>
<td>26.3±0.2</td>
<td>7.5±0.03</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>18.19</td>
</tr>
<tr>
<td>2</td>
<td>Furosemide 10</td>
<td>6.6±0.2**</td>
<td>568.3±1.6**</td>
<td>48.8±0.5**</td>
<td>7.8±0.1</td>
<td>6.6</td>
<td>1.18</td>
<td>1.85</td>
<td>11.63</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Bs.Cr. 10</td>
<td>1.3±0.01**</td>
<td>486.7±2.1</td>
<td>33.5±0.5**</td>
<td>7.5±0.01</td>
<td>1.3</td>
<td>0.20</td>
<td>1.01</td>
<td>1.27</td>
<td>14.52</td>
</tr>
<tr>
<td>4</td>
<td>Bs.Cr. 30</td>
<td>2.1±0.1**</td>
<td>510.3±0.3**</td>
<td>40.3±0.2**</td>
<td>7.7±0.1</td>
<td>2.1</td>
<td>0.30</td>
<td>1.06</td>
<td>1.53</td>
<td>12.65</td>
</tr>
<tr>
<td>5</td>
<td>Bs.Cr. 50</td>
<td>2.9±0.1**</td>
<td>553.3±3.3**</td>
<td>47.8±0.2**</td>
<td>7.7±0.1</td>
<td>2.9</td>
<td>0.44</td>
<td>1.15</td>
<td>1.83</td>
<td>11.56</td>
</tr>
</tbody>
</table>

Values given are Mean ± S.E.M of six observations. All the values are compared with control group (normal saline treated) and considered significant as *p < 0.01 and **p < 0.001.

Effect of plant extract on urinary electrolyte excretion and urinary pH

Bs.Cr. showed natriuretic effects in a dose-dependent manner (Fig. 2).

Fig. 2: The effect of Bs.Cr. on urinary sodium levels in urine excreted during 6 hours. Values shown are mean ± S.E.M of six observations and the values are compared with the control group and considered significant as *p < 0.01 and **p < 0.001.

The IP administration of Bs.Cr. also showed kaliuretic effects in a dose-dependent manner (Fig. 3). A similar finding was also evident through saliuretic index values. The pH of fresh urine samples, in the treated groups, was not significantly different from the pH of control group (normal saline treated) (Table 2).

Acute toxicity

Acute toxicity experiments in albino mice showed that Bs.Cr. was nontoxic even at the dose of 3000 mg/kg. The plant extract did not show any visible signs of toxicity, stress and/or adverse behaviors. Likewise, there was no sign of diarrhea, and none of the treated animals died in 24 hours.

Fig. 3: The effect of Bs.Cr. on urinary potassium levels in urine excreted during 6 hours. Values shown are mean ± S.E.M of six observations and the values are compared with the control group and considered significant as *p < 0.001.
DISCUSSION

In-vitro experimentation
The previous studies on phytochemical constituents of Bs.Cr. showed that it contains essential oils, gum and resin. Boswellic acid, a pentacyclic triterpene, was identified as active moiety of the resin portion (Sharma et al., 2007). In this study, the preliminary phytochemical investigation revealed the presence of flavonoids and saponins in Bs.Cr. (Table 1). Earlier studies demonstrated that there are numerous compounds (e.g., flavonoids, saponins or organic acids) which could be responsible for the plant’s diuretic effects (Singh et al., 2008). Similarly, some studies showed that certain flavonoids were found to exert diuretic activity by binding with adenosine A1 receptors associated with the diuretic action (Yuliana et al., 2009). Based on the fact that Bs.Cr. is rich in saponins and flavonoids, the diuretic activity of studied plant might be the consequence of such mechanisms. However, the precise site and cellular mechanisms of Bs.Cr. are unclear.

In-vivo experimentation
The diuretics are typically prescribed in clinical conditions like hepatic cirrhosis, congestive cardiac failure, nephritic syndrome and hypertension (Fink et al., 2003). It has long been known that hypertension accelerates the progression of renal disease (Whitworth, 2005). Progression of renal diseases associated with high blood pressure might be delayed by an adequate control of high blood pressure. The present study was performed on albino rats to scientifically prove the diuretic potential of Bs.Cr. The maximum diuretic effects were observed at the dose of 50 mg/kg. The further increase in dose resulted in the reduction of urine volume. This fact showed that the crude extract possess dual activity i.e. at lower doses the Bs.Cr. showed diuretic effects but as the dose increased, the diuretic activity was decreased. Similar to our study findings, Urtica dioica (Urticaceae) proved to act as diuretic and natriuretic agent at lower doses but no such effects were observed at the higher doses (Tahir et al., 2000).

The diuretic activity is considered to be good if the diuretic index values are greater than 1.50, moderate if the values are between 1.00 and 1.50, mild if the values lie between 0.72 and 1.00, and there is no diuretic activity if the value is < 0.72 (Abdala et al., 2008). In the present study, the diuretic index values of the treated groups were 1.31, 2.06 and 2.90, respectively. This indicated that crude extract showed good diuretic activity especially at the dose of 50 mg/kg. Lipschitz values were also calculated to compare the diuretic activity in treated groups with the reference standard (furosemide). The results showed that at the maximal dose (50 mg/kg), compared with furosemide, Bs.Cr. showed 44 % diuretic activity. These findings indicated that furosemide was much more potent than the crude extract. This phenomenon necessitates the isolation of pure phytochemical constituents of Bs.Cr. for the estimation of maximal diuretic activity.

Sodium is considered to an important external factor responsible for primary hypertension (Asif et al., 2013). Numerous studies showed that increased uptake of sodium adversely affects the arterial blood pressure (Horacio et al., 2007). Increased excretion of urinary sodium in our experimental animals, after the administration of Bs.Cr., showed that plant has a potential to be used as an antihypertensive agent.

In the toxicology study, even at the higher doses, no lethal effects were detected in all animals. It is therefore hypothesized that the plant is free from toxic effects. However, it is suggested that further studies should investigate hormonal, neural and metabolic parameters to study ultimate side effects.

CONCLUSION
Together with established renoprotective (Pandey et al., 2005) and antioxidant (Tripathi et al., 2004) properties, proved diuretic activity of Bs.Cr. in our study promotes this medicinally important plant as a potential candidate for a new diuretic agent. Further studies are encouraged to isolate the active phytochemical constituents, and to explore the exact mechanism of action.

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