Evaluation of Diuretic Activity of Extracts of Gentiana oliveri and Gentianine in Rats

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INTRODUCTION

The use of herbal medicine is undergoing a revitalization process to alleviate human sufferings. People are taking a more active role in their health and the health of their loved ones. As people turn to herbal supplements, professional, accurate information on their proper use needs to be circulated. The mountains of Balochistan are inhabited by a large variety of wild herbs and shrubs used for medicinal purposes including Gentiana oliveri [1].

Plants of the genus Gentiana have been the subject of interest from a medical point of view. Plants belonging to genus Gentiana are very well-known for their pharmacological properties. It is being used from centuries in traditional folk medicine for the treatment of skin disease ulcers, abscesses, arthritis, blood pressure, fever, stomach remedy and stimulant of appetite [2]. Extensive biological and pharmacological work was carried out on gentianine, an isoprene alkaloid commonly distributed in gentianaceae [3]. The alkaloid was reported to have diverse pharmacological activates and antihypertensive assays of the extracts of Gentiana oliveri and its pure alkaloid gentianine proved to be highly efficacious [4,5].

Evidence for its diuretic activity derives mainly from some experimental data [6] and traditional use [7]. Chinese species of Gentiana are still being used as diuretic herbs [8]. Many drugs currently in use are very expensive, cause undesirable side effects and people have developed resistance against them. These important properties of G. oliveri as hypoglycemic effect [9] must be utilized, by developing it into an efficacious drug, so that the people could be benefited by its therapeutic effects. However, the adverse effects or the side-effects of the drugs should be known before using it as a medicine. All drug regulatory agencies require that any drug that is to reach the market should be subjected to rigorous evaluation to determine its safety and efficacy. This study was conducted to determine the diuretic effect of the pure, aqueous and alcoholic extracts of G. oliveri in rats.

MATERIALS AND METHODS

Plant material

Fresh plant material of Gentiana oliveri was collected from Chiltan heights near Quetta, in April 2004 and was identified at the University of Balochistan and a sample specimen was deposited at the herbarium.
Extraction and purification of alkaloid

Three kg of air dried plant of *Gentiana oliveri* was grinded and soaked in ethanol for two weeks. The ethanolic extract was acidified and extracted with chloroform. The acidic aqueous layer was then basified with ammonia solution to pH 8.0 and further extraction was carried out with chloroform. Five g of this extract was then loaded on a silica gel column and elution was carried out first with hexane and then with increasing polarities of hexane-chloroform as explained by [4].

Diuretic assay

Male Wistar rats weighing 200-250 g were placed in four groups of six rats each. The fifth group was kept as a control. A water bolus of 2.5% body weight was administered 2 hrs, before the experiment. Extracts in a fixed dosage, based on dry weight in relation to body weight of each individual animal were re dissolved in water and applied by stomach tube. 100 mg/kg of pure gentianine was administered to the first group. The other two groups were dosed with 250 mg/kg of ethanolic extract and aqueous extract respectively. Furosemide (100 mg/kg) was given to the fourth group as a standard. Distilled water of 2.5% body weight was given to the control group. Animals were kept singly in metabolic cages and were allowed free access to water. Urine was collected for six days. The parameters taken for each individual rat were body weight before and after the test period, total urine volume (corrected for water intake during the test period), and urine concentration of Na⁺, K⁺ and Cl⁻ [6]. The electrolyte concentrations were measured by flame photometer.

The pure compound gentianine was also studied to examine the diuretic effect at different doses of 100, 200 and 400 mg/kg in three groups of six male Wistar rats each. The purpose of this series was to determine the renal excretory response to oral administration of gentianine in normal rats (n=6 each dose). Daily oral administration of gentianine was tested for 24 hours. The parameters taken for each individual rat were same as before. Urinary water and electrolytes excretion were determined after 24 hours. All animal studies were conducted in accordance with the criteria of the laws governing the use of experimental animals.

Statistical analysis

Statistical analysis of the results was performed by (SSPS) one-way ANOVA and Student's t-tests, as appropriate. Results are expressed as mean ± SEM and were considered significant at a *p* level of 0.05 or less.

RESULTS AND DISCUSSION

The pure compound isolated was identified with the help of UV spectrum, IR spectrum, High resolution mass spectrum, ¹H-NMR and ¹³C-NMR and was found to be gentianine (Mansoor, 2000).

The renal urinary excretory responses to gentianine, ethanolic extract, aqueous extract and furosemide are summarized in Table 1. Gentianine (100 mg/kg) increased urine flow by 211% (*p<0.02*) ethanolic extract of *G. olivieri* (250 mg/kg) by 217% (*p<0.02*) and aqueous extract significantly increased urine flow by 242.89% (*p<0.01*). Furosemide had the greatest effect on urine output and an increase of 454% (*p<0.001*) was determined.

There were significant changes in potassium excretion in rats treated with all the diuretics. All the fractions of the plant extract dosed, produced nearly similar effect on urinary excretion of potassium. Increase of 136% (*p<0.04*) at 100 mg/dose of gentianine and increase of 141% (*p<0.04*) at 250 mg/dose of ethanolic and 146% of aqueous extract were observed. However, furoseamide significantly increased potassium excretion by 188% (*p<0.01*) compared to vehicle. Observed on the sixth day in all the treated rats, which appear to be due to the body water loss in urine (Table 1).

<table>
<thead>
<tr>
<th>Measured parameters</th>
<th>Water control ± SEM</th>
<th>Gentianine (100 mg kg⁻¹) ±SEM</th>
<th>Ethanolic Extract (250 mg kg⁻¹) ±SEM</th>
<th>Aqueous Extract 250 mg kg⁻¹ ±SEM</th>
<th>Furosemide 100 mg kg⁻¹ ±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total urine volume (ml)</td>
<td>12.6 ± 2.8</td>
<td>26.7 ± 1.5*</td>
<td>27.4 ± 3.2*</td>
<td>29.6 ± 4.5*</td>
<td>32 ± 5.2*</td>
</tr>
<tr>
<td>Total sodium mEq/L</td>
<td>20 ± 3.8</td>
<td>54.2 ± 1.7</td>
<td>68.5 ± 2.2</td>
<td>80.0 ± 3.1</td>
<td>98 ± 2.5</td>
</tr>
<tr>
<td>Total potassium mEq/L</td>
<td>50 ± 4.2</td>
<td>68.2 ± 2.6</td>
<td>70.5 ± 3.6</td>
<td>78.5 ± 4.2</td>
<td>94 ± 4.4</td>
</tr>
<tr>
<td>Total chloride mEq/L</td>
<td>28 ± 2.9</td>
<td>80.1 ± 4.1</td>
<td>86.8 ± 2.4</td>
<td>90.0 ± 2.8</td>
<td>109 ± 5.4</td>
</tr>
</tbody>
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S.E.M is Standard error of mean.
*p<0.02; *p<0.02*, *p<0.01*, *p<0.001*

The aqueous sample preparation showed the highest diuretic activity among the extracts of *G. olivieri*. There was marked increase in Na⁺, K⁺ and Cl⁻ ion excretion in the aqueous extract as compared to control and furosemide (standard), although values of furosemide remain highest.

The urine output among the pure alkaloid. Extracts (ethanol and water) and the furosemide against the control was statistically significant (Table 1).

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Gentianine at different doses produced a significant and dose-dependent diuresis (Figure 1) and increase in electrolytes
excretion (Figure 2). The lowest dose (100 mg kg⁻¹) of gentianine caused urine output of 5.15 ± 0.35 ml/24 h which enhanced to 10.75 ± 0.65 ml/24 h at the dose of 200 mg kg⁻¹, and 8.01 ± 1.25 ml/24 h (p<0.001). At the highest dose of 400 mg kg⁻¹, a similar increase in all three ion concentrations was observed at the corresponding increasing doses (Figure 2). The increase of electrolytes in the urine on treatment with gentianine was dose-dependent. Excretions were determined in three separate groups (n=6 each) of rats, for 24 hours.

Figure 1. Dose dependent effect of oral administration of gentianine 100 mg kg⁻¹, 200 mg kg⁻¹ and 400 mg kg⁻¹ on urine volume in rats.

Figure 2. Dose dependent effect of oral administration of gentianine 100 mg kg⁻¹, 200 mg kg⁻¹ and 400 mg kg⁻¹ on sodium, potassium and chloride excretion in rats.

The pure aqueous and alcoholic extracts of G. olivieri appear to be quite active on the diuretic systems of rats. The data in Table 1 shows that urine volume in all the groups have considerably increased as compared to the standard. Ethanolic and alcoholic extracts proved to be slightly more effective than the activity of pure alkaloid. The single oral dose of aqueous extract of 250 mg kg⁻¹ seems to be the most effective diuretic. This indicates that besides gentianine, the other constituents of the plant, soluble in water also have similar diuretic property [6,10].

Ion excretion was also elevated in all the dosed groups, but was maximum with the aqueous extract. Sodium ion was greatly increased as compared to potassium. A high increase for C1 ions was also observed. Thus, the data presented supports the hypotensive and diuretic traditional use of this plant. Moreover, gentianine, dose-dependently increased urine flow, sodium excretion, potassium excretion and chloride excretion in normal rats. Greater diuretic activity of the aqueous extract might be due to the presence of other active water-soluble which might be diuretic in nature.

However, it has previously been observed by Englart and Harnischfeger that in some cases rats is a rather poor model for diuretic studies. Therefore this pharmacological action should also be studied in more appropriate model i.e. dogs [11,12]. Gentiana scabrae along with other diuretic herbs are also being used as antibacterial against various microbes in urine such as candida albicans and trichomonas vaginalis [8]. It is also known to treat and remove (with a combination of herbs) casts in urine, caused by a kind of cellular dysfunction. In Ayurveda system, a number of gentians including G. kurroo Royle (Indian gentian), have been reported as being used for stomach and urinary-tract medicine, and fever remedy [13,14].

Elevated blood pressure is a leading contributor to cardiovascular disease which has become the main cause of illness and death worldwide. Therefore, efficient inhibition of sodium reabsorption without reducing renal function is therapeutically advantageous in conditions such as chronic renal failure, congestive heart failure, and cirrhosis. It possesses analgesic, antihistaminic and anticonvulsant activity and anti-inflammatory [15] and antibacterial properties. Since, gentianine is a pure alkaloid, now with many known pharmacological effects, has a full potential to be developed as a drug. The safety and efficacy of G. olivieri and its alkaloid gentianine has been shown scientifically [4,6] therefore, measures should be taken to develop it as a drug. It is the need of the time to explore and evaluate this natural product from scientific perspective.

REFERENCES