Evaluation of Acute and Sub-chronic Diuretic, Saluretic and Kaliuretic Effects of Barleria lupulina

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Abstract: Background: This study evaluated the acute and sub-chronic diuretic, saluretic and kaliuretic effects of orally administered Methanol Extract of Barleria lupulina (MEBL) leaves in normal rats and thereby, confirm its ethno medical use. Materials and methods: Acute diuretic activity was estimated by single dose methanol extract of leaves of Barleria lupulina (200 and 400 mg kg⁻¹ b.wt. p.o.) and furosemide (10 mg kg⁻¹ b.wt. p.o.), duration of the study is 24 h. Sub-chronic diuretic activity was estimated by administered extract (200 and 400 mg kg⁻¹ b.wt. p.o.) and furosemide (10 mg kg⁻¹ b.wt. p.o.) for the period of eight days. Results: In acute study, the diuretic effect of MEBL at the higher dose i.e. 400 mg kg⁻¹ b.wt. was found to be highly significant at 1, 2, 4 and 6 h (p<0.01 vs. control). Both the doses of plant extract significantly enhanced the excretion of the Na⁺ and K⁺ versus control (p<0.01). In Sub-chronic study, both the doses of MEBL produced significant diuresis until day 3 (MEBL 200 mg kg⁻¹ b.wt.) and day 5 (MEBL 400 mg kg⁻¹ b.wt.). MEBL 400 mg kg⁻¹ b.wt. increased the excretion of Na⁺ and K⁺ significantly (p<0.01 vs. control) throughout the treatment from Day 1 to 8. Conclusion: The present study confirmed the ethno pharmacological use of Barleria lupulina as a diuretic agent based up on the experimental conditions tested.

Key words: Barleria lupulina, drugs, sub-chronic, pharmacological

INTRODUCTION

Diuretics are drugs that bring about an increase in urinary volume as well as in the electrolyte output. Due to this, they are used to regulate both volume and composition of the urine in different affections like high blood pressure, heart failure and nephritic syndromes (Peraz et al., 2011). There is wide stock of synthetic drugs, most of those drugs have the adverse effects like impotence, fatigue and weakness (Agus and Goldberg, 1971). Hence, search for the new diuretic agent become necessary. Medicinal plants can be important sources of unknown chemical substances with potential therapeutic effects and less adverse effects.

Barleria lupulina (Acanthaceae) is a small herb, distributed in the South Asia region. It has been traditionally used for mental tension, diabetes, rheumatoid arthritis and snake bite (Chopra et al., 1968). Seven iridoid glucosides have been reported from B. lupulina (Byrne et al., 1987). From the leaves a betaine compound was isolated (Suba et al., 2002). From the stem and leaves of B. lupulina, alkaloids were estimated (Suba et al., 2002). The extract of the plant showed high antiviral activity against HSV-2 and anti-ulcer activity (Wanikat et al., 2007). Barleria lupulina is well recognized in traditional medicine as having a diuretic effect but no scientific data has been published supporting the claimed ethno medical use. Therefore, the aim of this study was to evaluate the acute and sub-chronic diuretic, saluretic and kaliuretic effects of orally administered methanol extract of Barleria lupulina leaves in normal rats and thereby to confirm its ethno medical use.

MATERIALS AND METHODS

Plant collection and extraction: The leaves of Barleria lupulina were collected from B.I.T, Mesra, Ranchi in the month of December 2010. Previously the plant had been identified and authenticated by Dr. Tariq Husain, National Botanical Research Institute, Lucknow, India. The voucher specimens were retained in Department of Pharmaceutical Sciences, B.I.T. Mesra, Ranchi, for future reference. The leaves of Barleria lupulina were dried in the shade of about 30°C and crushed into a coarse powder. The dried and
powdered plant material (leaves) was subjected to successive hot extraction in a Soxhlet continuous extraction apparatus with methanol as a solvent. The average time period for extraction was 48 h. The extract was filtered and concentrated using a Rotary evaporator (Buchi US).

**Animals:** Forty-eight male Wistar Albino rats (150 to 200 g body weight) were used in this study. Animals were procured from Institutional Animal House (Reg no. BT/PH/IAEC/31/2010/dated 15-9-10) of Birla Institute of Technology, Mesra. All experiments involving animals comply with the ethical standards of animal handling and approved by Institutional Animal Ethics Committee.

**Drug:** Furosemide procured from IDPL (Indian Drugs and Pharmaceutical Laboratories) was used as reference diuretic drug.

**Acute diuretic activity**

**Treatment protocol:** Rats were numbered, weighed and then divided into 4 groups with 6 animals in each as follows:

- **Group I:** Served as the control group and received distilled water 10 mL kg⁻¹ b.wt., orally
- **Group II:** Received 200 mg kg⁻¹ b.wt. of methanol extract of *Barleria lupulina* (MEBL-200), orally
- **Group III:** Received 400 mg kg⁻¹ b.wt. of methanol extract of *Barleria lupulina* (MEBL-400), orally
- **Group IV:** Served as standard and received 10 mg kg⁻¹ body weight of furosemide, orally

Before treatment, the animals were fasted for 18 h with water ad libitum.

**Method:** Each animal was placed in an individual metabolic cage 24 h prior to commencement of the experiment for adaptation. Before treatment, all animals received physiological saline (0.9% Sodium chloride) at an oral dose of 5 mL/100 g body weight (BW), to impose a uniform water and salt load. Urine was collected and measured at 1, 2, 4, 6 and 24 h after the above treatment (Maghrani *et al.*, 2005). The sodium and potassium concentrations were determined in the 24 h urine samples as well as in the plasma of rats using flame photometer (Systronics flame photometer 128).

**Sub-chronic diuretic activity**

**Treatment protocol:** The animals were numbered, weighed and then divided into 4 groups with 6 animals in each as follows:

- **Group I:** Received orally distilled water 10 mL kg⁻¹ b.wt. for 8 days, orally and served as the control group
- **Group II:** Received orally 200 mg kg⁻¹ b.wt. of methanol extract of *Barleria lupulina* (MEBL-200) leaves for 8 days, orally
- **Group III:** Received orally 400 mg kg⁻¹ b.wt. of methanol extract of *Barleria lupulina* (MEBL-400) leaves for 8 days, orally
- **Group IV:** Received orally 10 mg kg⁻¹ b.wt. of Furosemide for 8 days and served as standard

Before treatment, the animals were fasted for 18 h with water ad libitum.

**Method:** For each rat, 24 h urine was collected daily and its volume measured. The Urinary sodium and potassium concentrations were measured in each urine specimen. Sodium and potassium levels were measured in plasma of rats on Day 8 using flame photometer (Systronics flame photometer 128). The Concentration of Creatinine in plasma and urine was determined for Day 8 by the Creatinine assay kit (Bio in vivo diagnostics Pvt. Ltd. Gujrat) using Jaffe alkaline picate method (Agurni *et al.*, 2005).

**Statistical analysis:** The values were calculated as Mean±SEM. The significance of the difference of mean value with respect to control group was analyzed by one way ANOVA followed by Dunnet’s t-test. p<0.05 or above was considered to be significant.

**RESULTS**

**Acute diuretic activity:** Treatment with a single dose of the *Barleria* extract increased diuresis at both the doses i.e., 200 mg kg⁻¹ and 400 mg kg⁻¹ b.wt. (Fig. 1a). This was significantly higher than in the control rats at 4 and 6 h after the dose of 200 mg kg⁻¹ b.wt. (p<0.05 vs. control) which became insignificant at the end of 24 h. The diuretic effect of MEBL at the higher dose i.e., 400 mg kg⁻¹ b.wt. was found to be highly significant (p<0.01 vs. control) at 1, 2, 4 and 6 h which at 24 h showed statistical significance (p<0.05 vs. control).

Both the doses of plant extract and furosemide significantly enhanced the excretion of the Na⁺ versus
controls (p<0.01). Furthermore, the natriuretic activity of higher doses of extract was higher than the furosemide which was higher than that at lower dose. The urinary K\(^+\) concentration was found to be significantly higher on administration of MEBL 400 mg kg\(^{-1}\) b.wt. versus controls (p<0.01) which was greater than produced by MEBL 200 mg kg\(^{-1}\) b.wt. There was no effect of furosemide or plant extract on plasma levels of Na\(^+\) and K\(^+\) levels (Fig. 2).

**Sub-chronic diuretic activity:** Administration of daily doses of both the doses of MEBL produced significant diuresis starting on day 1 (p<0.01 vs. control) which kept increasing until day 3 in case of MEBL 200 and until 5th day in case of MEBL 400 after that urinary output leveled off. The diuretic effect of furosemide was significant (p<0.01 vs. control) with reference to control from the day 1 to day 8 higher than produced by both the doses of the plant extract, on all the days (Fig. 1b).

When MEBL was administered at the dose of 200 mg kg\(^{-1}\) b.wt. it showed a significant increase in urinary excretion of Na\(^+\) on day 2 (p<0.05 vs. control) which became more pronounced (p<0.01 vs. control) from day 3 to 8. When MEBL was administered at the dose of 400 mg kg\(^{-1}\) b.wt., the excretion of Na\(^+\) increased significantly (p<0.01 vs. control) throughout the treatment from day 1 to 8. In case of furosemide there was significant increase (p<0.01 vs. control) in excretion of Na\(^+\) throughout the treatment from day 1 to 8 (Fig. 2).

When MEBL was administered at the dose of 200 mg kg\(^{-1}\) b.wt. it showed a significant increase in urinary excretion of K\(^+\) on day 5 (p<0.05 vs. control) which became more pronounced (p<0.01 vs. control) on 7th and 8th Day. When MEBL was administered at the dose of 400 mg kg\(^{-1}\) b.wt. the excretion of K\(^+\) increased significantly (p<0.01 vs. control) throughout the treatment from day 1 to 8. In case of furosemide there was no significant increase in excretion of K\(^+\) throughout the treatment (Fig. 2). Both MEBL and furosemide decreased the Na\(^+\) level in the plasma to a significant level (p<0.05 vs. control). MEBL at both the
Fig. 2(a-c): (a) Effect of MEBL and Furosemide on urinary electrolyte excretion and plasma electrolyte levels after single dose administration, (b) Effect on sodium excretion of sub-chronic administration of MEBL and Furosemide and (c) Effect on Potassium excretion of sub-chronic administration of MEBL and Furosemide. Values are reported as Mean±SEM for six rats in each group.

doses also reduced K⁺ level in the plasma though the level was statistically insignificant.

**DISCUSSION**

The diuretic effect of MEBL was confirmed, when it caused significant increase in the urine volume (diuresis) in rats; this effect was similar to that of furosemide, a standard diuretic. In parallel with increase in the volume of urine, MEBL also enhanced the urinary excretion of Na⁺ and K⁺. The mechanism of action by which diuresis was induced by this MEBL extracts was also assessed by comparing the effect with that of furosemide, a high-ceiling loop diuretic (Benjumea et al., 2005) and that of hydrochlorothiazide (Maghrani et al., 2005). The reference drug, furosemide, increases urine output and urinary excretion of sodium by inhibiting Na⁺/K⁺/2Cl⁻ symporter (co-transporter system) in the thick ascending limb of the Loop of Henley, while the thiazide diuretics inhibit the Na⁺/Cl⁻-symporter (co-transporter system) in the distal convoluted tubule. Hydrochlorothiazide has been reported to increase the urinary excretion of both Na⁺ and K⁺ by 50-60% over controls after a single oral dose in normal rats (Maghrani et al., 2005).

In our study we found that MEBL significantly increased the sodium and potassium in urine. Furosemide which was used as reference standard increased the sodium concentration in urine but not that of potassium unlike hydrochlorothiazide which has been reported to increase the urinary excretion of both Na⁺ and K⁺.

In the sub-chronic study MEBL and furosemide induced significant diuresis. Tolerance does not seem to develop to the diuresis stimulating activity of the plant extracts. Both MEBL and furosemide increased the sodium excretion. On the other hand, repeated administration of MEBL caused a significant increase in urinary excretion of K⁺ beginning on day 1 and continuing throughout the study period contrarily, furosemide virtually had no effect on urinary K⁺ excretion for the entire 8 days.
Further, there was decrease in plasma electrolyte levels both of sodium and potassium in the sub-chronic studies, suggesting that probably the active principle(s) in MEBL do not act like potassium-sparing diuretics. The results of the present studies suggest that the effect of one or more of the active components of MEBL have a thiazide-like action.

The present studies also support the ethnomedicinal use of *Barleria lupulina* for its diuretic effect. Although, the active components remain unidentified, based on the pattern of excretion of water, sodium and potassium, it appears that there are active principals present in its methanolic extract of its leaves, may have a thiazide-like activity. The plant extracts do not seem to have renal toxicity in rats at the dose studied. These findings suggest for the first time, mechanism(s) of diuretic action of *Barleria lupulina* used in traditional medicine.

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