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EVALUATION OF DIURETIC EFFECT OF *LYCIUM BARBARUM* LINN. (GOJI BERRY) IN RATS

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ABSTRACT: Wolf berries or Goji berries (*Lycium barbarum* L. Solanaceae) have a long tradition as food and medicinal plant. The bark of Goji berries is generally harvested in the winter season and then dried to be used for diuretic purposes. Hence, this study was designed to evaluate the possible diuretic effects of powdered *L. barbarum*. The study was conducted in saline primed Wistar albino rats (n=6) using frusemide (10 mg/kg) as the reference diuretic drug with two oral doses, 250mg/kg/day and 500mg/kg/day, of the test drug. Urine volume and electrolytes (Sodium, Potassium and Chloride) excretion was estimated at the end of 24 hours and data was analyzed by ANOVA followed by Dunnett's test. $P < 0.05$ was considered as statistically significant. *L. barbarum* statistically increased the volume of urine (8.08 ±0.35ml/100g/24hr and 10.05±0.51ml/100gm/24hr) in a dose dependent manner. There was a statistical significant increase in sodium ion excretion (143.17±9.5 m.mol/L at 250mg/kg and 182±8.25 m.mol/L at 500ml/kg) when compared to the normal control (107±2.11 m.mol/L). However, there was a statistically significant decrease in potassium ion excretion (47.17±4.1 m.mol/L and 30.17±2.4 m.mol/L) when compared to the control (55±4.1 m.mol/L). To conclude, these findings suggest that *L.barbarum* possesses diuretic activity and further studies elucidating mechanism of action are warranted.

INTRODUCTION: Plants are one of the most important sources of medicines. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective and their easy availability [1, 2]. However most of the claimed benefits need to be scientifically validated to be used in human clinical conditions.

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Diuretics play an important role in situations of fluid overload, like acute and chronic renal failure, hypercalciuria, cirrhosis of liver and also as an antihypertensive agent. Though a number of diuretics like mannitol, thiazides, furosemide, ethacrynic acid are used in practice, there is still a need for more effective and less toxic diuretic. Many indigenous drugs have been claimed to have diuretic effect in Ayurvedic system of medicine but most of them have not been properly investigated. Wolf berry is one such medicinal plant with many medicinal properties.

Wolf berries or Goji berries (*Lycium barbarum* L. Solanaceae) have a long tradition as food and medicinal plants in China and various other Asian

countries. *L. barbarum* L. is a deciduous shrub one to three meters high. The leaves are lanceolate to ovate. The oblong, orange to dark red berries measure up to 2 cm and possess a bitter to sweet taste. The berries are eaten raw, drunk as a juice, wine or tea. They can also be processed to tinctures, powders, and tablets. The recommended dosage of dried berries used as medicine varies between 5 and 12 g [3]. Fruit from *L. barbarum* L. has been claimed to possess a large variety of beneficial effects, such as reduction of blood glucose and serum lipids in alloxan induced diabetes models, anti-aging, anti-hyperlipidemic, immuno-modulating, anticancer, anti-fatigue, and male fertility-facilitatory actions [4-9].

Goji berries contain polysaccharides, vitamin C, vitamin B complex, vitamin E, 18 amino acids including the eight essential amino acids which the body does not produce**,** around 21 trace elements including (zinc, iron, copper, calcium, selenium, germanium sesquioxide and phosphorus) [10]. Some constituents of *Lycium barbarum* fruits have been chemically investigated, especially Lycium barbarum polysaccharide (LBP) components. Five polysaccharides (glycoconjugates) (LbGp1-LbGp5) were isolated and structurally elucidated [5, 6].

It has been reported that the bark of Goji berries is generally harvested in the winter season and is then dried to be used for diuretic and purgative purposes [11]. Hence this study was designed to evaluate the possible diuretic effects of powdered *L. barbarum.*

MATERIALS AND METHODS:

Experimental animal: Healthy adult Wistar albino rats of either sex, weighing 150-200 g obtained from Central Animal house, Bejai, Mangalore were used for the study. Rodents were housed in clean polypropylene cages, with dust free rice husk as a bedding material; three rats per cage; under controlled laboratory conditions. (Temperature: 25° ± 2°C, humidity (60% ± 10%) and 12 h light/dark cycle as per CPCSEA guidelines).

The experimental animals were fed with standard chow containing fat 4.15%, protein 22.15%, carbohydrates 4% (supplied by Amruth laboratory animal feed manufactured by Pranav Agro industries ltd., Sangli) and water *ad libitum.*

The rodents were allowed to acclimatize to these conditions for one week prior to the commencement of the study. The experimental work was approved by the Institutional Animal Ethics committee.

Drugs: Frusemide (Sanofi Aventis Co.) was used as a reference standard diuretic drug.

Test drug: Powdered *L. Barbarum* fruit extract (obtained from Gojiberry India Pvt. Ltd. Mumbai) was given at a dose of 250mg/kg/day and 500mg/kg/day, per oral, suspended in gum acacia.

Evaluation of diuretic activity: Each animal was placed in an individual metabolic cage 24h prior to commencement of the study for adaptation. The method of Lipschitz *et al* [12, 13] was employed for the assessment of diuretic activity. According to this method, the animals deprived of food and water for 18 hours prior to the experiment, were divided into 5 groups (n=6). Group I animals received normal saline (25 ml/kg, p.o.); Group II received the standard diuretic (10 mg/kg, p.o) and Groups III and IV received the test compound Goji berry (250mg/kg and 500mg/kg) respectively. The study drug dosages were selected on the basis of other studies conducted in our laboratory previously. Before treatment, all animals received physio-logical saline (0.9% NaCl) at an oral dose of 2.5ml/100g body weight to impose a uniform water and salt load[14]. All the drugs were freshly prepared prior to administration.

Immediately after administration, the animals were placed in metabolic cages (each animal per cage), specially designed to separate urine and faeces, kept at 20°C±0.5°C. The volume of urine collected was measured at the end of 5hrs and 24hrs. During this period, no food and water was made available to the animals. The following parameters were duly noted: body weight before and after test period, total urine volume, and concentration of Na^+, K^+ and Cl^- in the urine. Na^+, K^+, Cl^- concentrations were determined by Ion Sensitive Electrode; Roche Hitachi 917 automatic analyzer and bicarbonate ion was estimated with Blood gas analyzer: AVL compact-3.

The diuretic action of test drug was calculated by using the following formula:

Diuretic action =

$$\frac{\text{Urinary excretion of test drug group}}{\text{Urinary excretion in control group}}$$

Statistical Analysis: The results were expressed as mean ± SEM. The data was analysed by ANOVA followed by Dunnett's test. A value of $P < 0.05$ was considered as statistically significant. Statistical analysis were carried out using the software package SPSS (Version 17.0).

Effect on urine volume: There was no evidence of dehydration and the animals were found normal at the observed 5hr and 24hr intervals. As shown in table 1, the reference diuretic frusemide, significantly increased the urine output when compared to control ($P < 0.01$), the diuretic index being 2.74. The test drug at 250 and 500 mg/kg doses, showed a statistically significant increase in the urine volume when compared to the control. However, the increase in urine volume was not significant when compared to the control.

RESULTS:

TABLE 1: EFFECT OF ORAL ADMINISTRATION OF *L. BARBARUM* ON URINARY VOLUME EXCRETION

Group	Urine volume (ml/100g/24hr)	Diuretic index (24 hr interval)[†]
Control	3.33 ± 0.13	-
Frusemide	9.12± 0.25*	2.74
L. Barbarum (250mg/kg)	8.08 ± 0.35*	2.43
L. Barbarum (500mg/kg)	10.05 ± 0.51*	3.02

Values are expressed in mean ± SEM; [*]P <0.01 compared with control group (ANOVA followed by Dunnett's test) [†]Diuretic index = volume of test group/volume of control group

Effect on urinary electrolyte excretion: As indicated in **table 2**, frusemide significantly increased the electrolyte excretions when compared to the control (saluretic index Na/K being 1.81). The test drug, when compared to the control group, showed a significant increase in the excretion of sodium, potassium and chloride excretion in a dose dependent manner. This was also reflected in the saluretic index indicated by Na/K ratio which was 3.04 at the lower dose and 6.03 at the higher dose of the test drug. When compared to frusemide, the increase in sodium excretion was significant at 250mg/kg dose only; however, potassium and chloride excretion was significantly increased at both the doses employed.

TABLE 2: EFFECT OF ORAL ADMINISTRATION OF THE *L. BARBARUM* ON URINARY ELECTROLYTE EXCRETION:

Groups	Na^+	K^+	Cl^-	Saluretic index[‡]			Na/K
				Na	K	Cl	
Control	107±2.11	55±4.1	87.33±2.3				1.95
Frusemide	167.8±7.00*	92.5±2.1*	132.5±1.8*	1.57	1.68	1.52	1.81
L. barbarum (250mg/kg)	143.17±9.5*	47.17±4.1*	267.17±15.3*	1.34	0.86	3.06	3.04
L. barbarum (500mg/kg)	182±8.3*	30.17±2,4*	252.3±16.9*	1.7	0.55	2.89	6.03

Values are expressed in mean ± SEM; [*]P <0.01 compared with control group (ANOVA followed by Dunnett's test) [‡]Saluretic index = electrolyte concentration of test group/electrolyte concentration of control group

DISCUSSION: The present study revealed that L. barbarum fruit extract showed significant increase in urinary output and urinary electrolyte concentration when compared to the normal control. When compared to the reference diuretic, frusemide, the saluretic index was greater in the test groups than frusemide which could be attributed to a potassium retaining effect of the test extract. Diuresis has two components: increase in urine (water secretion) and a net loss of solutes (i.e. electrolytes) in the urine [15]. These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream.

The reference drug frusemide, increases urine output and urinary excretion of sodium by inhibiting Na^+ K^+2Cl^- symporter (co-transport system) in the thick ascending loop of Henle [15].

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 [16].

The regulation of Na^+/K^+ balance is also intimately related to renal control of acid-base balance.

The K^+ loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended [16].

In the present study, frusemide showed strong diuresis accompanied with high natriuresis, chloruresis, and kaliuresis ($P < 0.01$). Further there was low Na^+/ K^+ ratio, as it inhibits Na^+ K^+ and Cl^- co-transport at the thick ascending loop of Henle. K^+ excretion was increased perhaps due to high Na^+ load reaching the distal tube. However, *L. barbarum* fruits extract induced both marked natriuresis and kaliuresis ($p < 0.01$), but the Na^+/ K^+ ratio was more than that of frusemide, indicating the weak kaliuresis or K^+ saving property of the extract [17]. The above results raise the possibility of existence of diuretic activity by inhibiting tubular reabsorption of water and sodium ion. It is a good indicator for the efficacy of *L. barbarum* fruit extract as diuretics.

Phytochemical investigations of *L. barbarum* fruits have revealed the presence of polysaccharides which represent quantitatively the most important group of substances. A second major group of metabolites are the carotenoids, the content of which increases during the ripening process. The fruits further contain vitamins, in particular riboflavin, thiamin and ascorbic acid. The content of vitamin C of 42mg/100 g is comparable to that of fresh lemon fruits. Flavonoids are another important class of compounds. The fruit further contains 1–2.7% free amino acids with proline as the major constituent.

Finally, some miscellaneous compounds have been isolated including β-sitosterol and its glucoside daucosterol, scopoletin, p-coumaric acid, the dopamine derivative lyciumide A and L-monomenthyl succinate [3]. The diuretic action could be attributed to the presence of vitamin C [18] or flavonoids which have proven diuretic potential [19]. Certain flavonoids have also been found to exert their diuretic activity by binding with Adenosine A1 Receptor associated with the diuretic action [20] which could be a probable mechanism of action of the extract.

CONCLUSION: In conclusion, *L. barabrum* fruit extract showed good diuretic activity in the experimental model studied. Further studies to evaluate the mechanism of action and identification of the active ingredients could pave the way for therapeutic use of this ingenious herbal drug.

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