Original Article

Evaluation of Diuretic and Saliuretic Potential of *Beta vulgaris* (Beet Root) at Different Doses

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ABSTRACT

Background: Natural foods are presently being evaluated for their pharmacological activity. They are comparatively safer and possess fewer adverse effects. **Objective:** The current study was designed to evaluate the diuretic effect of different doses of lyophilized *Beta vulgaris*. **Methodology:** Aqueous solution of *Beta vulgaris* at doses of (500 mg/kg and 1000 mg/kg) was administered to rats. Furosemide 40 mg/70kg and distilled water were used as standard and control respectively. Metabolic cage was used for evaluation of diuretic effect. Diuretic effect was observed 24 hours after orally administering the drugs. The urine output, pH, sodium, potassium, chloride and phosphorus were measured. **Result:** *Beta vulgaris* showed highly significant ($p<0.001$) diuretic activity as compared to control. Both doses of *Beta vulgaris* also showed highly significant ($p<0.001$) effect on sodium, potassium, chloride and phosphorus excretion as compared to control. Both doses of *Beta vulgaris* showed alkalization of urine. **Conclusion:** From our study we came to conclude that *Beta vulgaris* at 500 mg/kg can be used as a diuretic agent where as at 1000 mg/kg dose it not only possesses diuretic potential but also doesn't cause hypokalemia and is also very useful in treating hyperphosphatemia as it increases excretion of phosphate in urine.

Key words: Chloride, Diuretics, Metabolic cage, Phosphorus, Potassium and Sodium.

INTRODUCTION

Diuretics are drugs used to induce diuresis i.e increase urinary outflow. It is usually also accompanied by increased excretion of electrolytes.[1] Diuretics are beneficial in many clinical conditions including hypertension, edema, congestive heart failure, nephritis and liver cirrhosis.[2] Traditionally many herbal drugs have been used in renal disorders because of their diuretic potential.[3] Although different classes of diuretics like loop, thiazide, osmotic and potassium sparing diuretics are still extensively used, they all possess adverse effects on long term exposure.[4] Recently naturally occurring medicinal plants and foods which have been known since centuries to possess therapeutic benefits for preventing and treating different disease conditions are being investigated.This is mainly due to the fact that naturally occurring food substances are considered safer and less expensive as compared to allopathic medicines.[5]

Naturally occurring food substances include vegetables as well. Vegetables are consumed by humans as a source of nutrition; however they can be used as therapeutic agents as well based on the phytochemicals present in them.[6] Beet root scientifically known as *Beta vulgaris* belongs to family Chenopodiaceae. It has a height of 1-2 cm and possesses leafy stems. It is a biennial, herbal plant having red roots.[7]

Beet root is highly rich in mineral and vitamin content. 100 gm of beet root contains 78 mg sodium (Na), 325 mg potassium (K), 40 mg phosphorus (P), 16 mg calcium (Ca), 23 mg magnesium (Mg), 0.8 mg Iron (Fe) and 0.35 mg zinc (Zn). Vitamin content per 100 gm of *Beta vulgaris* is as follows: Ascorbic

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acid 4.9mg, riboflavin 0.04mg, niacin 0.33mg, thiamine 0.031mg,pantothenic acid 0.155mg, pyridoxine 0.067mg, folate 109µgm and β-carotene 2µgm. *Beta vulgaris* is good source of nutrition. Consuming 100gm of *Beta vulgaris* gives 180 kilo joules of energy, 9.56gm carbohydrates, 6.76gm sugars, 2.8gm dietary fiber, 0.17 gm fat and 1.61gm protein is present in 100gm of beet root.[8]Traditionally various parts of beet root have been used for treating different respiratory, hepatic, cardiac, blood and neurological disorders.[9]

The main constituents of beet root are its pigments i.e. betalains which gives red colour to beet root. From pharmacological point of view beet root extract is used as diuretic and inhibitor of calcium oxalate crystal formation.[10] Beet root has also shown to possess anti-proliferative activity.[11] More over lyophilized aqueous extracts of *Beta vulgaris* has been shown to reduce cholesterol and increase high density lipoprotein (HDL) levels.[12]

Based on above literature review the current study was designed to explore the diuretic effect and effect on excreted urinary electrolytes by different doses of lyophilized beet root.

MATERIALS AND METHOD

Experimental animals

The experimental study was designed comprising of 150-170gm albino wistar rats of either sex. The rodents were taken from animal house of Department of Pharmacology, University of Karachi where they were housed at room temperature of 25 ± 2^0 C. They were then familiarized with laboratory environment and were kept at room temperature 25 ± 2^0 C under 12 hour light and dark cycle . The animals were given water and standard diet (food) freely. The rodents were separated into 4 groups, each group comprising of 10 rats.

Karachi University Board of Advanced Studies and Research vide Resolution approved the study and assigned it No10 (P) 18. The rodents were handled according to the specifications provided by Helsinki Resolution 1964.

Lyophilized Powder of *Beta vulgaris:*

Beta vulgaris lyophilized powder was purchased from Sun Rise NutraChem Group having Lot # Ctc 2015 0320. The powder was dark maroon in color and was packaged and stored in zip log Plastic bag which was further covered with aluminum foil. The instructions were to store it at room temperature protected from sunlight.

Dosing Protocol

Group I was taken as control and was given 0.1 ml distilled water. Group II and III were labeled as treated and given lyophilized *Beta vulgaris* in dose of 500mg/kg and 1000 mg/kg.[13] A stock solution was prepared by dissolving 6gm beet root powder in 50 ml distilled water and then the dose was calculated individually according to body weight of animal and administered once daily orally. Group IV was taken as standard and was given Furosemide (40mg/70 kg).[14] The dose was adjusted according to body weight of the animal and was given orally.

Procedure

Modified Lipchitz test was used for studying the diuretic activity in rat [15]. Single rat from each group was placed in a metabolic cage designed to keep the urine and feces separate. The urine samples were collected after 24 hours in cylinder and its volume was measured. The fresh urine samples pH was evaluated using PH meter. The urine samples were then diluted (1:1000 in deionized water) to estimate the concentration of electrolytes [Na, K, Cl and P) in urine.[16]

Diuretic action and activity

The diuretic action was calculated by using the following formula:

Diuretic action= Urine excreted by test group / urine excreted by standard group.

Diuretic activity= Urine excreted by test group / urine excreted by control group.

The diuretic activity was considered good if values were > 1.5, moderate if values lies between 1.00-1.5, little if the range is between 0.72-1.00 and nil if it <0.72.[17]

Saliuretic, Natriuretic and Carbonic anhydrase Inhibition

Saliuretic activity was evaluated by = Urinary Excreted Na^+ + Urinary excreted Cl^-

Natriuretic activity was calculated by taking ratio of Na^+ / K^+

Carbonic anhydrase inhibition was evaluated by taking ratio of Cl^- / (Na^+ + K^+).[18]

Statistical analysis

All values were expressed as Mean ± SD (standard deviation) and data was evaluated using SPSS version 21 and applying one way ANOVA (analysis of variance). Post hoc evaluation was done by Tukey's Test.

RESULTS

Table 1 shows the diuretic activity and action of *Beta vulgaris* at different doses. Our results show highly significant ($p<0.001$) increase in urination by both doses when compared with control. *Beta vulgaris* at 500mg/kg dose showed similar effects as (furosemide) standard after 24 hours. Low dose of lyophilized beet root showed better effects as compared to high dose. According to the set criteria of diuretic activity, both of our treated doses were good since the value is >1.5

Table 2 shows the effects on electrolyte excretion. Our results show highly significant ($p<0.001$) increase in Na excretion by 500mg/kg *Beta vulgaris* dose and Furosemide when compared with control. 1000mg/kg *Beta vulgaris* showed significant effect ($p<0.01$) as compared to control. Similarly highly significant ($p<0.001$) excretion of K is also observed by 500mg/kg dose of beet root and furosemide as compared to control. However 1000mg/kg *Beta vulgaris* show highly significant ($p<0.001$) decrease in K excretion when compared with control.

Chloride excretion was highly significantly ($p<0.001$) increased by furosemide as compared to control and was increased significantly ($p<0.01$) by 500mg/kg *Beta vulgaris* dose. 1000mg/kg *Beta vulgaris* highly significantly ($p<0.001$) reduced the Cl excretion as compared to control.

When compared with furosemide (standard) the electrolytes excretion was highly significantly ($p<0.001$) less by both doses of *Beta vulgaris*.

Table 3 shows the effect on saliuretic, natriuretic and carbonic anhydrase inhibition. Our results show highly significant ($p<0.001$) increase in saliuretic activity by 500mg/kg dose of *Beta vulgaris* and furosemide (Standard) as compared to control. However there was a highly significant ($p<0.001$) decrease in saliuretic effect at 1000mg/kg dose. Natiuretic activity was highly significantly ($p<0.001$) increased by 1000mg/kg dose of *Beta vulgaris* and standard as compared to control. Whereas at 500mg/kg dose there was a highly significant ($p<0.001$) decrease observed. There was no significant difference in carbonic anhydrase inhibition by 500mg/kg dose of *Beta vulgaris* and furosemide (standard) as compared to control. However highly significant ($p<0.001$) decrease in carbonic anhydrase inhibiton was observed at 1000mg/kg dose.

Table 4 shows the effect on urinary PH and phosphorus excretion. Our results show highly significant ($p<0.001$) increase in urinary PH by both doses of *Beta vulgaris* and standard as compared to control. The phosphorous excretion was also highly significantly ($p<0.001$) increased by both doses of *Beta vulgaris* and standard as compared to control.

DISCUSSION

Natural products have been used since ancient times because of their therapeutic benefits especially in managing symptoms of chronic diseases.[19,20] Diuretics are class of drugs used in treatment of hypertension, edema and other conditions in which there is abnormal retention of water and electrolytes in the body.[21]

The current study was conducted to assess the diuretic activity of different doses of *Beta vulgaris*. Our results showed that both doses of *Beta vulgaris* showed increased urination as compared to control. At 500 mg/kg dose the urination was similar to that of Furosemide (standard). According to the criteria set good urination was observed by both doses of *Beta vulgaris*.

Urine electrolyte evaluation is diagnostically important for evaluation of hyponatremia, hypokalemia and metabolic alkalosis.[22] 500mg/kg dose of *Beta vulgaris* significantly increased the electrolyte excretion (Na, K and Cl) as compared to control. At 1000mg/kg dose only Na was significantly excreted as compared to control. This suggests that unlike most plants which possess the diuretic effect promoting excretion of water only, *Beta vulgaris* diuretic effect promoted excretion of both water and salts.[19] Natriuretic effect was evaluated for aldosterone secretory activity.[23] Ratio greater than 2 shows good natriuretic potential indicating potassium sparing properties. The increase in natriuretic effect was dose dependent and *Beta vulgaris* at 1000mg/kg possesses good natriuretic effect showing effect like potassium sparing diuretics. Potassium sparing diuretics have slow onset of action, can cause alkalization of urine and are considered weak diuretics.[24] Natriuretic effect also leads to slight alkalization of urine which is also observed in our results as depicted in Table 4.

Our results indicate that *Beta vulgaris* causes increase excretion of sodium as compared to potassium, which is considered as a positive effect for a diuretic agent. One of the significant adverse effects of diuretics (furosemide) is hypokalemia which can be avoided by the use of 1000mg/kg dose of *Beta vulgaris*. [25]

The mechanism of diuresis involves increase water excretion (urine output) as well as increased excretion of solutes (electrolytes). This is usually achieved by inhibition of reabsorption of water and electrolytes from renal tubules into the blood stream.[26]

Thiazide diuretics increase excretion of sodium and chloride by competing for Cl binding sites by inhibiting the sodium/chloride co-transporter system in the

Table 1 : Comparison of Diuretic activity of *Beta vulgaris* at different doses			
Drugs	**24 hours (ml)**	**Diuretic Activity**	**Diuretic action**
Control 0.1ml Distill water	2.0±0.15	1	-
Beta vulgaris 500mg/kg	6.7±0.19***	3.35	1
Beta vulgaris 1000mg/kg	3.6±0.07***, ###,!!!	1.8	0.52
Furosemide (standard)	6.9±0.13***	3.45	1

Values are Mean ± S.D analysis by one way Anova, Post hoc Tukey's test
***p≤0.001= highly significant as compared to control
###p≤ 0.001 = highly significant as compared to standard
!!!p≤0.001 = highly significant, when compared among treated groups

Table 2 : Effect of different doses of *Beta vulgaris* on urinary electrolyte excretion							
Drugs	**Sodium (mEq/L)**	**Potassium (mEq/L)**	**Chloride (mEq/L)**	**Sodium Index**	**Potassium Index**	**Chloride Index**	**Thiazide Index**
Control 0.1ml Distill water	35.4±0.21	57.6±1.19	72.1±1.49	1.0	1.0	1.0	0.49
Beta vulgaris 500mg/kg	39.5±0.29***,###	74.9±1.49***,###	75.2±1.19**,###	1.11	1.3	1.04	0.52
Beta vulgaris 1000mg/kg	37.6±0.12**,###,!!!	26.8±1.47***,###,!!!	29.8±1.46***,###,!!!	1.0	0.4	0.41	1.26
Furosemide (standard)	79.5±0.27***	84.7±2.21***	134.3±1.37***	2.2	1.47	1.86	0.59

Values are Mean ± S.D analysis by one way Anova, Post hoc Tukey's test
p ≤ 0.01,*p≤0.001 = moderately significant and highly significant as compared to control
###p≤ 0.001 = highly significant as compared to standard
!!!p≤0.001= highly significant, when compared among treated groups

Table 3: Effect of different doses of *Beta vulgaris* on saliuretic, natriuretic and carbonic anhydrase inhibition

Drugs	Saliuretic effect ($Na^+ + Cl^-$)	Natriuretic effect (Na^+/K^+)	CAI ($Cl/ [Na^+ + K^+]$)	Saluretic Index	Natriuretic Index	CAI Index
Control 0.1ml Distill water	107.1± 0.22	0.61±0.014	0.67±0.022	1.0	1.0	1.0
Beta vulgaris 500mg/kg	114.7±0.25***###	0.52±0.017***###	0.65±0.014	1.07	0.85	0.97
Beta vulgaris 1000mg/kg	67.4±0.17***###!!!	1.4±0.026***###!!!	0.44±0.016***###!!!	0.62	2.29	0.65
Furosemide (standard)	213.8±0.28***	0.94±0.018***	0.63±0.023	1.99	1.54	0.94

Values are Mean ± S.D analysis by one way Anova, Post hoc Tukey's test
*p ≤ 0.05,***p≤0.001 = significant and highly significant as compared to control
###p≤ 0.001 = highly significant as compared to standard
!!!p≤0.001= highly significant, when compared among treated groups

Table 4 : Effect of different doses of *Beta vulgaris* on Urinary pH and Phosphorous excretion

Drugs	Phosphorous (mg/dl)	pH
Control 0.1ml Distill water	7.7±0.27	7.14±0.13
Beta vulgaris 500mg/kg	83.6±1.26***,###	8.18±0.18***
Beta vulgaris 1000mg/kg	300.7±1.49***,###,!!!	8.21±0.22***
Furosemide (standard)	109.7±1.4***	8.17±0.20***

Values are Mean ± S.D analysis by one-way ANOVA, Post hoc Tukey's test
***p≤0.001 = highly significant as compared to control
###p≤ 0.001 = highly significant as compared to standard
!!!p≤0.001= highly significant, when compared among treated groups

distal convoluted tubule.[27] Thiazide index indicates minor increase by both doses of *Beta vulgaris* showing the mechanism is not exactly similar to thiazide diuretics. High ceiling diuretics inhibit the Na/K/2Cl co transporter pump in thick ascending limb of loop of henle thereby increasing water and Na excretion.[28] They also inhibit the carbonic anhydrase enzyme. 500mg/kg dose of *Beta vulgaris* showed somewhat similar profile to furosemide but the electrolytes excreted were highly significantly less as compared to furosemide showing the mechanism of action is not similar to loop diuretics. Carbonic anhydrase inhibition is calculated by taking ratio of $Cl^-/ Na^+ + K^+$. At value of 0.8-1.0 the carbonic anhydrase inhibition is considered insignificant. Slight to strong carbonic anhydrase inhibition is assumed with decreasing ratios.[29] Our results show that 500mg/kg dose of *Beta vulgaris* possesses slight carbonic anhydrase inhibitory activity which was strong at 1000mg/kg dose. Beet root contains high source of nitrates as well as potassium. According to literature study 80 gm of beetroot contains 1.88 mmol of nitrate content.[30] Previous studies have shown that substances rich in potassium nitrate possess good diuretic activity.[31-32] Betalain and flavanoids are also important constituents of beet root, both of which are known to possesses diuretic activity.[33,34] Table 4 also shows that both doses of *Beta vulgaris* increase excretion of phosphorous and can be beneficial in treating hyperphosphatemia.

CONCLUSION

From the present study we can conclude that both doses of *Beta vulgaris* showed good diuretic activity. However 1000mg/kg dose of *Beta vulgaris* does not cause hypokalemia besides that it also cause increase excretion of phosphorus. 500mg/kg dose of *Beta vulgaris* showed better diuretic potential than 1000mg/kg. This makes it beneficial in treating renal disorders and edematous conditions. However further studies need to be conducted to evaluate the exact molecular mechanism responsible for diuretic effect.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS USED

Na: Sodium, **K**: Potassium, **Cl**: Chloride, **P**: Phosphorus, **HDL**: High density lipoprotein, **Ca**: Calcium, **Mg**: Magnesium, **Fe**: Iron, **Zn**: Zinc

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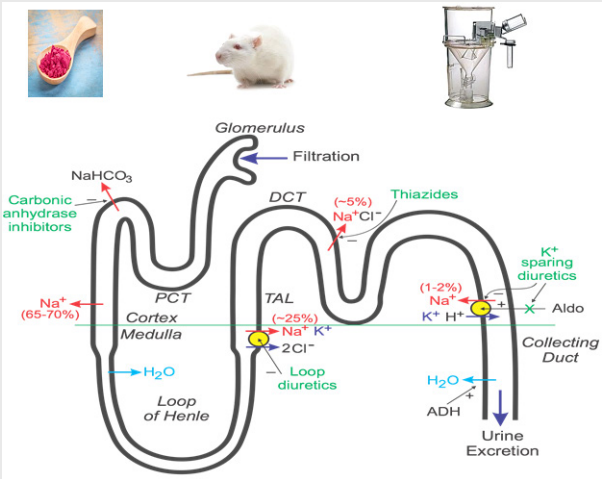
PICTORIAL ABSTRACT



SUMMARY

- Present study revealed that both doses of Beta vulgaris (500 mg/kg and 1000 mg/kg) possess diuretic activity.
- At 1000 mg/kg dose the side effect normally observed with loop and thiazide diuretic i.e hypokalemia can be avoided.
- Besides that both doses of Beta vulgaris also increased phosphorous excretion especially at 1000 mg/kg. This effect could be especially useful in diseases causing hyperphosphatemia.
- At both doses the pH of the urine was alkaline.
- *Beta vulgaris* could prove to be very beneficial in edematous conditions and renal disorders.

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