# Study of Diuretic Activity of Tukhm Karafs (Seeds of *Apium graveolens* L.) in Albino Rats

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## Abstract

Hydroalcoholic extract of Tukhm Karafs (seeds of *Apium graveolens* Linn) was studied for diuretic effect on Wistar albino rats divided into 4 groups of 6 animals each. Animals were treated with 1 ml of distilled water (Group I), 4 mg/kg of Furoseminde (Gropu II) and 150 mg/kg and 300 mg/kg of the test drug (Group II & IV, respectively) by oral route with the help of a gastric cannula. The animals were placed singly in metabolic cages and urine sample of each animal was collected after 12 hours to determine the diuretic activity. The volume of the urine and the concentration of sodium and chloride in it were found increased significantly showing diuretic activity. An increase in sum total of sodium and chloride and the sodium and potassium ratio demonstrated saluretic and natriuretic activity, respecyively. The study demonstrated that Tukhm Karafs possesses diuretic, saluretic and natriuretic activity.

**Key words:** Diuretics, *Apium graveolens* Linn., Furoseminde, Unani Medicine

## Introduction

Tukhm Karafs (seeds of *Apium graveolens* Linn. f., Apiaceae) known as celery in English, is an important drug of Tibbe Unani. It is used therapeutically as a single drug and as an important ingredient in many formulations/preparations such as Banadequl Bozoor and Jawarish Zarooni etc. It has been described to possess *mudir baul* (diuretic), *muhallil* (antiinflammatory), *mufattit hisat* (lithotryptic), *mufatteh sudad* (deobstruent), etc activities (Hussain, 1884; Dioscorides, d. 72 AD) and is used in the diseases of heart, kidney and liver etc where diuresis is an important part of therapeutic regimen. It is an erect, annual or biennial herbaceous plant native to Europe and now naturalized and occurring wild in the foot hills of North-Western Himalayas and the outlying hills of Punjab, Himachal Pradesh and Uttar Pradesh (Anonymous, 2003). Seeds and roots of this plant are equally popular for their medicinal values but the diuretic effect has been mainly attributed to the seeds. Its seed is light brown in color having a characteristic aroma with a warm bitter taste. The decoction prepared from it is frequently used as diuretic, emmenagouge and lithotriptic. A number of phytochemicals have been isolated from the seeds (Anonymous, 1997) and few of them have been reported to possess significant diuretic effect (Chandra *et al*., 2008). Synthetic diuretics although are very useful in the treatment of many diseases, yet serious side effects like hyperuricemia, acidosis, gastric irritation and high level of blood sugars associated with them

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have revived the interest in natural diuretics (Shahid *et al.*, 1999). A number of medicinal plants are being investigated for their diuretic and related activities (Serhat and Bora, 2006) and many of them have shown very promising results (Caceres *et al.*, 1987; Karim *et al.*, 2011). Therefore the age-old practice of Unani physicians to use Tukhm Karafs (TK) as a diuretic agent in a number of diseases without any report of major side effect makes it a promising candidate to be studied scientifically for diuretic activity.

## Materials and Methods

The study was undertaken in the Dept. of Ilmul Advia, National Institute of Unani Medicine (NIUM), Bangalore. The Institutional Animal Ethics Committee of National NIUM approved the protocol vide Reg. No. IAEC/ IV/IA.

### Experimental animals

The experiment was carried out on 24 healthy albino rats of Wistar strain weighing 150-200 gm of either sex. The animals were procured from Central Animal Research Facility (CARF) of National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore. Prior to the experiment, the animals were allowed to get acclimatized for at least one week. They were housed in clean polypropylene cages in an ambience maintained at a temperature of 25-30$^0$C and humidity of 45-55% with 12 hr light and 12 hr dark cycles and had free access to standard diet and water *ad libitum*. The animal care procedure and experimental protocol were adhered to, in accordance with the guidelines of CPCSEA.

### Preparation of extract

The test drug Tukhm Karafs was procured from local market of Bangalore. The sample was authenticated by Dr Siddamallaya at Regional Research Institute (Ay), Govt. Central Pharmacy, Bangalore. A voucher specimen has been stored vide no RRCBI/MCW/8 at the institute for future reference. The air dried seeds were put in drying chamber at 40$^0$C for about 30 min to remove the moisture if any, and were ground in an electric grinder to get the powder of the crude drug. The powdered drug was subjected to Soxhlet extraction with a mixture of water and alcohol (50% each) for about 6 hours to prepare the hydroalcoholic extract. The liquid extract was filtered using a filter paper (Whatman No. 40) and the filtrate was concentrated over a water bath at 80$^0$C. The resulting brownish-black residue was collected and stored for use. The yield percentage of the extract with respect to crude drug was found to be 23.64%.

## Dose

The dose of the crude drug for albino rats was calculated by multiplying its human dose by the conversion factor of 7 (Freidrich *et al.*, 1966) and was found to be 600 mg/kg. Another dose of 1200 mg/kg was also used to study the dose dependant effect if any. Since the drug was used in extract form therefore the dose of extract corresponding to 600 mg/kg and 1200 mg/kg of crude drug was calculated on the basis of yield percentage and was found to be 151.84 mg/kg and 303.68 mg/kg, respectively. The two doses however were rounded off to 150 mg and 300 mg, respectively. The doses appear to be appropriate as the $LD_{50}$ of 50% ethanolic extract of Tukhm Karafs has been reported to be 1000 mg/kg in rats (Tandon and Gupta, 2004).

## Drugs and chemicals

Ethanol (95%) of analytical grade (S.D. Fine chemicals), distilled water and Furosemide (Aventis) were used for the study. Elyte 3 kit of Crest Biosystems (Coral) laboratory reagent was used for the analysis of electrolytes.

## Test for diuretic activity

The method described by Taylor and Toplis (1962) and Afzal *et al.* (2004) was employed to study the diuretic activity. The animals were divided into four groups of six animals each. The animals in plain control group (Group I) received 1 ml of distilled water. The standard control group (Group II) received Furoseminde in the dose of 4 mg/kg. Whereas the two test groups i.e., Group III and Group IV were treated with 50% hydroalcoholic extract of Tukhm Karafs at the dose of 150 mg/kg and 300 mg/kg, respectively. The drugs were suspended in distilled water and administered once, orally with the help of a gastric cannula. On the day of experimentation food and water was withdrawn 6 hours before the treatment. At 8.00 p.m., immediately after dosing, all the animals were placed singly in metabolic cages and the urine passed overnight was collected next morning at 8.00 a.m. in a measuring jar. The total urine output was measured for the assessment of diuretic activity. The $Na^+$, $K^+$ and $Cl^-$ excretion were measured using Star 21 plus Auto analyzer (Aspen). The sum of $Na^+$ and $Cl^-$ excretion was calculated as a parameter of saluretic activity. The ratio $Na^+/ K^+$ was calculated to determine the natriuretic activity. The ratio $Cl^-/ Na^+ + K^+$ was calculated to estimate carbonic anhydrase inhibition (Nirupama *et al.*, 2005).

### Statistical Analysis

The data was analyzed using graph pad software. The results were analysed using ANOVA one way with post hoc Tukey Kramer comparison test. *P*<0.05 was considered significant.

## Results

### Total urine output

Furosemide treated rats (GP II) showed significant increase in the volume of urine ($p<0.01$) as compared to the plain control group, as it increased from 1.51ml in plian control to 3.65 ml in standard control. The rats in group III and IV treated with the test drug also produced a significant increase in the urinary volume of urine ($p<0.01$). It was measured to be 2.91 ml and 3.78 ml respectively. The urine output of rats in GP II and GP IV was found almost similar and did not show any significant difference (Table 1).

### Urinary Sodium

The concentration of sodium was measured as 41.82 mmol in plain control group which increased to 211.08 mmol/l, 121.81 mmol/l and 185.18 mmol/l in Group II, III, and IV, respectively showing a significant increase as compared to the plain control ($p<0.01$). A significant dose dependent effect ($p<0.01$) was also observed in the concentration of sodium of urine passed by rats in Group III and IV (Table 1).

### Urinary Potassium

No significant increase in the excretion of potassium in the urine of rats in standard or any of the test groups was observed. However, there was a significant decrease ($p<0.05$) in the excretion of potassium in the urine of rats in group IV as compared to the standard drug treated rats in (Table 1).

### Urinary Chloride

The three test groups II, III and IV showed increase in the excretion of chloride in the urine samples ($p<0.01$) as compared to the plain control group. The concentration determined as 41. 30 mmol/l in plain control group increased to 78.61 mmol/l, 55. 20 mmol/l and 84. 92 mmol/l in Group II, III and IV, respectively. A dose dependent effect was also observed in the concentration of chloride in the urine of rats in group III and IV ($p<0.01$) (Table 1).

**Table-1:** Diuretic activity of 50% hydroalcoholic extract of Tukhm Karafs.

| Groups / Parameter | GP-I Distilled water (1 ml) | GP-II Furosemide (4 mg/kg) | GP-III Tukhm Karafs (150 mg/kg) | GP-IV Tukhm Karafs (300 mg/kg) |
|---|---|---|---|---|
| Volume ml (mean ± SEM) | 1.51±0.24 | 3.65±0.27[a] | 2.91±0.20[a] | 3.78±0.24[a] |
| Sodium mmol/l (mean ± SEM) | 41.82±3.37 | 211.08±9.42[a] | 121.81±4.80[a,c] | 185.18±11.23[a,d] |
| Potassium mmol/l (mean ± SEM) | 32.09±1.53 | 34.60±0.46 | 28.73±0.08 | 25.99±3.29[b] |
| Chloride mmol/l (mean ± SEM) | 41.30±2.69 | 78.61±3.26[a] | 55.20±3.17[a,c] | 84.92±1.41[a,d] |
| $Na^+ + Cl^-$ (mean ± SEM) | 83.13±4.39 | 289.70±11.83[a] | 177.02±6.92[a,c] | 270.11±10.25[a,d] |
| Na+/K+ (mean ± SEM) | 1.31±0.12 | 6.09±0.24[a] | 4.23±0.16[e] | 7.91±1.42[a,d] |
| $Cl^-/Na^+ + K^+$ (mean ± SEM) | 0.56±0.05 | 0.29±0.02[a] | 0.36±0.01[a] | 0.40±0.02[e] |

N=6 in each group, Test used: ANOVA one way with Tukey Kramer multiple comparison test

a- $p<0.01$with respect to GP-I, b- $p<0.05$ with respect to GP-II, c- $p<0.01$ with respect to GP-II, d- $p<0.01$ with respect to GP-III, e-$p<0.05$ with respect to GP I.

## Discussion

Tukhm Karafs produced significant diuretic effect as it increased the urine output significantly. The significant increase in the concentration of sodium and chloride in the urine collected after the treatment further demonstrated dieresis inducing ability of the test drug. Since the excretion of electrolytes is as important as the water excretion for many pathological conditions therefore the excretion of the two electrolytes along with the fluid content makes Tukhm Karafs a good diuretic agent. Diuretics relieve pulmonary congestion and peripheral oedema and are useful in reducing the syndrome

of volume overload, including orthopnoea and paroxysmal nocturnal dyspnoea. They also decrease plasma volume and subsequent venous return to the heart (Jain *et al*., 2002). 50% hydroalcoholic extract of Tukhm Karafs by demonstrating significant diuretic effect has emerged as a promising candidate for the treatment of peripheral oedema, ascites, congestive cardiac failure and hypertension etc (Vogel, 2002). The control of plasma sodium is important in the regulation of blood volume and pressure (Guyton and Hall, 1998) therefore saluretic drugs and potassium sparing diuretics have been developed to deal with the situations of volume overload. In the present study it can be observed that the sum of $Na^+$ and $Cl^-$ increased significantly indicating saluretic effect possessed by test drug. Further, despite changes in $Na^+$ and $Cl^-$ value no alteration occurred in the level of potassium (Table 1). This phenomenon is also important therapeutically and renders an edge over the diuretics that induce hypokalemia. The control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles (Guyton and Hall, 1998). The loss of $K^+$ that occurs with many diuretics lead to hypokalemia giving rise the chances of derangement in cardiac and skeletal muscles functioning (Stuart, 2008). By demonstrating significant saluretic effect without inducing any alteration in potassium concentration the test drug indicated its potential as an effective and safe diuretic agent (Table 1). The mechanism of this effect may be assumed to be due to the aldosterone antagonistic action as well as the $Na^+$ channel blockage in collecting ducts (Jayashree *et al.*, 2011) however, this requires further elucidation. The regulation of $Na^+/K^+$ balance is also intimately related to renal control of acid-base balance. For this reason generally potassium sparing diuretics are recommended (Stuart, 2008). Values greater than 2.0 indicate a favorable natriuretic effect whereas the ratio of greater than10.0 indicates potassium-sparing effect. In the present study $Na^+/K^+$ ratio of 6.09, 4.23, 7.91 was found with respect to group II, III and IV, respectively (Table 1) showing highly significant natriuretic effect, but the values are little short of being categorized to possess the potassium-sparing diuretic effect therefore the findings warrant further investigation at higher doses. $Na^+ + Cl^-$ and $Na^+/K^+$ ratio showed dose dependent effect which suggested that the diuretic effect is intrinsic and causal and possibly receptor mediated (Jayakody et al., 2011). Though the receptors for many clinically important diuretics are yet unknown (Odlind, 1984) but Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8. With decreasing ratios, slight to strong carbonic anhydrase inhibition can be assumed (Vogel, 2002). The present study however does not have evidence in favour or otherwise of such a mechanism. On the other hand, in addition to the above features, the diuretic action of the test drug appears to be identical with the standard drug

Furosemide, a high ceiling diuretic which acts by inhibiting the $Na^+/K^+/2Cl^-$ co-transporter in the thick region of ascending limb of loop of Henle (Lahloo *et al.*, 2007; Rang, et al., 2003). However no conclusion can be arrived at with regard to the mechanism of action of Tukhm Karafs. In such an equivocal situation multiple mode of diuretic action reported with some of herbal medications (Chandra *et al*., 2008; Wright, et al., 2007) cannot be ruled out. The seeds of Tukhme Karafs have been reported to contain glycosides, steroids, phenols, flavonoids, saponins etc (Anonymous, 1997). These active phytochemicals may be responsible for its diuretic activity as some of the phytochemicals such as flavonoids, saponins, volatile oils, sterols and triterpenes etc are known diuretic agents (Chandra *et al*., 2008) but the cumulative effect is more likely. The findings also suggested that bothe doses of Tukhm Karafs are effective but the higher dose is more efficacious than the lower dose. Thus, the study demonstrated that the two doses of 'Tukhm Karafs' possess dose dependant diuretic effect and that the effect of higher dose is comparable with that of the standard drug.

## Conclusion

In view of the above findings it can be concluded that 50% hydroalcoholic extract of Tukhm Karafs possesses significant diuretic effect. The study thus validated the age-old practice of this plant drug as a diuretic agent by the physicians of Unani medicine.

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