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DIURETIC ACTIVITY OF CAROB (*CERATONIA SILIQUA* L.) HONEY: COMPARISON WITH FUROSEMIDE

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Abstract

Background: Honey has wide range of biological activities. It has effect on renal function, and urinary nitric oxide and prostaglandins level. The present study was conducted to evaluate diuretic potential of carob honey, collected from Morocco, in normal rats and the results were compared with use of furosemide.
Materials and methods: Adult male Wister rats weighing between 230 and 278 g were used. The animals were divided into three groups; group 1 received oral administration of distilled water (10 ml/kg BW), and served as control group, group 2 received oral administration of furosemide (10 mg/kg BW), and group 3 was treated with oral administration of carob honey (100 mg/kg BW). Urine volume, and urine and plasma sodium and potassium were measured after single dose of the interventions and after daily administrations of the interventions.
Results: After the single dose of carob honey, urine output was significantly increased at all time intervals (1-6 hrs and at 24 hrs after administration). The daily dose of carob honey for nine days significantly increased urine volume as compared to control group. Carob honey increased urinary levels of sodium and potassium, and did not cause hypokalemia, while furosemide increased urinary sodium and potassium and caused hypokalemia.
Conclusion: Carob honey has diuretic, natriuretic and kaliuretic activity without side effects of hypokalemia that was observed with use of furosemide.

Key words: Carob honey, furosemide, diuresis, sodium, potassium, creatinine clearance

Introduction

Honey contains various biological active substances including proteins, free amino acids, enzymes, vitamins, organic acids, flavonoids, phenolic acids and other phytochemicals (Lee et al., 2012, Je-Ruei *et al*., 2013). Honey has antioxidant, antiviral, antimicrobial, antiparasitic, and antimutagenic properties (Al-Waili et al., 2011). This might explain its wide range of the therapeutic and biological activities. Honey has been mentioned in Holy Books as remedies for cure. In this regard, honey has been mentioned in the Talmud, the old and new testaments of the Bible, and the Holy Qur'an as remedies for cure. There is a large chapter (SORA) in Holey Quran named Al Nahl (BEE)) and part of it says (And thy LORD taught the bee to build its cells in hills, on tree and in men's habitations, then to eat of all the produces (fruits) of the earth and find with skill the spacious paths of its LORD, there issues from within their bodies (bellies) a drink of varying colors, wherein is healing for men, verily in this is a sign for those who give thought).

Honey was found to alleviate renal function during food restriction in rats and protect liver and kidney when used in sheep treated with CCl4 (Al-Waili *et al*., 2003, AL-Waili *et al*., 2006). Honey solution increases total nitrite concentration in different biological fluids collected from humans, including saliva, plasma, and urine (Al-Waili and Boni 2004). Honey ingestion by normal individuals increases urinary nitrite excretion, free water clearance, filtered sodium and creatinine clearance. It decreases PGF2 alpha, TXB2 and PGE2. However, artificial honey decreases urinary nitrite and increases urinary prostaglandins concentration (Al-Waili 2005b). In sheep, we found that intravenous infusion of various kinds of honey elevated urinary nitric oxide metabolites. In addition, it was found that honey contains various concentrations of nitric oxide metabolites and intravenous infusion increased plasma and urinary nitric oxide metabolites (Al-Waili 2003).

Honey composition depends highly on the type of flowers utilized by the bees (Wang and Li 2011). A recent study has revealed the physicochemical characterization and antioxidant activity of carob honey collected in Moroccan (Smail et al., 2013). *Ceratonia siliqua* L., the scientific name of the carob tree, is famous tree in Morocco.

Clinically, diuretics are used in the treatment of hypertension, congestive heart failure, ascites and pulmonary edema. The two widely used diuretics, thiazides and furosemide, have been associated with a number of adverse effects, such as electrolyte imbalance, development of new-onset diabetes, and activation of the renin–angiotensin systems (Alberto 2005, Iqbal and Javaid 2014). New diuretic intervention that has no or less side effect would be warranted.

Although, various types of monofloral honey are well recognized in Moroccan traditional medicine as having a diuretic effect, no scientific data have been published supporting the claimed ethno-medical use. In addition, honey affects urinary prostaglandin and nitric oxide that play an important role in the renal physiology and urine formation. Therefore, the aim of this study was to investigate diuretic, saliuretic, and kaliuretic effects of orally administered carob honey in normal rats, and compared the results with use of furosemide.

Materials and Methods

Honey and Furosemide

Carob honey samples were obtained from Beekeepers Associations, Fez-Boulemane , Morocco. Honey was dark yellow in color and it was of monofloral origin. It was stored in dark containers at room temperature. Honey`s sample was subjected for biochemical tests that

included water content (20%), pH (3.9), free acidity (19.7 mEq kg1), HMF 3.25 mg Kg1) , and electrical conductivity (640 S/cm). Furosemide was used as the reference drug. It was dissolved in water prior to administration.

Experimental Animals

Adult male Wister rats weighing between 230 and 278 g were obtained from animal house breeding center, Faculty of Sciences, Dhar Al-Mahraz Fez, and were housed under normal environmental conditions (25 ± 1 ◦ C 55 ± 5% humidity and 12 h/12 h cycle light / dark). The animals were allowed free access to tap water and standard laboratory rat food. The care and handling of rats were in accordance with internationally recognized standards guidelines for the use of animals. The protocol was approved by our institutional committee on animal care, Faculty of Sciences, DharMahraz, University Sidi Mohamed Ben Abdallah, Fez.

Effect of Honey, Furosemide or Distal Water on Urine Volume

The animals were placed in metabolic cages for a period of three days to adapt them to the new conditions before experimentation. The rats were randomly divided into three groups of five animals each; group 1 received oral administration of distilled water at a dose of 10 ml/kg .B.W, and served as the control group, group 2 was treated with an oral dose of 10 mg/kg. B.W of furosemide, and group 3 received oral administration of 100 mg/kg. B.W of carob honey. Urine was collected at 1, 2, 4, 6, and 24 h after the oral dose of honey, furosemide or distal water to measure urine volume.

Effect of Daily Oral Doses of Carob Honey, Furosemide or Water on Urine Volume, Urine and Plasma Sodium and Potassium, and Creatinine Clearance

Carob honey, furosemide and distal water were administered to three groups of rats for nine days at doses similar to the experiment 1. The collection of urine was performed every 24 hours in graduated cylinders to calculate urine volume for each rat and to analyze urine sodium, potassium and creatinine. Blood samples were withdrawn before and after treatment, centrifuged at 4000 RPM for 10 minutes, and then the plasma were decanted and stored at -20 ° C until analysis.

Biochemical Methods

The plasma and urinary levels of sodium and potassium concentrations were measured in all the urine and plasma specimens by flame spectrophotometry. Concentration of creatinine in plasma and urine was determined by the Jaffe alkaline picrate method, and creatinine concentration was measured by spectrophotometer at 500 nm. Creatinine clearance was calculated from plasma and urinary creatinine levels.

Statistical Analysis

The results were expressed as mean values ± S.E.M (standard error of mean). Statistical analysis of the data was performed with one-way analysis of variance followed by Tukey's Multiple Comparison Test (ANOVA Followed by Tukey's test) (Graph Pad Prism, version 5.03). Significant differences were indicated by *P* values less than 0.05.

Results

Effect of Single Dose of Carob Honey or Furosemide on Urine Volume

Figure 1 demonstrates changes in urine volume during 24 hrs following administrations of the interventions. The treatment with carob honey increased urine volume at each time interval that was obvious from the first hour after the administration. This effect was significant at 6 hrs ($P< 0.05$) and also significant at 24 hrs ($p<0.001$) as compared to distal water.
Furosemide caused significant increase in the urine volume as compared to distal water: however, its effect was insignificant as compared to carob honey.

Table 1: The effect of carob honey and furosemide on urinary electrolyte excretion in normal rats

	Dose (mg/kg BW)	Urinary [Na+] (mmol/l)		Urinary [K+](mmol/l)	
		D1	D9	D1	D9
Water	10	55.34+0.7	58.52+0.7	47.50+0.8	53.82+0.5
Furosemide	10	62.50+1.7*	98.78+0.8***	50.54+1.1	51.10+1.3
Carob honey	100	95.50+1.7***	124.67+1.2***	63.22+0.8***	86.9+0.4***

Values are expressed as Mean ± SEM, *P < 0.05 compared to control, **P<0.01 compared to control, ***P <0.001 compared to control

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Effect of Daily Administration of The Interventions on Urine Volume

Carob honey caused a significant increase in the urinary volume ($p < 0.001$) that was observed at the first day after the administration (carob honey; 10.0 ±0.7ml, control; 5.375±0.2 ml. Similar results was observed every day for the nine days of experimentation (**Figure 2**). Furosemide increased urine volume, and its effect was significant from the 5th day of treatment (furosemide; 8.25±1.02ml, control ; 5.37±0.2ml, $p < 0.05$). The difference in the diuretic effect between honey and furosemide was insignificant.

Effect of Daily Administration of the Interventions on Urinary Sodium and Potassium

Effect of the daily dose of carob honey or furosemide on the urinary excretion of Nsodium and potassium were measured in the first, sixth and ninth day of experimentation. In the rats treated by carob honey, the urinary excretion of sodium and potassium was significantly increased during each time interval ($p<0.001$) (**Figure 3**, **Table 1**). The increment in urinary excretion of sodium and potassium was higher with use of Carob honey than with use of furosemide.

Table 2: Effect of sub-chronic diuretic effect of carob honey or furosemide on plasma electrolytes level in normal rats Wistar rats

Treatment	Dose(mg/kg BW)	Plasma electrolyte level			
		D1		D8	
		Na+ (mmol/L)	K+ (mmol/L)	Na+ (mmol/L)	K+ (mmol/L)
Control	10	155.7 ± 2.1	4.3,6± 0.2	145.5 ± 2.4	4.2 ± 0.1
Furosemide	10	134.5 ± 1.7***	3.5 ± 0.2**	141.5 ± 2.8	3.3 ± 0.4
Honey	100	148.1 ± 1.7	4.7 ± 0.3	147.3 ± 1.3	4.6 ± 0.3

Data are presented as Mean ± SEM, ***P* < 0.05 compared to control, ***P*<0.01 compared to control, ****P* <0.001 compared to control.

4-Effect of Daily Administration of the Interventions on Plasma Sodium and Potassium and Creatinine Clearance

Furosemide caused significant hypokalemia as compared to carob honey or distal water. It caused an increase in the plasma sodium level as compared to day one (**Table 2**). Carob honey did not cause significant change in the plasma sodium or potassium Carob honey and furosemide increased creatinine clearance that was insignificant as compared to distal water (carob honey; 3.47 mL/min±0.25, furosemide; 3.26 mL/min±0.17, distal water; 2.64 mL/min±0.66, $p<0.05$) (**Figure 5**).

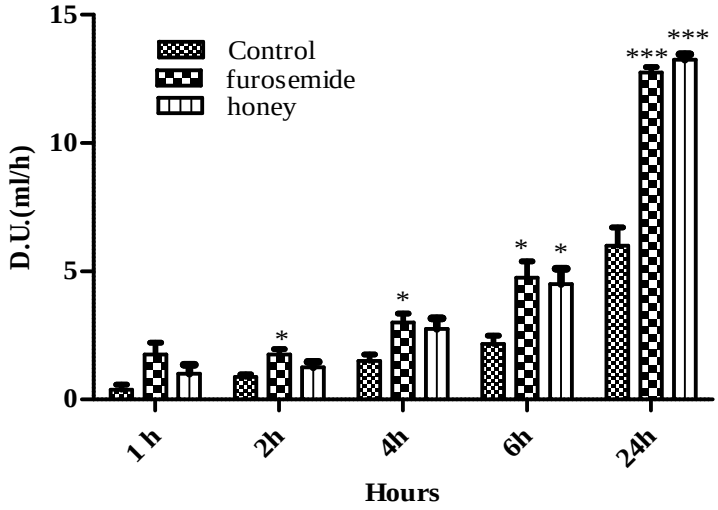

Figure 1: Acute diuretic effect: diuretic activities of single oral doses (100 mg/kg BW) of carob honey and furosemide (10 mg/kg BW). The volume of excreted urine was measured at 1, 2, 4, 6 and 24 h after the treatment; cumulative values are reported as Mean ± SEM **P*<0.05 compared to control, ***P*<0.01 compared to control, ****P*<0.001 compared to control . DU-urine output.

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Discussion

In the present work, the diuretic effect of carob honey was evaluated in normal Wister rats after single dose and after daily administration for nine days. The response was compared with that produced by furosemide, a widely used diuretic in clinical practice. The effect on the major electrolytes, sodium and potassium, and creatinine clearance was also studied. The results showed that carob honey and furosemide significantly increased urine volume and urinary level of sodium and potassium. Furosemide caused significant hypokalemia while carob honey did not cause significant change in plasma level of either potassium or sodium. This demonstrated that carob honey is better than furosemide concerning hypokalemia that is a common finding with use of diuretics. Furthermore, daily administration of carob honey or furosemide increased creatinine clearance.

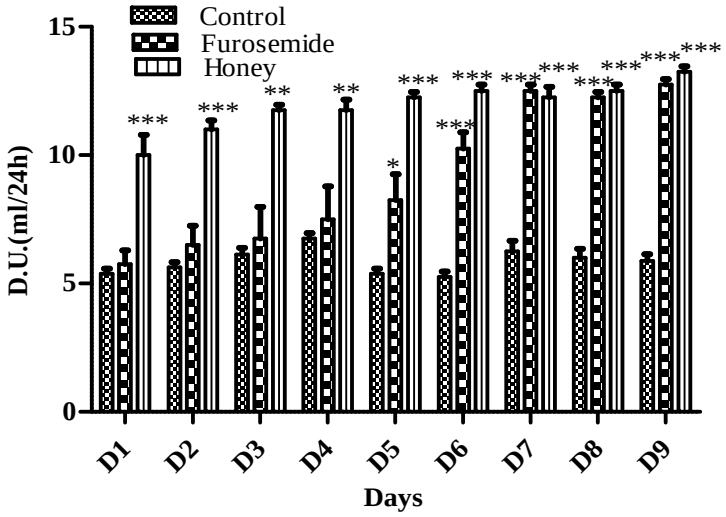

Figure 2: Sub-chronic diuretic effect: diuretic activities of daily oral doses (100 mg/kg BW) of carob honey and furosemide (10 mg/kg BW) administered for nine days. The volume of 24 h urine was measured on Days one through nine of treatment and reported as Mean ± SEM, **P*<0.05 compared to control, ***P*<0.01 compared to control, ****P*<0.001 compared to control. DU-urine output

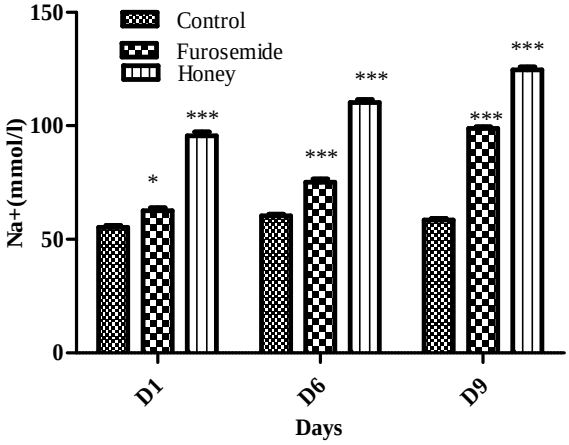

Figure 3: Sub-chronic natriuretic activity: effect of daily oral doses (100 mg/kg BW) of carob honey or furosemide (10 mg/kg BW) administered for nine days on urinary excretion of Na+. The reported Na+ levels (mean±S.E.M.) are in pooled 24 h urine for 1, 6 and 9 days for each group. **P*<0.05 compared to control, ***P*<0.01 compared to control, ****P*<0.001 compared to control.

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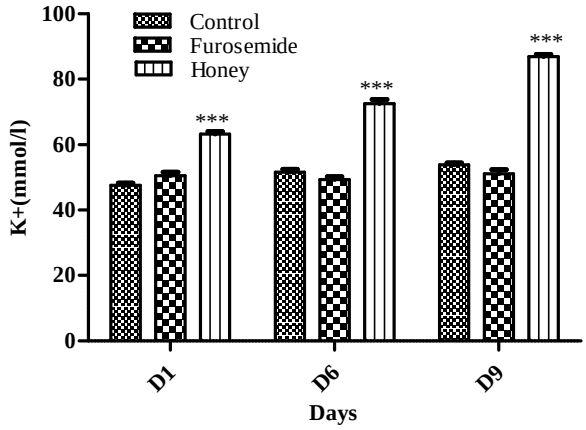

Figure 4:. Sub-chronic kaliuretic activity: effect of daily oral doses (100 mg/kg BW) of carob honey or furosemide (10 mg/kg BW) administered for nine days on urinary excretion of K+. The reported K+ levels (mean±S.E.M.) are in pooled 24 h urine for 1,6 and 9 days for each group. *P < 0.05 compared to control, **P<0.01 compared to control, ***P <0.001 compared to control.

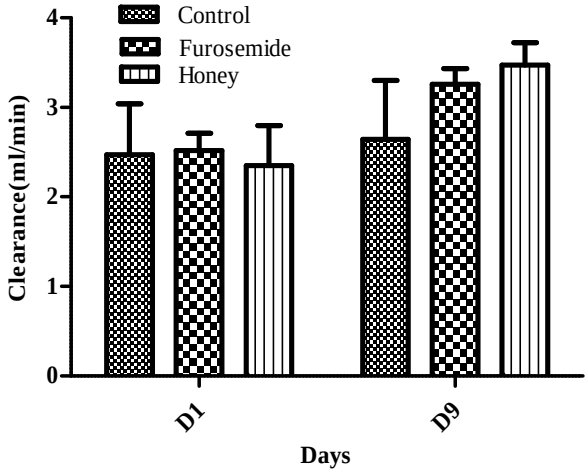

Figure 5: Effect of sub-chronic oral administration of carob honey or furosemide on creatinine clearance in normal rats. The data are presented as mean ± SEM, *P < 0.05 compared to control, **P<0.01 compared to control, ***P <0.001 compared to control

Diuresis means an increase in the production of urine volume by the kidney and a net loss of electrolytes in the urine**.** Aberrantly, both honey and furosemide have diuretic effect and they increased urinary sodium and potassium. The mode of action might be similar. Furosemide increases urine output and urinary excretion of sodium by inhibiting Na+/K+/2Cl− co-transporter system in the thick ascending limb of the Loop of Henley. However, the exact mechanism of action of honey is not clear.

Honey contains many biological active substances such as trace substances, vitamins, amino acids, enzymes, flavonoids, phenols and various carbohydrates (Al-Waili *et al*., 2011, Je-Ruei *et al*., 2013, Smail *et al*., 2013). In addition to antioxidant activity, several studies showed that flavonoids have been linked to diuretic effects (Emura *et al*., 2007, Wu and Muir 2008, Jouad *et al*., 2011). This might explain, in part, the diuretic activity of honey. Regarding carbohydrates, diuresis might be related to glucose and fructose in the honey. However, we found that artificial honey caused insignificant increase in urine volume in normal individuals (Al-Waili 2005b).

Endothelium derived nitric oxide inhibits sodium reabsorption, mediating pressure natriuresis and diuresis (Shultz and Tolins 1993, Noonan and Banks 1999, Mount and Power 2006). Nitric oxide decreases ADH and inhibits fluid reabsorption (Herbert et al., 1993, Garcia , Pomposiella, and Govin 1996). Studies showed that prostaglandins decrease aldosterone secretion, and cause natriuresis and diuresis (Hockel and Cowley 1979, Herbert *et al.*, 1993, Csukas *et al.*, 1998). We have hypothesized that prostaglandin overproduction is associated with enuresis and frequency of micturition (Al-Waili 2002, Al-Waili 2005b). However, PGE2 has a direct inhibitory effect on sodium chloride transport in the collecting ducts (Funder 2005, Wang *et al*., 2006, Chen et al., 2008). PGE2 has dual opposing effects on maintenance of vascular tone, water absorption, and Na+ absorption (Breyer & Breyer 2000). Honey increases urinary NO and decreased urinary prostaglandins in normal individuals (Al-Waili 2005a). This might explain diuretic effect of honey.

In general, the natriuretic effect of carob honey might be related to effects on the Na-K-2Cl cotransporter in the ascending limb of Henle's loop, effect on the expression of AQP2, AVP, sympathetic nervous system, natriuretic peptide or the renin-angiotensin-aldosterone system. This possibility needs further experimentation. The result of this study suggested that a possible diuretic effect of honey on various clinical entities such as liver, renal or cardiac diseases warrant further investigation.

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