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EVALUATION OF THE DIURETIC EFFECTS OF CROCIN (ACTIVE CONSTITUENT OF SAFFRON) IN RATS

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ABSTRACT

Aim of this study is to confirm and investigate the diuretic effect of crocine which is the pharmacologically active component of *Crocus sativus* L. (saffron) and to study the possible mechanism of action in relation to urinary nitrite. Twenty four rats were divided into 3 groups; control, hydrochlorothiazide and crocine treated groups. Assay of the diuretic activity of crocine was performed by determination of urine volume, urine sodium and chloride. Creatinine clearance or urinary nitrites were measured. Hydrochlorothiazide is chosen as a standard diuretic because it has moderate potency and an intermediate duration of action. oral administration of Both Hydrochlorothiazide and Crocin produced a significant increase of the 5 hours urine volume, urine sodium and chloride. Oral administration of crocin produced a significant increase of creatinine clearance and urinary nitrites. Crocine has a diuretic effect in rats. It induces its diuretic effect partially through simulation of Nitric oxide..

KEY WORDS: Crocine, saffron Hydrochlorothiazide, Diuresis.

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INTRODUCTION

Strict care and hygiene were observed to keep rats in normal and healthy conditions. Rats were allowed free access to food and water. All experiments were performed in accordance to the guidelines of the care of animals and approved by the Research and Ethics Committee of Faculty of Medicine, Mansoura University. Assay of the diuretic activity was performed according to the method of Lipschitz (8) and described by Timmerman et al (9).The animals fasted for 18 hours before each experiment and were deprived of both food and water throughout the 5 hours test to reduce the basal urinary excretion to minimum. At the day of experiment, animals received orally a suspension of the drug and a hydrating dose of water 25 ml/kg. Animals of each group were subdivided into 2 subgroups of 4 rats, where each subgroup was housed in a single metabolic cage (NALGENE metabolic cage, Nalge Company, Rochester, New York-14602- 0365 USA) designed for urine collection. The collected urine was considered as one reading for each of control, crocine, and hydrochlorothiazide treated groups. Then samples were analyzed for their Na^+, Cl^- , K^+, creatinin and nitrate concentration. From each subgroup, blood sample was obtained at the end of 5 hours (10) Centrifuged and serum was separated for determination of serum creatinin. Three groups (each containing eight rats) were divided as follow:

Group I (control group)
each rat received 25ml/kg saline orally.

Group II (Hydrochlorothiazide -treated group)
Rats received hydrochlorothiazide powder (Memphis Company) orally at a dose of 10 mg/kg (8) suspended in saline at a total volume of 25 ml/kg.

Group III (Crocin- treated group)
Rats received crocin (Sigma Chemical Co., St. Louis, MO, USA) orally at a dose of 20mg/kg/day (11) dissolved in normal saline at a total volume of 25 ml/kg.

Saffron is the most expensive spice in the world and consists of the dried stigmas of Crocus sativus. It is a perennial plant widely cultivated in different parts of the world, particularly in Iran. The name of saffron is derived from Arabic word of za-faran meaning “be yellow.” Saffron it is used in folk medicine as antispasmodic, carminative, stomachic, expectorant, aphrodisiac and cardiotonic. Pharmacological studies have reported that saffron extract has antitumor, anticonvulsant, antidepressant, anti-inflammatory, anti-hyperlipidemic, free radical scavenging and antioxidant properties. (1, 2) Saffron is the dried stigma of the flowers of the saffron crocus (Crocus sativus L. Iridaceae), which can be classified as a potent plant antioxidant. Numerous studies that indicated the health promoting properties of saffron are attributed primarily to crocin, a unique carotenoid with powerful antioxidant capacity (3) .Thus, Crocin is a pharmacologically active component of *Crocus sativus* L. (saffron) that has been used in traditional Chinese medicine. (4) Crocin (crocetin glycoside), crocetin and safranal are the main active constituents of saffron (5). In addition, saffron comprises protein, sugars, vitamins, flavonoids, amino acids, vital minerals and other chemical compounds (6). Crocin is a carotenoid isolated from *C. sativus* and is responsible for the red color of saffron. It is the water soluble and considered a pharmacologically active component of saffron (8). Modern pharmacological studies have demonstrated that crocin can be used as a new therapeutic agent. (2) Considering the various uses of *Crocus sativus* L in Iran's folk medicine as a diuretic, this study was designed to confirm and investigate the diuretic effect of crocine and to study the possible mechanism of action in relation to urinary nitrite.

MATERIALS AND METHODS

Male albino rats weighing 150-200 g were obtained from the animal house of faculty of medicine, Mansoura University. Rats were housed under similar conditions and fed simi

The urine of each group of rats was collected at 5, 24 hours post dosing. Blood samples were taken from retro-orbital sinus for estimation of serum creatinine. (12) Urine samples of each group of rats were measured and were subjected to the following biochemical tests:
1-Urinary excretion of sodium in m mol/litre (13) and calculated as m mol/kg/5hours.
2- Urinary excretion of chloride in m mol/litre (14) and calculated as m mol/kg/5hours.
3- Urinary excretion of potassium in m mol/litre (13) and calculated as m mol/kg/5hours.
4- Measurement of Urinary Nitrites (NO2s); Total nitrate and nitrite concentrations were estimated by the conversion of nitrate into nitrite. Total nitrite content was then measured by the Greiss reaction using NaNO2 as standard with detection limit of 1 mM (15)
6- Urinary excretion of creatinine (16).
7-Creatinine clearance was calculated according to the following equation:

Creatinine clearance $=\frac{u \times v}{p}$

Where:
U=concentration of creatinine in 1 ml of urine.
V=volume of urine per minute.
P= concentration of creatinine in 1 ml of blood.

Statistical Analysis

Data are expressed as mean value ± SD. Comparisons were carried out by analysis of variance followed by Tukey's test, using SPSS for Windows (17.0 Version). Differences were considered statistically significant when $P < 0.05$. Pearson correlation was used to assess relations between variables.

RESULTS

Effect of hydrochlorothiazide and crocin on 5 hours urine volume, urine sodium, chloride and potassium

As shown in table (1), oral administration of hydrochlorothiazide at a dose of 2 mg/kg produced a significant increase of the 5 hours urine volume, urine sodium, potassium and chloride as compared to control group. While, oral administration of Crocin at a dose of 20mg/kg produced a significant increase of the 5 hours urine volume, urine sodium and chloride as compared to control group. The output of potassium while increased in hydrochlorothiazide treated group, it was not affected in Crocin treated group. In comparison with hydrochlorothiazide treated group, Crocin orally produced significantly more effect on urinary sodium and chloride. As regard to the effect of crocin on 5 hours urinary volume excretion, it showed higher urine output when compared to the control group. Comparing it to hydrochlorothiazide group, crocin increased urine volume by 85% as compared to control group.

Effect of hydrochlorothiazide and crocin on 5 hours creatinine clearance, serum creatinine and urinary nitrites

Table 2 illustrated that Oral administration of hydrochlorothiazide at a dose of2 mg/kg did not significantly affect creatinine clearance or urinary nitrites in comparison to the control group.On the other hand, Oral administration of crocin produced significant increase of creatinine clearance and urinary nitrites in comparison to the control or hydrochlorothiazide group. While no effect on serum creatinine in crocin treated group as compared to control group. (Table2).

Table 1
Effect of hydrochlorothiazide (10 mg/kg) and Crocin (20mg/kg) on 5 hours urine volume, urine sodium, potassium and chloride.

	Control group	Hydrochlorothiazide – treated group	Crocin- treated group	P
Urine Volume	4.40±.16	14.50±.07 [a]	12.53±.04 [bc]	<.001
Sodium (m mol/litre)	2.54±.27	4.49±.20 [a]	6.18±.34 [bc]	<.001
Potassium (m mol/litre)	.30±.02	.80±.03 [a]	.26±.02 [c]	<.001
Chloride (m mol/litre)	3.61±.31	6.60±.33 [a]	8.65±.29 [bc]	<.001

Data are expressed as mean±SD. P: Probability
Test used: ANOVA followed by tukey for multiple comparisons
a: significance between Control and Hydrochlorothiazide groups
b: significance between Control and Crocin groups
c: significance between Hydrochlorothiazide and Crocin groups

Table 2
Effect of hydrochlorothiazide (2 mg/kg) and Crocin (20mg/kg) on Serum creatinine, Creatinin clearance and Urinary nitrites.

	Control group	Hydrochlorothiazide – treated group	Crocin- treated group	P
Serum creatinine (mg/dl)	.64±.03	.66±.02	.66±.04	0.33
Creatinin clearance (ml/minute)	1.14±.03	1.15±.02	2.23±.28 [bc]	<.001
Urinary nitrites (nmol/h)	218.50±2.45	216.50±2.45	323.25±2.12 [bc]	<.001

Data are expressed as mean±SD. P: Probability
Test used: ANOVA followed by tukey for multiple comparisons
a: significance between Control and Hydrochlorothiazide groups.
b: significance between Control and Crocin groups.
c: significance between Hydrochlorothiazide and Crocin groups.

DISCUSSION

Considering the various uses of *Crocus sativus* L in Iran's folk medicine as a diuretic, this study was designed to confirm and investigate the diuretic effect of crocine and to study the possible mechanism of action in relation to urinary nitrite.In the present study, hydrochlorothiazide was chosen as a standard diuretic because it has moderate potency and an intermediate duration of action. This makes comparative study with a potential agent more expressive. The aim of this work is to investigate the diuretic effect of crocin and to study the possible mechanism of action in relation to urinary nitrite. Crocin is a pharmacologically active component of *Crocus sativus* L. (saffron) that has been used in traditional Chinese medicine. The results of the present study showed that Oral administration of crocin to rats produced a significant increase of urine volume, urine sodium and chloride. While , output of potassium is not affected. A similar study is in consistent with these results was done by Shariatifar et al., (17) who showed that saffron has a strong diuretic activity, hypernatremic but hyperkalemic activities.Comparing the effect of crocin and hydrochlorothiazide on urinary volume, urinary sodium, Potassium and chloride, it is evident that crocin produced a significantly more pronounced effect on the 5 hours urinary sodium and chloride. While, the diuretic efficacy of crocin was almost comparable (diuretic activity 85%) to hydrochlorothiazide, a widely used thiazide diuretic in clinical practice. This could be interpered on bases that crocin has more pronounced effect on chloride excretion than volume excretion.The results of the present study showed that there was a significant increase of urinary excretion of urinary nitrite in crocin treated rats when compared to either control or the hydrochlorothiazide group (Table 2).In the present study, The significant increase of urinary nitrite excretion of crocin treated rats suggests the possibility of the involvement of the renal nitric oxide (NO) in the diuretic activity of crocin. As whole body NO synthesis was estimated by measuring the urinary excretion of the NO metabolites nitrite and nitrate (18). As urinary nitrite is believed to be a waist product of

nitric oxide**.** (19) This can be explained as, in vivo studies generally indicate that NO inhibits fluid and sodium reabsorption by the proximal tubule. However, the final effect of NO on proximal tubular reabsorption appears to depend on the concentration of NO and involve interaction with other regulatory mechanisms. NO regulates Na^+-K^+-ATPase, Na^+/H^+ exchangers, and paracellular permeability of proximal tubular cells, which may contribute to its effect on proximal tubular transport. (20) Endothelial NOS (eNOS)-derived NO plays an important role in determining and maintaining aspects of normal renal function, for instance proximal tubule sodium reabsorption (20, 21). He et al., (22) found that Crocin could reduce the level of serum TC, TG, LDL-C and inhibit the formation of aortic plaque. Crocin could reduce MDA and inhibit the descending of NO in serum. Another possible mechanism of the diuretic effect of crocin could be attributed to its effect on renal haemodynamics. The haemodynamic effect of crocin could be attributed to stimulation of NO. crocin (the crocetin digentiobiosyl-ester) converts to crocetin . Crocetin, significantly restored the EDR (endothelium-dependent relaxation) of the thoracic aorta in hypercholesterolemic rabbit, which might be explained by its action to increase the vessel eNOS activity, leading to elevation of NO production. Crocetin increased serum level of nitric oxide (NO). (7&23) The study of MCKEE, et al., (24) indicates that NO through generation of c GMP mediates the action of several intracellular messengers to regulate renal tubular Na, K –ATP ase and Inhibition of the sodium pump. That would be expected to decrease intracellular water and sodium reabsorption from the tubular lumen, resulting in diuresis and natriuresis. (24) Crocin scavenges free radicals, mainly the superoxide anions, and so may defend cells against oxidative stress. Research results have shown that crocin is beneficial for sperm cryo-conservation; therefore, it could be helpful in the treatment of neurodegenerative disorders due to its great antioxidant activity (25). Its high radical scavenging activity may be due to their ability to donate a hydrogen atom to the diphenyl-picrylhydrazyl (DPPH) radical (26). Crocine neutralized augmented serum levels of inflammatory mediators likeTNF-α, IL-1β, NF-κB, IL-6, COX-2, PGE_2 and ROS. (27) Crocin ($C_{44}H_4O_{24}$) is a di-ester which is formed from the disaccharide gentiobiose and the dicarboxylic acid crocetin and is considered as one of the few naturally occurring carotenoids easily soluble in water. Crocin has shown various pharmacological activities such as anti-oxidant, anti-cancer, learning and memory enhancer in the medicinal field (28, 29)

CONCLUSION

The results of the present study showed that crocine has a diuretic effect in rats. It induces its diuretic effect partially through simulation of Nitric oxide. The diuretic effect is not accompanied by a significant effect on potassium. Consequently, it is not advisable to be tried clinically as a diuretic with a high degree of safety.

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