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Effect of Saffron (*Crocus Sativus*) Administration on Kidney Function in Normal Cats as Determined by Use of ^{99m}Tc-DTPA Renal Scintigraphy

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Abstract

Objective- To evaluate and compare kidney function with and without saffron administration and consider the diuretic and nephroprotective effects of aqueous extract of this substance in normal cats.
Design- Descriptive study
Animals- 6 female healthy cats (age: 2 to 4 years, average weight: 4 kg) - no clinical sign of renal disorders and prior to presentation
Procedures- The scintigraphy of right and left kidney was performed using Gamma camera (Piker Model) and 99mTc-DTPA as radiopharmaceutical with and without saffron administration. The average quantities of Total and individual GFR, Time to peak activity (TOP), Emptying half time and 3-min activity are the determined parameters in this evaluation.
Results- A significantly higher amount of total GFR was present at the end of the study with Saffron administration compared with control study with saline administration (P= 0.017). Also T1/2 of the treatment renograms was significantly shorter than that of the control renograms (P=0.002 for right kidney and p=0.001 for left kidney).
Conclusion and Clinical Relevance- Administration of saffron increased the glomerular filtration rate and shortened the emptying half-time of radiopharmaceutical. This study shows that Saffron as a harmless substance can play as a diuretic substance, therefore can play a significant role in the diagnosis and treatment of many diseases.
Key Words- Saffron, Kidney, Scintigraphy, 99mTc-DTPA, Cats

Introduction

Saffron is a valuable spice derived from the dried red stigmas of *Crocus sativus* Linné which was known by ancient nations and different cultures.[1,2] This plant is cultivated in southern Europe, India and mainly in Iran[2] and has remained among the world's costliest substances throughout the history.[1]
Saffron has been used for different purposes such as a spice, a dye and a perfume[1] but recent clinical studies have shown that saffron extract and its components have a potential to promote health. These studies have already revealed the antitumor, anti-bacterial, anti-fungal, free radical scavenging, anti-inflammatory and antioxidant effects of this precious plant.[3,4,5,6,7]

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Saffron is also believed to have the potential to heal swellings and wounds, treat keratitis and cataract, improve circulation and prevent coagulation.[1] In urinary tract and kidney, saffron can act as a diuretic and purifies kidney and bladder. Also it has a significant efficacy in curing infection of urinary tract and facilitates passage of renal stones.[1]
Since there have been so many researches proving the diuretic role of saffron and since this substance improves circulation and increases blood flow[1], in present study we aimed to evaluate and compare kidney function in normal cats with and without saffron administration in order to consider its properties and usages to be a background conducive to future studies. We believe this is the first time that the effect of saffron on kidney function is investigated by ^{99m}Tc-DTPA scintigraphy method in normal cats.

Materials and methods

Animals

Six female cats with a range of 2-4 years old and an average weight of 4 kg were selected. Animals were all healthy based on history, physical examination, complete blood count, serum biochemistry profile and urinalysis. All cats were detected negative for feline immunodeficiency virus (FIV) antibody and feline leukemia virus (FeLV) antigen (using respective commercial enzyme-linked immunosorbent assay test kits). Also thoracic and abdominal radiographs as well as ultrasound examination were within normal limits in all cats. Food was withheld for 12h before scintigraphy but water was accessible at all times and animals were in a moderately hydrated state before the examination because dehydration could lead to decrease in blood volume and as a result the radiopharmaceuticals will be washed out with a delay which is concerning with kidney function.[8]

Extract preparation

Aqueous extract of saffron was prepared using Soxhlet procedure [9] and stored at -80 ^{0}C. To do this, 15g of ground saffron was put in flask containing 150 ml distilled water and was gradually warmed up. Procedure was continued overnight till the water became colorless.

Experimental groups

Cats were randomly divided into 2 experimental groups as follows: control and treatment. In treatment group, 90 mg/kg BW of saffron extract was administered intra-peritoneally 15 min before scan for each cat since it takes about 15 minutes for saffron to reach kidney and start its function[5]. For the control scan, each cat received an equivalent volume of saline intra-peritoneally 15 min before scan.

Renal scintigraphy

Since the main purpose of this study was only to compare the kidney function with and without saffron administration, no baseline was considered for normal ranges of renal parameters. ^{99m}Tc-DTPA renal scintigraphy was performed twice in each cat with a 14-day-period between the two experiments. Intraperitoneal saline and saffron were injected 15 min before radiopharmaceutical administration and scans for the control and treatment groups, respectively. A dynamic acquisition was performed for 8 min. An intravenous (IV) catheter was placed in the cephalic vein for radiopharmaceutical injection. Animals were anaesthetized with a combination of Ketamin 10% (30 *mg/kg*) and Diazepam (0.2 *mg/kg*) and placed in dorsal recumbency, with the gamma camera positioned dorsal to the kidneys. The choice of dorsal recumbency was based on the setup of the room and easy access to the patient. The gamma camera (Piker Model) was fitted with a low-energy all purpose (LEAP) parallel hole collimator. A multi-phase dynamic frame-mode acquisition was controlled by an imaging computer and was initiated simultaneously with IV injection of 4mCi ^{99m}Tc-DTPA. A 128×128×128 matrix size was used with a frame rate of one frame every 6 s for 3 min. Then one frame every 3 s for an additional 5 min. Both kidneys were evaluated resulting in 12 kidneys per group.

Regions of interests (ROIs) were drawn manually around each kidney. A TAC was then created by applying the ROI to the dynamic data. The numeric data from the TACs were imported into a spreadsheet to determine individual and global glomerular filtration rate (GFR) using a modified Gates technique.

The average quantities of the following parameters in each cat were evaluated for conventional and treatment scan: Total and individual GFR, Time to peak activity (TOP), Eptying half time and 3-min activity.

For comparison between control scan and treatment scan and comparison within groups, statistical evaluation was performed using Student's *t*-test. *P*-value of <0.05 was considered significant.

Results

Scintigraphic data with saline and saffron administration have been summarized in Table 1 and 2.

Table 1. ^{99}mTc-DTPA renal scintigraphy data in control scan (saline)

Measured parameters	Average quantities
Total GFR (*ml/min*)	124.5±5.3
Individual GFR (*ml/ min*)	Left 63.6±4.9 Right 61.13±4.3
Time to peak activity (*min*)	Left 2.95±0.8 Right 3.35±0.3
Emptying half time(T1/2) (*min*)	Left 7.6±1 right 8.1±0.7
3-min activity (*min*)	Left 229.2±8 Right 187.9±8

A significantly higher amount of total GFR was present at the end of the study in treatment renograms (142.75±1.1ml/min) compared with control renograms (124.5±5.3ml/min) ($P_=$0.017).

Also T1/2 of the treatment renograms was significantly shorter (6.12±0.6 min for left kidney and 6.15±0.6 for right kidney) than that of the control renograms (7.6±1 min in left kidney and 8.1±0.7 min in right kidney) ($P_=$0.002 for right kidney and $p_=$0.001 for left kidney).

The TOP of the treatment renogram curves did not differ from that of the control renograms. Also comparison of Individual GFR and 3-min activity in control and treatment scans did not show any significant difference.

Table 2. ^{99}mTc-DTPA renal scintigraphy data in treatment scan (saffron)

Measured parameters	Average quantities
Total GFR (*ml/min*)	142.75±1.1
Individual GFR (*ml/ min*)	Left 72.6±3.3 Right 69.1±4.3
Time to peak activity (*min*)	Left 2.9±0.7 Right 3.2±0.2
Emptying half time(T1/2) (*min*)	Left 6.12±0.6 Right 6.15±0.6
3-min activity (*min*)	Left 199.6±2.8 Right 182.8±10

Discussion

Saffron is popular because of its unique aroma and attractive color and can by all means be considered a new introduction to 21st century cuisine and medicine.[2,10]

Nowadays, many researches are being done on applications of this precious spice in the treatment of numerous human and animal ailments.[2,5] Some of them such as anticarcinogenic effect, decreasing blood pressure, and controlling tonic-clonic and absence seizures have been reported so far.[11]

Feizzadeh *et al.* (2008) investigated the inhibitory effects of saffron on tumoral cells. They evaluated the cytotoxic effect of aqueous extract of saffron on human transitional cell carcinoma (TCC) and mouse non neoplastic fibroblast cell lines and proved the inhibitory effect of saffron aqueous extract on the proliferation of those tumoral cells.[12]

Karimi *et al.* (2010) found Saffron stigmas to possess antioxidant activity. They suggested that saffron stigma could play a role as antioxidant source, which might enhance the quality of the products in functional foods, beverages, drinks, pharmaceutical and cosmaceutical industries.[13]

El daly (1998) investigated the anti-oxidative effects of saffron in rats intoxicated by cisplatin and found that daily supplement of the aqueous extract reduced the nephrotoxicity of the chemotherapy, slowing down the renal excretion.[8]

Furthermore, animal studies suggest ethanol *Crocus Sativua* extract produce vasodilation, and anti inflammatory effects, and, therefore, prevents renal ischemia reperfusion-induced oxidative injury in rats.[14]

Derakhshanfar *et al.* (2008) have recommended the utility of this valuable herbal plant in ischemic situations. They reported that saffron at the dose of 10mg/kg could increase renal blood flow without any considerable tissue side effect. Therefore it can be a suitable substance in these conditions.[5]

We also aimed to assess the diuretic effects of saffron in this study and consider the effects of this spice on renal function. Results showed that the administration of saffron increase the Glomerular filtration rate and can shorten the emptying half-time of radiopharmaceutical. We also utilized the scintigraphy method with diethylenetriaminepentaacetic acid (^{99}mTc DTPA) for assessing the renal function with and without saffron administration. Dynamic scintigraphy is a non-invasive method of measuring glomerular or tubular filtration rate and thereby offers additive and complementary information to that gleaned by radiography and ultrasonography.[15,16]

Hecht *et al* (2008) in a study on ten healthy adult female cats conducted by the radiopharmaceutical 99mTc-DTPA, investigated some parameters such as total and individual GFR, $T_{1/2}$ and T_{mox} by conventional scintgraphy and diuretic scintigraphy with furosemide. They administered 2.8±0.2 mCi IV ^{99m}Tc-DTPA to each cat. We also used 4 mCi of the same radiopharmaceutical for each cat.[17] These researchers obtained the total and individual values according to gate technique which is the same technique we used. Normal values for total and individual GFR are defined respectively ≥2.5 ml/min/kg and ≥1.25 ml/min/kg in this technique. The resultant amounts of this study were respectively 2.79±0.83 ml/min/kg for total GFR and 1.39±0.43 ml/min/kg for individual GFR of each kidney.

Also comparison of T_{mox} in control group (3.01±0.61min) and diuretic group (3.06±0.5min) did not show any significant difference, but $T_{1/2}$ of the diuretic group was significantly shorter (5.15±0.08min) than that of the control group (6.31±1.5min) ($P \leq 0.05$).

This similarity in results of Hecht's study and our study and considering that furosemide exerts its diuretic pharmacokinetic by inhibiting reabsorption of sodium and chloride in the proximal tubule and ascending limb of loop of henle, it seems logical that saffron exerts its diuretic effect by the same mechanism.[17]

Overally, scintigraphy is a noninvasive and safe method. Besides it's a fast procedure to perform in cats and many different renal diseases, such as nephrolithiasis, hydronephrosis, ureteral obstructions, renal aplasia/hypoplasia or trauma can be recognized and differentiated relatively cheaply using this technique.[18] On the other hand, saffron as a harmless substance can increase renal blood flow and can play

the diuretic role in the diagnosis and treatment of many diseases like glomerulonephritis or localization of antigen-antibody complexes in the renal glomerulus.[5] We therefore concluded that saffron can act as a diuretic and purifies the blood, kidney and bladder, and may regulate the BUN and sCr rate in blood.

Nowadays, many researches and studies are being done on medicinal and other applications of Saffron.[1] We also suggest more investigations on saffron, especially when it comes to the therapeutic uses of this amazing plant, as there is little known about it.

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چکیده

بررسی اثر تجویز زعفران (*Crocus Sativus*) بر عملکرد کلیه با استفاده از سینتی گرافی با رادیو داروی تکنسیوم دی اتیلن تریامین پنتاستیک در گربه نرمال

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هدف- ارزیابی و مقایسه عملکرد کلیه با و بدون تجویز زعفران و بررسی خاصیت ادرارآور عصاره آبی این ماده در گربه نرمال

طرح مطالعه- مطالعه توصیفی

حیوانات- این مطالعه بر روی ۶ گربه ماده سالم با محدوده سنی ۲ تا ۴ سال و متوسط وزن ۴ کیلوگرم انجام شد. گربه‌ها هیچ گونه علائم بالینی دال بر درگیری و اختلالات کلیوی قبل از بررسی‌ها نشان ندادند.

روش کار- سینتی‌گرافی کلیه چپ و راست با استفاده از دستگاه گاما اسکنر (مدل piker) و رادیو داروی دی اتیلن تریامین پنتاستیک، با و بدون تجویز زعفران انجام شد و پارامترهای میزان کلی فیلتراسیون گلومرولی (Total GFR)، میزان فیلتراسیون گلومرولی هر کلیه (Individual GFR)، زمان بیشینه فعالیت (TOP)، نیمه عمر تخلیه دارو (T1/2) و فعالیت سه دقیقه‌ای (3 min activity) اندازه‌گیری شدند.

نتایج- در انتهای مطالعه میزان فیلتراسیون گلومرولی کلی در زمان تجویز زعفران به طور قابل توجهی از زمان تجویز سالین بیشتر بود (P=۰/۰۱۷) . همچنین نیمه عمر تخلیه دارو در رنوگرام‌های گروه درمان به طور معناداری کوتاهتر از این مقدار در گروه کنترل بود (برای کلیه راست: p= ۰/۰۰۲ و برای کلیه چپ: p= ۰/۰۰۱).

نتیجه‌گیری و کاربرد بالینی- تجویز عصاره زعفران در حیوانات، میزان فیلتراسیون گلومرولی را افزایش و نیمه عمر تخلیه‌ی رادیو دارو را کاهش داده است. این مطالعه نشان می‌دهد که زعفران به عنوان یک ماده بی ضرر می تواند به عنوان یک ماده ادرارآور عمل کرده و بنابراین می‌تواند نقش مهمی در تشخیص و درمان بسیاری از بیماری ها ایفا کند.

کلمات کلیدی- زعفران، کلیه، سینتی‌گرافی، تکنسیوم دی اتیلن تریامین پنتاستیک، گربه.