IRANIAN JOURNAL OF
VETERINARY SURGERY
(IJVS)
WWW.IVSA.IR

Pathological and Doppler Ultrasonographic Study of Kidney Hemodynamic Response in Saffron (*Crocus Sativua*) Pretreated Rats

Amin Derakhshanfar[1*], PhD
Dariush Vosough[2], DVSc
Arash Bidadkosh[3], DVM

[1] Department of Pathobiology and [2] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran,
[3] Health Department, Veterinary Supervision of Shiraz, Shiraz, Iran.

Abstract

Objective- To evaluate kidney hemodynamic response including blood flow velocity in segmental arteries shortly after administration of various dose of saffron extract (10, 40 and 90 mg/kg).
Design- Technical assessment, experimental study.
Animals- 20 healthy male Sprague-Dawley rats.
Procedures- In this study, using a real-time pulsed doppler analysis, kidney hemodynamic response including blood flow velocity in segmental arteries shortly after administration of various doses of saffron (10, 40 and 90 mg/kg) was investigated and eventually the data represented for each group were interpreted into tissue changes blindly.
Results- Saffron at a dose of 10 mg/kg significantly increased renal blood flow with minimum tissue side effects, while at the higher doses it was remarkably associated with tissue lesions such as ATN and glomerulopathy.
Conclusion and Clinical Relevance- Saffron is a scarce and valuable crop because of the stigma's charming fragrance and pleasant flavor, the aroma of which is naturally furnished by a desirable golden color when dried. Due to innate therapeutic properties, it has been a focus of interest for modern pharmacological studies to comprehensively recognize the advantages of using saffron, in the light of its low toxicity and powerful anti-oxidant properties. Beyond direct effects on the epithelial cell function, saffron significantly enhances vascular blood flow, resulting in an indirect control towards cardiovascular system. In conclusion, saffron therapy is recommended in ischemic conditions. The preferred doses ranged between 10 to 40 mg/kg. At the dose of 10 mg/kg lower tissue side effects is expected.
Key Words- Saffron, Kidney, Doppler Ultrasonography, Pathology, Blood Flow.

[*] **Corresponding author:**
A. Derakhshanfar, PhD
Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
E-mail address: damin@mail.uk.ac.ir

Introduction

Known since ancient times, saffron is still used as a spice for flavoring and coloring food preparations, as a perfume, and also as a dye or ink.[1] In traditional medicine, as well as in modern pharmacy, it has been reputed to be useful in the treatment of numerous human ailments. According to medieval literatures, the dry stigma was used by folks as instant sedative in pain relief and an effective remedy in gastro-intestinal complications.[2] At the turn of the century, with rise in demographic trends toward curative power of herbs, the potential therapeutic properties of saffron have attracted a great deal of research's attentions as a remarkable nutritional supplement. Through such extensive investigations spanning four decades, clinical studies have already proved the potent anti-microbial, anti-convulsant, anti-depressant, anti-tumor, radical scavenger, chemo-preventive properties as well as learning and memory improving capacity of saffron.[3] El daly et. al. (1998) investigated the anti-oxidative effects of saffron in rats intoxicated by cisplatin and found that daily supplement of the aquas extract reduced the nephrotoxicity of the chemotherapy, slowing down the renal excretion.[4] In a parallel point of view, Boroushaki et. al (2007) corroborated the anti-oxidative effects of saffron with intracellular events of hexachlorobutadiene to lipid peroxidation and showed that the protective effect of safranal might be related to invigorated activity of glutathione-s-transferase and cysteine conjugate ß-lyase to prevent toxic thiol formation.[5] 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.[6] These effects could, at least in part, be a result of the antioxidant activity, because it has been reported that saffron is a powerful scavenger of oxygen free radicals, restoring the intracellular level of glutathione.[2,3,6] Despite all these efforts undertaken so far, we are still short of having brought an explanation to that denoting how saffron promotes the diffusion of oxygen across tissues. These observations indicate that more than one mechanism of protection is operating. Increased blood flow due to vasodilation was considered as one possible explanation to this.[2] With respect to the latter viewpoint, in a leading pioneer study, utilizing a real-time pulsed doppler analysis, kidney hemodynamic response including blood flow velocity in segmented arteries shortly after administration of various dose of saffron extract (10, 40 and 90 mg/kg) was investigated, and eventually the data represented for each group were interpreted into the tissue changes blindly. We believe this is the first time that the effect of saffron on kidney is monitored by a developed method of pathology and real-time ultrasonography.

Materials and Methods

A total of 20 healthy male Sprague-Dawley rats were randomly divided into four equal groups, each given intraperitoneally a corresponding dose of saffron as G1 (10 mg/kg/bw), G2 (40 mg/kg/bw) and G3 (90 mg/kg/bw), or of normal saline as control,G4. To avoid interference from overlying bowel gas, rats got fasted for at least 12 hours prior to the starting of sonography. The study protocol was set up and conducted by reviewing similar previous studies concerning ultrasonographic examinations. We obtained approval for the study from the University College Kerman Committee on the Ethics of non-human research. A day before to the beginning of study, saffron extract was prepared according to the method instructed by Hosseinzadeh et. al.[3] In this method, 10 mg of the purified powder of *Crocus Sativua* is dissolved in 500 ml ethanol (80 v/v)

and then microfiltered under reduced pressure at 40 C˚. After onset of saffron, animals were put into the deep anesthesia of haloperidol (5 mg/kg/bw). 15 minutes later, imaging of the kidney vasculature was started by a GE Voluson ultrasonography machine integrated with an 8-12 MHz linear array transducer. For ease in pulsed-waved doppler ultrasonography and to reduce the size of error, rat's skin at site of operation was shaved, scrubbed, and prepared by squeezing a plenty of acoustic gel over the skin. The kidney was initially localized by 2D scan, lying up the probe superficially beneath the abdominal wall. Doppler-shifted signals were calibrated and tuned exactly to emit sound wave at less than a 50-degree angle to the artery. For each mentioned group, peak systolic velocity (PSV), mean velocity (MnV), resistive index (RI) and end diastolic velocity (EDV) in segmented artery were obtained. Kidneys were removed at the end of the experiment and fixed in 10% neutrally buffered formalin. Paraffin embedding was performed following routine procedures. Paraffin sections were stained with hematoxylin and eosin. A minimum of 20 microscopic fields per each block was assessed at 400× magnification. Mean velocity in segmented artery for all of the groups were analyzed using sample t-test ($P<0.05$).

Results

Histopathological findings: G1: mild hyperemia, G2: severe hyperemia and mild ATN, G3: severe hyperemia, severe ATN, hyaline and cellular cast formation, glomerular atrophy and necrosis (Fig 1), Group4: normal tissue structure.
Ultrasonographic data have been summarized in Table 1 and Fig 2. As can be seen from the figures, saffron at all therapeutic doses (10, 40 and 90 mg/kg) significantly increase the sonographic indices of PSV, MnV and EDV roughly twice those of controls, and these changes were similar in magnitude and pattern. Statistically, changes in RI index of rats in the first, second and third group was not significant when compared to those littermates in controls.

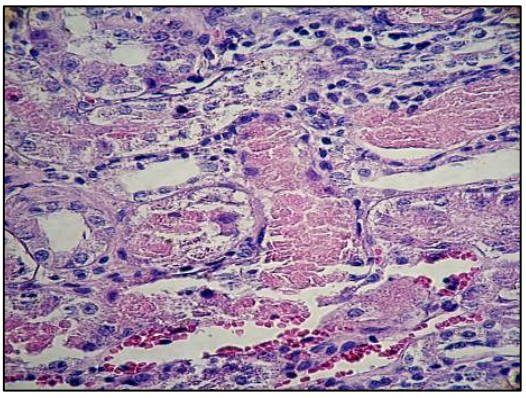

Figure 1. Severe ATN and hyperemia in group 3.× 400, H&E.

Figure 2. Pulse wave Doppler of the segmented artery in group 1.

Table 1. Results of Waveform Analysis in segmented artery. There was a significant difference in mean velocity between rats in group 4 and those in another groups ($p<0.05$).
No significant difference of the mean velocity was found among groups1, 2 and 3 of study ($p>0.05$).

		Segmented Artery				
		Doppler angle	PSV cm/s	MnV cm/s	EDV cm/s	RI
Groups	G1	41-48	8.94±0.78	7.03±0.06	5.13±0.78	0.73±0.06
	G2	41-48	9.03±0.62	7.11±0.16	5.21±0.58	0.73±0.07
	G3	41-48	9.09±0.59	7.21 ±0.39	5.33±0.12	0.71±0.05
	G4	41-48	5.91±0.31	4.51±0.93	3.12±0.34	0.89±0.04

Discussion

In some diagnostic and treating indications of the kidney such as transplantation, tumors, ischemic nephropathy and renal artery stenosis, most often coincided with a secondary hypertension, it would be necessary to clinically provide detailed characterization of the renal vasculature.[7] Although contrast angiography remains the gold standard, because of its invasiveness and attendant risks, it is not considered as an appropriate screening test for all patients thought to have renovascular problems.[8] Consequently, many noninvasive modalities have been developed that are divided into tests relying on an evaluation of the "physiologic" sequelae of renovascular disease (e.g. captopril test, captopril scintigraphy, and renal vein renins), and those directly imaging the renal artery (e.g. magnetic resonance angiography) and sequential helical computed tomography. An ideal noninvasive renal artery imaging study would be accurate enough to delineate renal vasculature anatomic pattern. Other attributes of the ideal imaging technique are minimal expense, good reproducibility, and low complication rate. Nowadays lots of less invasive renal artery imaging strategies have been established. Each has its cons and pros but much safer than conventional angiography.[9]
Unfortunately, few trials of ultrasonography have considered kidney hemodynamicity in human and animal subjects. In series of experimental works by Rooma et al (2005), conducted in in-vivo and in-vitro system, the effects of sever acute normohypovolomic anemia on parameters of doppler renal artery in dogs were investigated. Authors reported that in severe acute normohypovolomic anemia, the amounts of PSV, MnV and RI increased and EDV decreased significantly. Pulsed-waved doppler ultrasonography is changed with hypertensive or

antihypertensive medications for example, ketamine as a cardiovascular stimulant increases blood pressure and the velocity in renal artery. In contrast, acepromazine as with most alpha-receptor blockers causes partly vasodilatation and hypotension when decreasing blood velocity.[10] Although in some previous studies GFR or RBF, per se or together, were considered as trustable markers to monitor renal blood flow, in this attempt, we subjected PSV, MnV, EDV and RI as real-time indices to the kidney performance. To our knowledge, this is likely the first of clinical series in which pulse wave doppler sonography has employed for study of kidney hemodynamicity in rat models of saffron inoculation.

Collectively, saffron at the doses of 10, 40 and 90 mg/kg increase renal blood flow, enhancing PSV, MnV, EDV and RI index. Increased tissue injuries strengthen by dose would be accounted as a glimmer of huge changes in renal circulation, predisposing rat kidneys to hypertensive nephropathy. These findings also support the suggestion that the biomechanical behavior of saffron is likely a combined activity of the cardiovascular system, and that the amount of transient loading pressure, to which kidneys are normally subjected to, is substantially determined by changes in PSV, MnV and EDV other than RI when these are compared to control. In addition, the observed increase in PSV, MnV, EDV and non-significantly in RI, by saffron extract in the segmented artery of rats in the first three groups (G1, G2 and G3) may be caused by a direct or indirect effect on ROS afflicting the activity of endogenous vasodilators, prostaglandins and nitric oxide.[3,6] Some authors have earlier proposed that the crocins, a carotenoid isolated from saffron, may protect the mice's brain against excessive oxidative stress, attenuating lipid peroxidation by-products as well as increasing antioxidant power values, and therefore, constitutes a potential therapeutic candidate in transient global cerebral ischemia.[11] Crocetin, the main metabolite of crocins in the living organism, has also been shown to have cardiovascular protective effects. In line with this view, Shen et al injected mice for a dose of 60 mg/kg of saffron and observed although the crude extract could only moderately inhibit acetylcholinesterase activity, up to 30%, directly, crocetin inhibited the bioactivity in a dose-dependent manner.[12] This observation would potentially be translated into the notion that therapeutic advantages of saffron on blood circulating system was principally attained by other chemical compositions than crocetin where collecting figures released from sonographic analyzing showed saffron increased hemodynamic variables of sonography regardless of the dose. This supposition is further substantiated by the results of the experiments indicating therapeutic preference of the aqueous extract of saffron, to crocin alone on ischemia reperfusion injury in anesthetized rats. The authors cited that this might be due to the water-soluble constituents which their quenching of free radicals and antioxidant effects would have role in protective effect of saffron on ischemia reperfusion injury.[6] Other recent studies, therefore, suggests that the aqueous saffron extract are useful agents for the prevention of renal ischemia reperfusion-induced oxidative injury in rats6. These findings have not yet been verified by clinical studies in humans and comprehensive, in-depth studies still need to put in trials to define mechanisms involved in the therapeutic properties of saffron.

In conclusion, saffron at the dose of 10mg/kg can increase renal blood flow without any considerable tissue side effect. Therefore, the utility of this ancient and valuable herbal plant is recommended in ischemic situations. However, further studies are needed to clarify the exact mechanism of anti-ischemia action of this remedy.

References

1. Abdullaeve FI. Cancer chemopreventive and tumoricidal properties of Saffron (Crocus sativus L.). *Exp Biol M* 2002;227: 20-25.
2. Abdullaev FI, Espinosa-Aguirre JJ. Biomedical properties of Saffron and its potential use in cancer therapy and chemoprevention trials. *Cancer Detect Prev* 2004;28: 426-432.
3. Hosseinzadeh H, Modaghegh MH, Saffari Z. Crocus Sativus L. Saffron extract and its active constituents (Crocin and Safranal) on Ischemia-Reperfusion in rat skeletal muscle. *eCAM* 2007;10: 1-8.
4. El Daly ES. Protective effect of cysteine and vitamin E, Crocus sativus and Nigella sativa extracts on cisplatin-induced toxicity in rats. *J Pharm Belg* 1998;53: 87-95.
5. Boroushaki MT, Mofidpour HH, Sadeghnia HR. Protective effect of safranal against Hexachlorobutadiene-induced nephrotoxicity in Rat. *Iran J Med Sci* 2007;32: 173-176.
6. Hosseinzadeh H, Sadeghnia HR, Ziaee T, Danaee A. Protective effect of aqueous saffron extract (Crocus sativus L.) and crocin, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats. *J Pharm Pharm Sci* 2005;8: 387-393.
7. Bech K, Hilden T. The frequency of secondary hypertension. *Acta Med Scand* 1975;195: 65-79.
8. Mailloux LU, Napolitano B, Bellucci AG. Renal vascular disease causing end-stage renal disease, incidence, clinical correlate, and outcomes: A 20-year clinical experience. *Am J kidney Dis* 1994;24: 622-629.
9. Burns PN. The physical principles of Doppler and spectral analysis. *J Clin Ultrasound* 1987;15: 567-590.
10. Katzung T, Trevor AJ. Examination and board review pharmocology. 3rd ed. New Jersey:Prentice-Hall, 1993; 143-162.
11. Zheng YQ, Liu JX, Wang JN, et al. Effects of crocin on reperfusion-induced oxidative/nitrative injury to cerebral microvessels after global cerebral ischemia. *Brain Res* 2007;23: 86-94.
12. Shen XC, Qian ZY. Effects of crocetin on antioxidant enzymatic activities in cardiac hypertrophy induced by norepinephrine in rats. *Pharmazie* 2006;61: 348-352.

چکیده:

مطالعه پاتولوژیک و اولتراسونوگرافی داپلر بروی پاسخ همودینامیک کلیه در موش های صحرائی درمان شده با زعفران

امین درخشانفر[1]، داریوش وثوق[2]، آرش بیدادکش[3]

[1] گروه پاتوبیولوژی و [2] گروه علوم درمانگاهی، دانشکده دامپزشکی، دانشگاه شهید باهنر کرمان، کرمان، ایران.
[3] بخش بهداشت، اداره کل دامپزشکی استان فارس، شیراز، فارس.

هدف - پاسخ همودینامیک کلیه شامل سرعت جریان خون در سرخرگ های قطعه ای پس از تجویز دوز های مختلف عصاره زعفران(۱۰ و ۴۰ و ۹۰ میلی گرم به ازاء هر کیلوگرم وزن بدن) مورد تحقیق قرار گرفته و نتایج حاصله در هر گروه در ارتباط با تغییرات بافتی تفسیر گردید.

طرح مطالعه - ارزیابی تکنیکی ، مطالعه تجربی.

حیوانات - ۲۰ سر موش صحرائی نر از نژاد اسپراگ - داولی.

روش کار - در این مطالعه برای نخستین بار با استفاده از آنالیز اولتراسونوگرافی داپلر پالسی به هنگام ، پاسخ همودینامیک کلیه شامل سرعت جریان خون در سرخرگ های قطعه ای پس از تجویز دوز های مختلف عصاره زعفران(۱۰ و ۴۰ و ۹۰ میلی گرم به ازاء هر کیلوگرم وزن بدن) مورد تحقیق قرار گرفته و نتایج حاصله در هر گروه در ارتباط با تغییرات بافتی تفسیر گردید.

نتایج - زعفران با دوز ۱۰ میلی گرم به ازاء هر کیلوگرم وزن بدن بطور معناداری باعث افزایش جریان خون کلیوی گردید. در این حال حداقل عوارض جانبی در بافت کلیه مشاهده شد ، در حالی که دوز های بالاتر ضایعات بافتی همچون نکروز حاد لوله ای و گلومرولوپاتی را بدنبال داشتند.

نتیجه گیری و کاربرد بالینی- زعفران گیاهی نادر و ارزشمند است که از کلاله ای با بوی مطبوع و طعمی جذاب برخوردار بوده ، رنگ طلائی و عطر طبیعی آن پس از خشک شدن مورد استفاده قرار می گیرد. خواص درمانی زعفران سبب شده تا در کانون مطالعات دارو شناسی نوین قرار گرفته و از این رهگذر خواصی همچون مسمومیت پائین و قدرت آنتی اکسیدانی بالای آن مورد تحقیق واقع شود. گذشته از اثرات مستقیم بر سلول های پوششی ، زعفران جریان خون عروقی را تحت تاثیر قرار داده و بطور غیر مستقیم بر دستگاه قلبی – عروقی موثر واقع می گردد. از اینرو ، بکارگیری زعفران در شرایط ایسکمیک توصیه می شود. دوز های ۱۰ تا ۴۰ میلی گرم به ازاء هر کیلوگرم وزن بدن ترجیح داده می شوند ، هر چند که کمترین آن با حداقل ضایعات بافتی همراه است.

کلید واژگان- زعفران ، کلیه ، اولتراسونوگرافی داپلر ، پاتولوژی ، جریان خون.