DOI: 10.7860/JCDR/2017/23266.9491

Original Article

Ophthalmology Section

Effect of Intraperitoneal Injection of Saffron on the Treatment of Experimental Endotoxin Induced Uveitis in the Rabbit

MOHAMMAD REZA TALEBNEJAD[1], KAZEM SOLTANZADEH[2], EGHTEDARI MASOOMEH[3], MASOUD YASEMI[4], MOHAMMAD REZA KHALILI[5], SHERAFAT MARYAM[6]

ABSTRACT

Introduction: Saffron with scientific name of "*Crocus sativus*" from the family "Iridaceae" is a plant without stem and root. Antioxidative and immunomodulatory effects of saffron has been demonstrated in different studies.

Aim: To assess the effects of saffron in the treatment on Lipopolysaccharide (LPS) induced uveitis in the rabbit.

Materials and Methods: Thirty healthy female New Zealand white rabbits were prepared. LPS induced uveitis was confirmed via slit lamp examination 24 hours after intravitreal injection of *Salmonella typhimurium* LPS endotoxin in the right eye of all rabbits. Then the animals were randomly divided into two equal groups - group 1 (controls) and group 2 (saffron). After 24 hours of intravitreal injection of LPS, 60 mg/body weight of saffron was injected intraperitoneally for Group 2. On the seventh day after intraperitoneal injection, all rabbits of both groups were euthanized with an overdose intraperitoneal injection of sodium thiopental. Immediately after euthanasia, enucleation of both groups of rabbits was done. Clinical Scoring System (CSS) and pathological severity score of uveitis was assessed for each eye.

Results: No statistical difference in the CSS was found in days 3,5, and 7 after intraperitoneal injection of saffron. Also, Pathological Severity Score (PSS) did not show a significant difference between two groups. Additionally, the maximum PSS of both groups was very closed but the number was observed in the saffron group (20%) more than control group (10%).

Conclusion: Intraperitoneal injection of saffron was ineffective in the management of experimental uveitis. Further studies with different dosages and follow up and using other routes of administration are needed.

Keywords: Crocus sativus, Immunomodulatory, Intravitral, Lipopolysaccharide

INTRODUCTION

Medicinal herbs are considered as valuable potential replacement or adjunctive for synthetic drugs because of their availability, minimal side effects, and reasonable prices. Various properties and constituents of saffron have been interesting for researchers in the biosciences over the years [1,2].

Saffron with scientific name of "*Crocus sativus*" from the family "*Iridaceae*" is a plant without stem and root. Some of beneficial properties of saffron are antispasmodic, sedative, antiflatulent by facilitation of digestion, and libido stimulant. It has also been used as an analgesic. Chemical analysis has shown more than 150 primary ingredients in the saffron [3,4].

Saffron contains three types of pharmacological active metabolites. Combinations of saffron are "crocin" (substance of α-crocin or digentiobiosyle ester) which is part of crocetin that is main ingredient of saffron. The second part is "picrocrocin", which is responsible for bitter taste of saffron and "safronal" (that is a kind of evaporative oil), which makes the specific odour of saffron. In addition to the mentioned ingredients, there are other components such as sugar, vitamins, and minerals in the saffron. Recent studies have shown anti-tumour properties, anti-inflammatory and anti-depressant effects that increase the ability of learning and memory [5,6]. One of the important characteristics of saffron is its anti-oxidation effect that has been confirmed in a recent research. Also, it has been shown that short-term use of saffron as a supplement can have beneficial effects on protection of the retina from light [7,8].

Bacterial LPS is a component of bacterial cell walls of most Gram-negative bacteria. It is also called as endotoxin, which is an important trigger of the innate immune system. It contains three component of lipid A and O and core oligosaccharides. Lipid A is responsible for most inflammatory effects of LPS. It contains two units of glycosaminoglycan with attached many fatty acid chains that can activate effector cells in the small amounts (pictograms) (pg) [9]. Pleiotropic effects of LPS activates monocytes and neutrophils leading to upregulation of various cytokine genes, including Interleukin (IL) like IL1, IL6, and Tumour Necrosis Factor (TNF) and also activate the complement system via its alternate pathway that can damage endothelium of vessels. LPS injection into mice foot induces anterior uveitis. The animal model is known as "Experimental immune". The main mechanism of LPS-induced ocular effects after systemic administration is still unknown. One possibility is binding of LPS to vascular endothelium after entrance of it into blood or other parts of the anterior uvea. Alternatively, LPS may activate macrophages and circulating leukocytes, which make them preferable to bind to the endothelium of anterior uvea. The first histological change in the LPS-induced uveitis is degranulation of platelet. Intravitreal injection of LPS, leads to dose-dependent infiltration of monocytes and neutrophils in the uveal tract, retina and vitreous [9-11].

Antioxidative and immunomodulatory effects of saffron has been reported in different studies [8,12-14]. We did not find any published study about anti-inflammatory effects of saffron in the treatment of uveitis. So, in the present study, it was evaluated the effects of saffron in the treatment of LPS- induced uveitis in the rabbit.

MATERIALS AND METHODS

The experimental in vivo study was done in the experimental and comparative medical center of Shiraz University of Medical Sciences,

Shiraz, Iran, in the September 2015. The research project has been approved by Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran. For doing the study, firstly required saffron was extracted from "*Crocus sativus*" plant using the following method. At first, 60 grams of "*Crocus sativus*" was powdered and inserted inside a glass Erlen. Then, water was added to the powder. Then homogenizer device was used as mixer. Obtained mixture was filtered by using vacuum Erlen system. The filtered fluid was changed to the powder form after inserting inside "frizzier dryer system" and the powder was held at the 3ºC temperature. All of the study steps were done in the dark.

Then 30 female healthy New Zealand white rabbits weighting between 2.5 to 3.5 kg were prepared. The rabbits were treated in accordance with the association of research in vision and ophthalmology statement for the use of animals.

All procedures were performed under general anaesthesia with an intramuscular injection of a mixture of ketamine hydrochloride and xylazine hydrochloride (10 mg/kg). Ophthalmic solution of tetracaine was used for topical anaesthesia. A Total of 2 µg LPS endotoxin of *Salmonella typhimurium* (L6511: Sigma Chemica, St. Luis, MO, USA) was injected intravitreally in the right eye of all the rabbits. LPS-induced uveitis was confirmed by slit lamp examination after 24 hours. Then the animals were randomly divided into two groups consisting of 15 rabbits each. Group 1 and 2 were considered as control and cases (saffron) groups, respectively. The intensity of intraocular inflammation was graded using CSS [10,15]. Iris hyperaemia was scored for absent (0), mild (1), moderate (2), or severe (3) hyperaemia. Pupil was scored as miotic (0) or normal (1). Anterior chamber exudation was scored for absent (0), mild (1), or severe (2), and hypopyon was scored for absent (0) or present (1). The maximum possible PSS (sum of the four parameters scores) was assessed. Group 1 did not receive any further procedure or drug (control group).

After 24 hours of intravitreal injection of LPS, 60 mg/body weight of saffron was injected intraperitoneally for Group 2. Slit lamp examination of the two groups was done one day after intraperitoneal injection and also every other day by an ophthalmologist who was not aware of the treatment protocol.

On the seventh day after intraperitoneal injection, the rabbits were euthanized with an overdose intraperitoneal injection of sodium thiopental. Immediately after euthanasia, enucleation of both groups of rabbits was done. The whole eye was fixed in 10% formaldehyde for three days and processed in the standard manner for light microscopy, using haematoxylin and eosin. Sagittal sections were cut through the papillary optic nerve axis. Two masked observers counted all infiltrating inflammatory cells in three random, non-contiguous fields at ×200 magnification in both the anterior and posterior segments including (the iris, anterior chamber, ciliary body, vitreous, and the retina). Also, PSS of uveitis [10,15] that is the sum of the four parameters of iris, vitreous and retinal inflammation and presence of Proliferative Vitreoretinopathy (PVR) were evaluated. A semi-logarithmic grading scale, modified from Verma MJ et al., [15], was used to compare the median inflammatory cell infiltrate among the two groups. The grades were as follows; grade 0: no cells, Grade 1: 10 to 20, Grade 2: 20 to 30, Grade 3: 31 to 100, and Grade 4: 101 to 300 cells per field.

STATISTICAL ANALYSIS

Finally the data was analyzed with statistical package for social science (SPSS-version 21.0) software. Data is presented as mean and percentages. Chi-square and student t-tests were used for comparison of data. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

One day after saffron injection: No statistically significant difference was found between the two groups in terms of iris hyperaemia, pupil condition, presence of exudates inside the anterior chamber, and CSS (p>0.05). Also, hypopyon was not observed among the rabbits of two groups. In addition, in terms of pupil condition, 13% of the rabbits in Group 2 had transformed from "miotic" to "normal state", while the pupils of all rabbits in the control group (100%) had remained miotic. However, this difference was not statistically significant (p=0.1).

Clinical severity score: The highest percentage (33%) of CSS was related to score 5 that were same in the both groups.

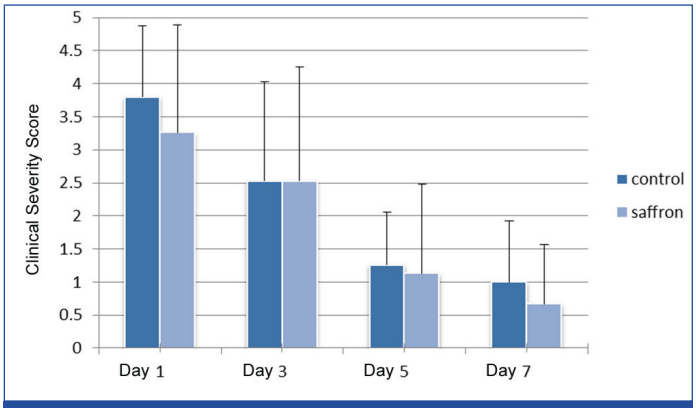
[Table/Fig-1] shows a comparison between the trends of CSS variations from day 1 to day 7 in the two groups.

Three days after saffron injection: After 72 hours of injection of saffron, there was a significant difference between pupil condition and exudates level in the anterior chamber of two groups (p= 0.02). In terms of CSS, this parameter was 6 in 6.7% of the Group 1 and 5 in 13% of Group 2, but this difference was not statistically significant (p=0.08).

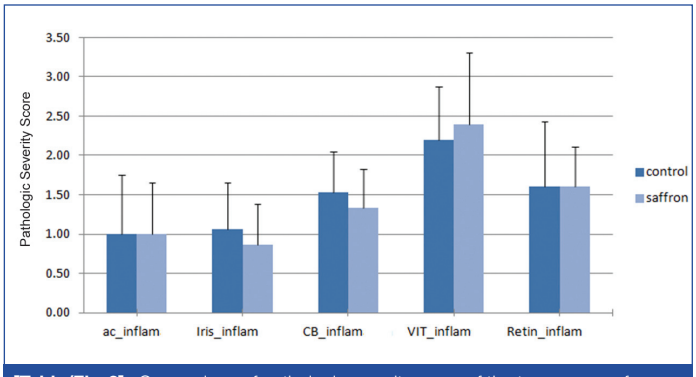
Five and seven days after saffron injection: No statistical difference was found in days 5 and 7 between two groups (p-value 0.1). Also, 86% and 80% of the rabbits receiving saffron did not have iris hyperaemia at the days 5 and 7, respectively. But the rate was 65% and 62 % in the control group. In addition, 80% of saffron group did not have exudates in the anterior chamber, while this value was 33% in the control group.

Histological evaluation: Level of inflammation in the iris tissue, anterior chamber, vitreous body, ciliary body, and retina did not show a statistically significant difference between the two groups (p=0.09). In addition, PVR was found in 66% of the rabbits in the second group, while this value was observed in 33% of the control group.

Pathological severity score: Also, PSS was calculated separately for both groups that the highest percentage in the control group (26.7%) and the saffron group belonged to scores 9 and 8, respectively. Additionally, PSS was 11 for 6.7% of the rabbits of saffron group, while the maximum PSS in the control group was 10



[Table/Fig-1]: Comparison of clinical severity score of two groups receiving saffron and controls at days 1, 2, 3, and 7.



[Table/Fig-2]: Comparison of pathologic severity score of the two groups of controls and saffron receivers based on severity of inflammation.
ac-anterior chamber, CB-Cilliary body, inflam-Inflammation, VIT-Vitreous, Retin-Retina

(20%). In [Table/Fig-2], PSS of the control group has been compared with the saffron group.

Comparison of pathologic severity score of the two groups of controls and saffron receivers based on severity of inflammation was shown in the [Table/Fig-2].

DISCUSSION

Saffron has been introduced and evaluated as an important herb in most pharmacopoeias because of having significant alkaloids such as lycopene and crocin in the growth framework and its root [3,6]. Cytotoxicity, antioxidant properties, and the capability of inducing apoptosis in cells with high division rate are regarded as the most important pathways of this valuable plant to fight against malignant cells and modifying the immune system activity [16]. The anti-cancer effect of saffron as well as its antioxidant property in various tissues has been investigated experimentally and has been proven in different studies. Few experimental studies have assessed the effects of this plant in the management of different eye diseases [5,7,9].

The inflammation of uveal tissue, as a harmful disorder with severe ocular complications, is an important topic of discussion in the ophthalmology societies. In this regard, experimental models of uveitis have been used for the identification and assessment of different parameters involved in eye inflammation [11].

In several studies, anti-inflammatory effects of saffron have been reported [12,17,18]. In the present study, for the first time we tried to evaluate anti-inflammatory effect of saffron on the induced uveitis after intravitreal injection of bacterial LPS. So far no similar study has been done to investigate the anti-inflammatory and therapeutic effects of *Crocus sativus* in uveitis. Thus, there were considerable limitations in designing the most suitable method for this study. For example, considering the lack of previous reports in terms of ocular side effects of intravitreal saffron injection, intraperitoneal injection was used in the present study. The injected dose of saffron was according to the study done by Makri OE et al., in which positive effects of intraperitoneal injection of saffron in preventing the selenite-induced cataract was reported [9]. However, in the former study, more attention was paid to the antioxidant property of saffron, while in the present study, inflammatory reaction of uveal tissue was assessed. Clearly, oxidative materials result in intense immune system response and can lead to severe tissue destruction. Thus, oxidative and inflammatory mechanisms are in close relationship and control one of these could be definitely effective in reducing the tissue damaging trend [12,13,19]. However, the effective dosage for breaking damage cascade of tissue oxidation is not necessarily similar to the standard dosage for reducing the tissue inflammation. Therefore, the application of various dosages to determine the best immunity response is of great importance [17,18].

In terms of suitable time for enucleation of rabbits, no definite time could be concluded based on the published studies [13]. In the present study, similar to the study of Marki OE et al., about the effect of saffron in preventing selenite-induced cataract [9], enucleation was done seven days after intraperitoneal saffron injection.

Also in this study, evaluation of iris tissue, anterior chamber, ciliary body, vitreous body, and retina was done in terms of inflammation intensity. In addition, the tissue PSS index, which is the sum of various inflammation levels in the mentioned tissues as well as the presence or absence of PVR, was used [14]. The PSS of the two groups did not show a statistically significant difference. This might be attributed to the timing of enucleation.

The therapeutic effect of saffron on the uveitis was not proved in this study. However, it has been shown to be effective in preventing cataract formation [9]. Also, the rising level of superoxide dismutase, glutathione peroxidase, and catalase enzymes as well as the increase of glutathione level as a result of injecting intraperitoneal saffron in the selenite-induced cataract in rats has been proven. The important point is the link between these enzymes and various activities of the immune system. Moreover, other studies reported that consumption of food complements containing saffron can retard the degeneration of photoreceptor cells, preventing more decrease in the function of retina and the tearing of its vascular network [14,15].

Saffron is also useful in retarding pathological changes of retina among patients with retinitis pigmentosa. This may be caused by decreased inflammatory response of the tissue or the antioxidant feature of the plant. However, inflammatory and oxidative reactions have not been proven in the pathology of the disease and further studies are required [14,15,20].

In another study, the positive effect of saffron was proved in reduction of ocular pressure in patients with primary open angle glaucoma compared with the placebo group. In the mentioned study, the positive effect of saffron has been attributed to its antioxidant feature, resulting in possible decline of tissue inflammation [21].

LIMITATION

Since so far, it has not been done any study about effect of saffron on uveitis, we encountered with many questions in different parts of the study design such as selection of proper dosage of saffron and the appropriate time of enucleation.

CONCLUSION

Intraperitoneal injection of saffron was ineffective in the management of experimental uveitis. Considering the mentioned problems such as the effective saffron dosage, and the timing of enucleation, no firm conclusions can be made about the ineffectiveness of saffron in uveitis. However, based on the numerous studies indicating the antioxidative and anti-inflammatory effect of saffron there is a demand for further studies with different dosage, follow up, and other routes of administration including intravitreal injection to clarify the possible beneficial effects of this herb in the management of uveitis.

ACKNOWLEDGEMENTS

The study was done under support of Poostchi Eye Research Center and Shiraz University of Medical Sciences.

REFERENCES

[1] Bolhasani A, Bathaie S, Yavari I, Moosavi-Movahedi A, Ghaffari M. Separation and purification of some components of Iranian saffron. Asian Journal of Chemistry. 2005;17(2):725.
[2] Schmidt M, Betti G, Hensel A. Saffron in phytotherapy: pharmacology and clinical uses. Wiener MedizinischeWochenschrift. 2007;157(13-14):315-19.
[3] Boskabady MH, Farkhondeh T. Antiinflammatory, antioxidant, and immunomodulatory effects of crocus sativus l. and its main constituents. Phytother Res. 2016;30(7):1072-94.
[4] Bolhassani A, Khavari A, Bathaie SZ. Saffron and natural carotenoids: Biochemical activities and anti-tumour effects. Biochim Biophys Acta. 2014;1845(1):20-30.
[5] Nassiri-Asl M, Hosseinzadeh H. Neuropharmacology effects of saffron (*Crocus sativus*) and its active constituents. bioactive nutraceuticals and dietary supplements in neurological and brain disease. Prevention and Therapy. 2014:29-39.
[6] Nair SC, Kurumboor S, Hasegawa J. Saffron chemoprevention in biology and medicine: a review. Cancer Biotherapy & Radiopharmaceuticals. 1995; 10(4):257-64.
[7] Abdullaev FI. Inhibitory effect of crocetin on intracellular nucleic acid and protein synthesis in malignant cells. Toxicology Letters. 1994;70(2):243-51.
[8] Khandhadia S, Lotery A. Oxidation and age-related macular degeneration: insights from molecular biology. Expert Reviews in Molecular Medicine. 2010;12(34):01-28.
[9] Makri OE, Ferlemi A-V, Lamari FN, Georgakopoulos CD. Saffron administration prevents selenite-induced cataractogenesis. Molecular Vision. 2013;19:1188-97.
[10] Caspi R. Animal models of autoimmune and immune-mediated uveitis. Drug Discovery Today: Disease Models. 2006;1(6):03-09.
[11] Del Sole MJ, Sande PH, Felipe AE, Fernandez DC, Sarmiento MIK, Aba MA, et al. Characterization of uveitis induced by use of a single intravitreal injection of bacterial lipopolysaccharide in cats. American journal of veterinary research. 2008; 69(11):1487-95.
[12] Boskabady MH, SeyedhosseiniTamijani SM, Rafatpanah H, Rezaei A, Alavinejad

A. The effect of Crocus sativus extract on human lymphocytes' cytokines and T helper 2/T helper 1 balance. Journal of Medicinal Food. 2011;14(12):1538-45.
[13] Poma A, Fontecchio G, Carlucci G, Chichiricco G. Anti-inflammatory properties of drugs from saffron crocus. Antiinflamm Antiallergy Agents Med Chem. 2012;11(1):37-51.
[14] Mruthyunjaya P, Khalatbari D, Yang P, Stinnett S, Tano R, Ashton P, et al. Efficacy of low-release-rate fluocinolone acetonide intravitreal implants to treat experimental uveitis. Archives of Ophthalmology. 2006;124(7):1012-18.
[15] Verma MJ, Mukaida N, Vollmer-Conna U, Matsushima K, Lloyd A, Wakefield D. Endotoxin-induced uveitis is partially inhibited by anti-IL-8 antibody treatment. Investigative Ophthalmology and Visual Science. 1999;40:2465-70.
[16] Boskabady M, Tabatabaee A, Byrami G. The effect of the extract of *Crocus sativus* and its constituent safranal, on lung pathology and lung inflammation of ovalbumin sensitized guinea-pigs. Phytomedicine. 2012;19(10):904-11.
[17] Xuan B, Zhou Y-H, Li N, Min Z-D, Chiou GC. Effects of crocin analogs on ocular blood flow and retinal function. Journal of Ocular Pharmacology and Therapeutics. 1999;15(2):143-52.
[18] Bonyadi MH, YazdaniS, Saadat S. The ocular hypotensive effect of saffron extract in primary open angle glaucoma: a pilot study. BMC Complementary and Alternative Medicine. 2014;14(1):399.
[19] Mérida S, Sancho-Tello M, Navea A, Almansa I, Muriach M, Bosch-Morell F. An anti-interleukin-2 receptor drug attenuates T-helper 1 lymphocytes-mediated inflammation in an acutemodel of endotoxin-induced uveitis. PLoS One. 2014;9(3).
[20] Falsini B, Piccardi M, Minnella A, Savastano C, Capoluongo E, Fadda A, et al. Influence of saffron supplementation on retinal flicker sensitivity in early age-related macular degeneration. Investigative Ophthalmology & Visual Science. 2010;51(12):6118-24.
[21] Serrano Diaz J, Sanchez AM, Maggi L, Martinez Tome M, Garcia Diz L, Murcia MA, et al. Increasing the applications of crocus sativus flowers as natural antioxidants. Journal of Food Science. 2012;77(11):1162-68.

PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Ophthalmology, Poostchi Ophthalmology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Ophthalmologist, Department of Ophthalmology, Poostchi Ophthalmology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
3. Associate Professor, Department of Ophthalmology, Poostchi Ophthalmology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
4. Ophthalmologist, Department of Ophthalmology, Poostchi Ophthalmology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
5. Assistant Professor, Department of Ophthalmology, Poostchi Ophthalmology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
6. Faculty, Department of Traditional Persian Medicine, School of Traditional Persian Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Masoud Yasemi,
Ophthalmologist, Department of Ophthalmology, Poostchi Eye Research Center, Shiraz University of Shiraz, Fars, Iran.
E-mail: masoodyasemi@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Aug 02, 2016**
Date of Peer Review: **Aug 26, 2016**
Date of Acceptance: **Nov 28, 2016**
Date of Publishing: **Mar 01, 2017**