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Endemic saffron (*Crocus sativus* L.): A promising therapeutic agent for retinal dystrophies

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A comprehensive approach was applied to study the mechanism underlying saffron stigmas extract therapeutic effect on the retina by employing biochemical, pharmacological and electrophysiological methods. It was found that saffron extract inhibits free radical processes in the retina and reduces the accumulation of lipid peroxidation products. The activity of key "protective" enzymes such as catalase, superoxide dismutase and glutathione peroxidase was preserved while the activity of transport ATPases was increased as well. In addition, the study demonstrated better preservation of retinal electrogenesis and its restoration following parabulbar injection of a 0.5% saffron extract solution in animals with experimental dystrophy.

Keywords: *Saffron, free radical processes experimental dystrophy of retina, antioxidant enzymes, transport ATPases, electroretinogram, antioxidant*

INTRODUCTION

One of the most significant challenges modern pharmacology faces is identifying and studying plant-derived bioactive substances (phytochemicals) to have potential in disease treatment and health promotion.

In the past century, advances in chemical technology have hastened progress in synthetic drug discovery and development. However, the last decade has renewed interest among biologists, pharmacologists and physicians in plant-based medicines. The side effects resulting from the combined action of some synthetic drugs and the quick drug resistance development are challenging issues that make searching for effective pharmaceutical compounds from natural sources even more relevant.

In this regard, saffron (*Crocus sativus* L.) is of significant interest in practical medicine. There are 12 species of saffron in the Caucasus, 5 species of which grow wild in Azerbaijan and a species has been introduced into culture, where it is used as a spice, a coloring agent and in traditional medicine. The medicinal properties of saffron have been known since ancient times, and it was believed that saffron cures more than 100 diseases (Karomatov et al., 2018; Charles et al., 2013; Lim et al., 2014; Pandita, 2021). Recently, scientists from different countries have been studying the beneficial effects of saffron on a scientific basis, relying on empirical information (Abdullaev, 2003; Abdullaev et al., 2004; Butnariu et al., 2022; Bukhari et al., 2022).

Despite clinical trials being conducted in Azerbaijan to study the therapeutic effects of saffron water extract on various eye diseases, there is as yet insufficient data on its pharmacological, physiological, and biochemical activity (Nesrullaeva et al., 2001a; Nesrullaeva et al., 2001b; Nesrullaeva et al., 2002a; Nesrullaeva et al., 2002b).

The purpose of this work was to elucidate the possible mechanisms behind the therapeutic effects of saffron stigma extract in experimental ocular pathology.

To achieve this goal, on the model of experimental dystrophy of the retina the intensity

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of lipid peroxidation (LPO), changes in the activity of antioxidant enzymes (SOD, CAT, and GPx), transport ATPases in photoreceptor cells and the electroretinogram (ERG) were performed after saffron extract administration.

MATERIALS AND METHODS

The study was conducted on rabbits kept under standard vivarium conditions. The experimental animals were divided into the following groups (each consisting of 4-5 animals): 1) an intact control, which included intact animals and served as background measurements; 2) a control group, which included animals in which experimental retinal dystrophy was modeled, but the saffron extract was not administered; 3) experimental group, which included animals with experimental retinal dystrophy to which saffron extract was administered.

Experimental retinal dystrophy (pigmentary retinitis) of moderate severity was induced by a single intravenous injection with a 4% solution of monoiodoacetic acid (MIA) at a dose of 0.5 ml per 1 kg body weight of the animal (23-25 mg/kg).

The saffron used in the study was grown in the village of Bilgyah in the Apsheron Peninsula. Saffron extract was administered to the animals in the experimental group as a 0.5% aqueous solution (at a dose of 5 mg/kg) by parabulbar injection for 20 days. The control group received a physiological saline solution alone.

The subject of the study was the retina of the eyes of the experimental animals. The material was collected on the 5th, 10th, 15th, and 20th days of the experiment after the decapitation of the animals.

Animals of all three groups were subjected to decapitation. All experimental procedures were carried out in accordance with international and institutional regulations and guidelines relating to the use of animals for scientific purposes (Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes, 2012) and complied with the rules for the use of animals in the study of the eye and vision, developed by the Association for Research in Vision and Ophthalmology (ARVO), as well (Zhiqing et al., 2008).

The intensity of lipid peroxidation was judged by the level of primary product - hydroperoxide (HP) and the secondary product malondialdehyde (MDA), which were determined by the method of T.Asakawa, S.Matsushita (Asakawa et al., 1980). The enzymatic activity of superoxide dismutase (SOD) in the retina was determined by the method of C.Beauchamp, J.Fridovich (Beauchamp et al., 1971), catalase activity was determined using the method of H.U.Bergmeyer (Bergmeyer, 1956) and glutathione peroxidase activity was determined by the method of D.Paglia, W.Valentine (Paglia et al., 1967).

The sum of the activities of Na, K and Mg-ATPase in retinal tissues was determined by the method of S.L.Bonting in Sobota modifications (Bonting et al., 1964). Electrophysiological studies were conducted as described in the study (Abdullaev et al., 1975).

The difference between the means of groups was compared using Student's t-test.

RESULTS AND DISCUSSION

Research studies have demonstrated that experimental dystrophy is accompanied by a significant accumulation of lipid peroxidation products in the retina. Administration of a 0.5% aqueous solution of saffron extract via parabulbar injection to the animals in the experimental group for 20 days prevented the intensity of lipid peroxidation processes.

On the 15th day of saffron extract administration, the level of glutathione peroxidase (GP) in the rabbit retina tissue decreased to 2.3 ± 1.2 relative units (RU), while the level of malondialdehyde (MDA) remained stable at 2.1 ± 0.5 nmol/mg protein.

By the 20th day, the GP content in the rabbit retina had decreased to 2.0 ± 0.6 RU, which was statistically significant compared to the control group (7.1 ± 2.3 nmol/mg protein, $p < 0.05$). The MDA level had also decreased to almost intact control levels, reaching 1.7 ± 0.6 nmol/mg protein (Table 1).

It is worth noting that a low level of free radical-mediated lipid peroxidation process activity is necessary for normal life to occur. Cells

possess a multi-component antioxidant defense system to prevent lipid peroxidation reactions from to be going out of control.

Table 1. Dynamics of changes in hydroperoxides content in the retina of animals with experimental dystrophy under saffron extract administration (M±m, n=5)

Intact control	Day of experiment	Control group (experimental dystrophy of the retina)	Experimental group (experimental dystrophy + saffron extract)
2,45±0,12	5th day	3.4±1.0	2.8±0.8
	10th day	5.6±1.8*	3.6±1.2°
	15th day	4.3±1.3*	2.3±0.6°
	20th day	7.1±2.3**	2.0±0.6°°

Note: Significant difference: *p<0.05, ** p<0.01 - compared to intact control; °p<0.05, °° p<0.01 - compared to the control group.

Meanwhile, in the course of experimental retinal dystrophy, the variation of peroxidation processes in the photoreceptor cell should influence the functioning of its antioxidant system (AOS). To prove that we have studied the dynamics of the activity of antioxidant enzymes - catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) in the retina during experimental moderate dystrophy and saffron extract administration.

Analysis of the experimental data obtained in this series of experiments showed that a slight decrease in CAT and SOD activity was observed in the control group animals from the first days of the experiment. On later observation there was a tendency for an increase in enzyme activity but for catalase, it was statistically insignificant (Table 2).

This, in turn, can be caused by the imbalance or damage in the systems responsible for maintaining - lipid peroxidation reactions at a low, steady-state level in the cells. Saffron extract reactivates the studied antioxidant reserves in the retina. Thus, the activity of SOD doubles on the 5th day of observation, increases by 1.5 times on the 10th day of the experiment, and by 2.2 times on the 15th day of observation. By the 20th day of administration of the saffron extract, the activity of SOD increased by 3.2 times, almost reaching intact levels. A similar trend is observed when analyzing the activity indicators of GPx, which correlates with the data on the nature of changes in thiol metabolism in the retina under the influence of saffron extract in experimental toxic dystrophy (Table 2).

Table 2. Effect of saffron extract on the activity of antioxidant defense system in the retina behind moderate-severity experimental toxic retinal dystrophy (M±m, n=5)

Indicators	Intact control	Control group (experimental dystrophy of the retina)	Experimental group (experimental dystrophy + saffron extract)
5th day			
Catalase, units/mg protein	209.6±1. 2	199.3±0.22	201.4±0.24
Superoxide dismutase (SOD), units/mg protein	192.0±17.3	81.0±7.4***	160.0±15.5 *°°°
Glutathione peroxidase (GPO), nmol of NADPH per mg protein per min	23.64±0.19	18.85±0.45*	18.92±0.12°
10th day			
Catalase, units/mg protein	209.6±1. 2	200.0±0.2	205.2±0.22*
Superoxide dismutase (SOD), units/mg protein	192.0±17.3	97.0±9.5***	143.0±13.4*°°
Glutathione peroxidase (GPO), nmol of NADPH per mg protein per min	23.64±0.19	17.49±0.54***	22.84±0.19°°°
15th day			
Catalase, units/mg protein	209.6±1. 2	198.6±0.18	208.4±0.24°°°
Superoxide dismutase (SOD), units/mg protein	192.0±17.3	78.0±6.2***	174.0±16.5°°°
Glutathione peroxidase (GPO), nmol of NADPH per mg protein per min	23.64±0.19	14.16±0.57***	25.18±0.48°°°
20th day			
Catalase, units/mg protein	209.6±1. 2	198.0±0.14	209.5±0.2
Superoxide dismutase (SOD), units/mg protein	192.0±17.3	65.0±4.7***	204.0±19.2°°°
Glutathione peroxidase (GPO), nmol of NADPH per mg protein per min	23.64±0.19	10.18±0.48***	27.63±0.25°°°

Note: Significant difference: *p<0.05, ** p<0.01 - compared to intact control; ° p<0.05, °° p<0.01 - compared to control group

It is known that ion movements in the outer segments of retinal photoreceptor cells, particularly rods, constitute the basis of photoreceptor functioning. Some authors assign a special role in the primary processes of photoreceptor cell excitation and the generation of electrical potential to the ATPase systems of the retina, which provide a directed flow of potential-forming ions (Bonting et al., 1964). Thus, the processes of generating and propagating bioelectrical potentials in photoreceptor cells are closely related to the functioning of energy-dependent ion transport ATPases fixed on plasma membranes.

Studies on rabbits have shown that the toxic effects of MIDA lead to a suppression of the activity of transport enzymes in the rabbit retina, although the sensitivity of enzymes to toxic exposure varied: on the 10th day of the experiment, the activity of Na^+, K^+-ATPase decreased by 39%, while the activity of Mg^{2+}-ATPase decreased by 10%, compared to intact control data. On the 20th day of the experiment, the activity of Na^+, K^+-ATPase in the examined eye structure decreased by an additional 10%. As it is seen in Fig. 1, retrobulbar injection of saffron extract contributed to the preservation of the activity of transport ATPases in the rabbit retina. Thus, on the 5th day of the experiment, the activity of Na^+, K^+ -ATPase was 19% lower than the corresponding value in the intact control (the difference is statistically significant $p<0.01$), and 5% higher than the value in the control group. In turn, the activity of Mg^{2+} -ATPase decreased by 33%.

Starting from the 10th day of the experiment the activity of transport enzymes remained stable and continuously increased throughout all the subsequent observation periods (see: Fig. 1 and Fig. 2).

Electrophysiological methods, such as electroretinography, play an important role in the differential diagnosis of eye diseases. These methods are valuable tools in the clinical-physiological arsenal that enables an objective and more precise assessment of the functional status of various parts of the visual system to be one of the leading methods in the objectification of the functional state of the retina (Abdullaev et al., 1975).

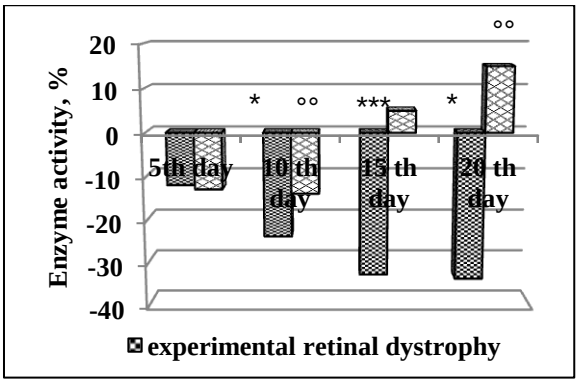
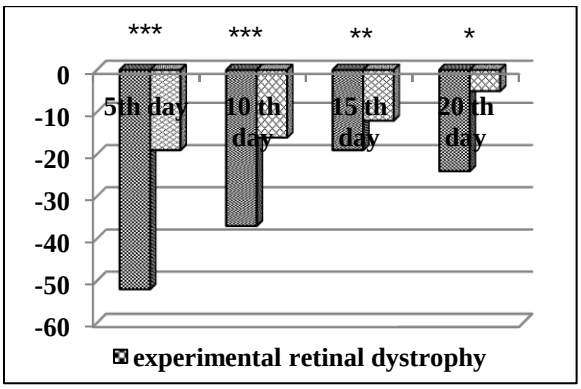


Fig. 1. Effect of saffron extract on changes in the activity of Na^+, K^+-ATPase (μmol Pi/mg protein/h) in the retina of animals with moderate-severity experimental toxic retinal dystrophy.



Note: Significant differences:
*p<0.05, ** p<0.01 - compared to intact control;
° p<0.05, °° p<0.01 - compared to control group.

Fig. 2. Changes in the activity of Mg^{2+}-ATPase (μmol Pi/mg protein/h) in the retina of animals with moderate-severity experimental toxic retinal dystrophy.

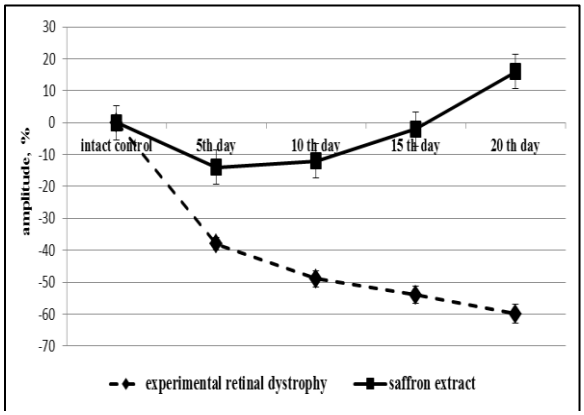


Fig. 3. Dynamics of the a-wave of the electroretinogram (ERG) upon parabulbar administration of saffron in animals with moderate-severity experimental toxic retinal dystrophy.

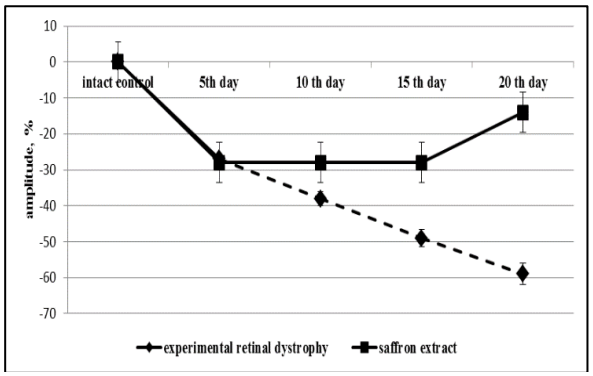


Fig. 4. Dynamics of the b-wave of the electroretinogram (ERG) upon parabulbar administration of saffron in animals with moderate-severity experimental toxic retinal dystrophy.

During dynamic observation of the control group animals, simultaneous depression of the amplitude parameters of all components of the ERG was observed; its nature objectively reflected the topography of the damage, which can be considered as a result of the initial stage of the visual act being affected. Analysis of the data suggests that the depression of the amplitude of the a- and b-waves of the ERG in experimental medium-severity dystrophy can be attributed to the toxic effects of MIDA; it leads to an increase in the level of lipid peroxidation products occurring in the retina behind the development of pigmentary retinitis.

A moderate depression of retinal functional activity was detected after parabulbar injection of saffron extract in the experimental group of animals. The stabilization of the a-wave has maintained (remained) until the end of the experiment (on the 20th day after injection of the saffron extract), as shown in Figure 3. Similarly, better dynamics of the b-wave were found in the ERG under saffron extract treatment (Fig. 4).

The analysis of the electrical responses of the retina to a single light stimulus also revealed an increase in the c-wave of the electroretinogram under saffron extract administration. The available literature presents interesting evidence that the c-wave of the ERG cannot be registered without normal physical and biochemical relationships between the pigment epithelium and the outer segments of the photoreceptors, without disc renewal, photochemical transformations of visual pigments and normal retinal nutrition.

CONCLUSION

Therefore, registration of the local ERG c-wave in our experiments is objective evidence of the stimulating effect of saffron on the biochemical processes in the retina; it manifests in the normalization of metabolic processes and as a result, improves visual function and functional activity parameters.

In this regard, it is plausible to hypothesize that the relatively superior preservation of retinal electrogenesis and its subsequent recovery in the experimental group animals following the administration of saffron extract may be attributed to the neuroprotective and retinoprotective properties of saffron compounds on retinal function during the progression of moderate-severity experimental toxic retinal dystrophy.

In summary, it can be inferred that saffron exerts a normalizing and stimulating influence on certain biochemical processes within the retina. By mitigating oxidative stress, saffron prevents the suppression of transport ATPase enzymes and antioxidant defense enzyme activity within the organism promoting the normalization of metabolic processes and consequently improving the functional state of the retina.

REFERENCES

Abdullaev F. (2003) *Crocus sativus* against cancer. *Archives of Medical Research*, **34:** 354.

Abdullaev F., Espinosa-Aquirre J. (2004) Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. *Cancer Detect Prev*., **28(6):** 426-432.

Abdullaev Q.B., Efendiev N.M., Gadjieva N.A. et al. (1975) Electrophysiological and morphological studies in experimental retinal dystrophy. *Proceedings of the Academy of Sciences of the Azerbaijan SSR, a series of biological sciences*, **2:**111-132.

Asakawa T., Matsushita S. (1980) Coloring condition of TBA test for detecting lipid hydroperoxidases. *Lipids*, **15(3):** 137-140.

Beauchamp C., Fridovich J. (1971) Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.*, **44(1):** 276-287.

Bergmeyer H. (1956) Test for detecting of catalase. *J.Biochem*., **237:** 255-262.

Bonting S., Cavaggio L., Canady M. (1964) Studies on sodium-potassium adenosine thriphosphatase X occurrence in retinal rode and relation to rhodopsin.*Exp.Eys.Ros*., **3(1):** 47-56.

Bukhari S.I., Manzoor M., Dhar M.K. (2018) A comprehensive review of the pharmacological potential of *Crocus sativus* and its bioactive apocarotenoids. *Biomedicine & Pharmacotherapy*, **98:** 733-745.

Butnariu M. et al. (2022) Pharmacological activities of *Crocus sativus* L.: a review based on the mechanisms and therapeutic opportunities of its phytoconstituents. *Oxidative Medicine and Cellular Longevity*, **2022:** Article ID 8214821.

Charles D.J., Charles D.J. (2013) Saffron. *Antioxidant Properties of Spices, Herbs and Other Sources*, p. 509-520.

Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes (2012) SPb.

Karomatov I.D., Murodova M.M. (2018) Saffron condiment and medicinal plant (review). *Biology and Integrative Medicine*,**3:** 147-177.

Lim T.K., Lim T.K. (2014) Crocus sativus. *Edible Medicinal and Non-Medicinal Plants*, **Volume 8:** Flowers, p. 77-136.

Nesrullaeva Q.M., Abdullaeva İ.M., Saidova Y.Kh. et al. (2002a) Therapeutic effect of an aqueous solution of whole saffron flowers on eye hemodynamics in retinal vein thrombosis. *Modern Problems of Ophthalmology*, p.267-269.

Nesrullaeva Q.M., Guseynova S.K., Abdullaeva I.M. (2002b) Therapeutic effect of an aqueous solution of saffron flowers on some functions of the organ of vision in various forms of retinal dystrophies. *Materials of the VIII International Congress on immuneo-rehabilitation "Allergy, Immunology and the Global Network"*, France: Cannes, p.173.

Nesrullaeva Q.M., Guseynova S.K., Kasumova Kh.Kh., Abdullaeva I.M. (2001a) Blood supply of the uveal tract in retinal dystrophies. *Achievements of Medical Science and Practical Healthcare in Azerbaijan*, **2:** 399-402.

Nesrullaeva Q.M., Guseynova S.K., Kasumova Kh.Kh., Abdullaeva I.M. (2001b) Effectiveness of an aqueous solution of whole saffron flowers in atherosclerotic macular degeneration.*I-st International conference "Clinical trials of drugs"*, Moscow, p.194.

Paglia D., Valentine W. (1967) Studies on the quantitative characterization of erytrocyteglutatione peroxidase. *J. Lab. Clin. Med.*, **70(1):** 158.

Pandita D. (2021) Saffron (*Crocus sativus* L.): Phytochemistry, therapeutic significance and omics-based biology. *Medicinal and Aromatic Plants*. Academic Press, p. 325-396.

Sepahi S. et al. (2021) Pharmacological effects of saffron and its constituents in ocular disorders from in vitro studies to clinical trials: a systematic review. *Current Neuropharmacology*, **19(3):** 392-401.

Zhiqing Ch., Ke Yao, Wen Xu, Renyi W. (2008) Inhibition of calpain expression by E-64d in the rat retina subjected to ischemia/reperfusion injury. *J. Molecular Biology*, **42(2):** 258-264.

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