

Effects of saffron (*Crocus sativus* L., *Iridaceae*) on the testosterone level in the blood and sexual behaviour of male rats

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The aim of the present research was to study the effects of Azerbaijan-grown saffron *Crocus sativus* L. *Iridaceae* on the testosterone level in the blood and sexual behaviour of normal male rats. The data obtained showed that saffron increased the blood testosterone level as compared to untreated controls, which did not receive saffron. *Per os* administration of saffron also led to marked stimulation of the proceptive and receptive components of the sexual behavior in male rats.

Keywords: Saffron extract, sex hormones, testosterone, sexual behaviour, male rats, phyto-geroprotector

INTRODUCTION

The objective of modern physiology, gerontology and medicine is to extend the active period of life and maintain reproductive health. Reproductive system is one of the most important systems of entire body, and age-related decline in reproductive functions is one of the manifestations of biological ageing in humans and animals (Anisimov, 2008; Colman et al., 2009; Svendsen et al., 2008; Wu et al., 2010). The activity of the reproductive system is directly related to age, and the level of sex hormones reflects and determines the biological age of a person. As the body ages, the function of the sex glands gradually decreases until it fades completely. Reproductive aging in males is characterized by a diminution in sexual behavior beginning in middle age.

Prevention and correction of premature aging are the key issues facing anti-ageing medicine and preventive geriatrics. They make the studies of properties of plant-derived medicines extremely relevant; according to WHO the global market for these substrates is steadily growing, in Europe and Central Asia, in particular (WHO monographs, 2010).

Saffron is of particular interest in this context, as it is known for its healing properties since ancient times. Modern pharmacological research methods make it possible to study the molecular mechanisms of effects of saffron, widely applied in ancient phytotherapy. By now, sufficient data has been accumulated to indicate the stimulating effects of saffron extract and its elements on sexual behavior of experimental animals (Agmo, 1997; Heidary et al., 2008; Hosseinzadeh et al., 2008; Shamsa et al., 2009). For example, H.Hosseinzadeh et al. studied the effects of the aqueous extract of saffron and its main components, safranal and crocin, on the sexual behavior of male rats. It was shown that safranal did not affect the sexual behavior of male rats, while *Crocus sativus* stigma aqueous extract and another safranal element, crocin, enhanced male sexual activity (Hosseinzadeh et al., 2008). M.Modaresi et al. showed the effectiveness of the saffron extract at a dose of 100 mg / kg on the pituitary-testis axis in mice (Modaresi, 2008). J.Ai et al. in the study of the effects of the aqueous extract from the stigmas of saffron on serum level of follicle-stimulating hormone, luteinizing hormone, progesterone and estrogen, as well as folliculogenesis in 45 adult rats, revealed that administration of the aqueous extract from the stigmas of saffron at a dose of 80 mg/kg significantly raised the serum level of all studied hormones as well as a number of basic, secondary and tertiary follicles in treated rats (Ai et al., 2009). Clinical studies also show the effectiveness of saffron in the treatment of premenstrual syndrome (PMS) (Agha-Hosseini et al., 2008). For example, the double-blind and placebo-

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controlled trial on women aged 20-45 years, who have regular menstrual cycle and PMS symptoms, revealed that oral administration of the saffron capsules at a dose of 30 mg/day (15 mg twice a day, morning and evening) for the duration of two menstrual cycles reduced the severity of PMS symptoms (Agha-Hosseini et al., 2008). Our study showed that the *per os* administration of alcoholic extract of saffron was able to decrease the FSH levels in blood of the 12-month-old rats as compared to that in the control group, involving the animals of the same age which have not received the saffron extract, and was close to the FSH levels reported for the 6-month rats. There was also an increase in number and body weight of pups from rats receiving the saffron extract prior to pairing with the intact males (Gashimova et al., 2017).

The objective of the research was to study the effects of the *Crocus sativus* L. *Iridaceae* stigma extraction some parameters of sexual behavior and blood level of testosterone in male rats.

MATERIAL AND METHODS

In the present study the saffron grown in the Bilgah village of the Absheron peninsula was used. The saffron stigma extract was obtained by a percolation method. The ethanol extract was filtered, the residue was washed with 75% alcohol and filtered again, then distilled off alcohol. The obtained liquid extract was further vacuum dried to concentrate to a dry residue. The yield of the active extract as viscous gum like substance constituted 56% of total mass of row material.

The tests was conducted on 45 Wistar rats, kept in standard cages (10 animals per cage) at a room temperature of $22 \pm 2^\circ$ C. All animals were fed ad libitum with standard laboratory chow, and had an access to tap water.

The work was carried out in accordance with the international principles of the Helsinki Declaration on Humane Treatment of Animals, the Principles of Humanity set out in the European Community Directive (86/609/U), Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Directive 2010/63/EU, 2012).

Male rats were divided into 3 groups: the 1st group included intact animals, the 2nd group

(control) included animals that received physiological saline, and the 3rd group (experimental) included animals that received the saffron extract at the dose of 120 mg / kg for 21 days. The saffron extract and the physiological saline were administered to animals *per os* using a thin metal probe.

Sexual behavior in male rats was studied via standard 15-minute paired test (Buresh et al., 1991; Agmo, 1997) on the second day following the last administration of the saffron extract. The male rats were placed in the test chamber (measuring 50×35×25 cm) for 5 minutes prior to an introduction to a sexually receptive female rat. The experiments were performed under dim red light. Before being tested, the animals were kept in the dark for 4-5 hours. Each male was placed with 2 females. The females were returned to their cages after 10-minute exposure. The components of sexual activity were recorded visually for 15 minutes. We registered both the proceptive phase of sexual behavior (the latent period of the onset of sexual activity (LPS), the duration of sexual activity and the number of "emotional" approaches to the female) and the receptive sexual behavior (the number of female coverage's with/without intramission - the number of mounts (NM).

Upon completion of the experiments with saffron extract administration, the blood samples were collected from animals of the control and experimental groups. The blood testosterone level was studied on the 7th, 14th and 21st days of administration of the saffron extract. For the indicated time intervals, the blood sampling was carried out in the male rats of the control and experimental groups. Procedure was conducted in the morning (9-10 a.m.), blood was obtained from the tail vein under light (1 min) diethyl ether anesthesia. The level of testosterone was determined in the blood serum using hormonal test kits for enzyme immunoassay *in vitro* for mammals ("Pishtaz", Iran).

Data analysis was performed using Microsoft Excel statistical package. Statistical significance of differences was proven by Student's t-test.

RESULTS AND DISCUSSION

Analysis of the results of the study of saffron extract effect on sexual behavior of male rats re-

vealed positive dynamics in the proceptive and receptive sexual behavior. It was manifested by decrease of LPS and increase in the number of "emotional" approaches of the male towards the female and NM. The latent period before the initiation of the elements of courtship (licking, sniffing, grooming) in the experimental group dropped down compared to the controls (the experiment time lapse was 95.0 ± 4.0 sec.; the control time lapse was 100.0 ± 0.13 sec., $p < 0.05$). Likewise, NM in saffron-treated male rats averaged 14.1 ± 1.1 , or 1.2 times higher than in the controls (Table 1).

The sexual behavior assessment shows that in male rats treated with saffron extract for 21 days, the proceptor behavior was activated at 15%, $p < 0.05$. The indicators of receptive sexual behavior in experimental animals displayed a tendency towards growth compared to controls ($p < 0.05$).

Hence, the results of the tests allow to conclude that saffron extract had the stimulatory effects on the sexual behavior of animals.

The male rats treated with saffron extract at a dose of 120mg/kg displayed a significant decrease in the temporal parameters of sexual behavior and an increase in the qualitative parameters of sexual motivation (Table 1). Consequently, it appeared rational to investigate the effects of saffron on male sex hormones content. For this purpose, blood samples were collected to register testosterone level in male rats on days 7, 14 and 21 of saffron extract administration.

Table 1. The effects of the saffron extract on the sexual behavior in male rats ($M \pm m$, $n=10$)

Groups of animals	Time, days	Sexual behavior		
		Duration of sexual activity, sec.	Number of "emotional" approaches	Number of mounts (NM)
Intact		83.0 ± 3.6	15.3 ± 0.26	10.4 ± 1.0
Control	7	75.0 ± 3.9	14.1 ± 0.14	10.2 ± 0.9
	14	78.0 ± 6.4	13.0 ± 0.35	6.6 ± 0.28
	21	95.0 ± 4.0	16.2 ± 0.24	11.2 ± 1.0
Experiment	7	83.0 ± 3.4	20.6 ± 1.23	10.2 ± 0.9
	14	88.0 ± 4.5	21.5 ± 1.13	13.2 ± 2.1
	21	100.0 ± 3.1	22.2 ± 0.17	14.1 ± 1.1

The results revealed, that the saffron extract application for 21 days manifested a statistically significant increase of the total testosterone level

in experimental animals at the end of the treatment period. The initial testosterone content in control animals was 1.35 ± 0.44 IU/L. After a 7-day administration of the saffron extract, it reached 1.38 ± 0.22 IU/L ($p < 0.05$) (Table 2).

Consecutive changes in the total testosterone content occurred as follows: on day 14 of saffron extract treatment, the testosterone concentration was 1.42 ± 0.14 IU/L ($p < 0.05$); on day 21 of saffron extract administration it reached the point of 2.27 ± 0.28 IU/L ($p < 0.01$). It is noteworthy that the difference in testosterone levels on day 7 and day 21 of the treatment are statistically significant ($p < 0.001$) (Table 2).

Table 2. The serum testosterone level in male rats under saffron extract treatment

Groups	Testosterone, IU/L		
	7th day	14th day	21st day
Intact	1.35 ± 0.44		
Control group (saline)	1.35 ± 0.44	1.38 ± 0.22	1.4 ± 0.24
Experimental group (the saffron extract)	1.38 ± 0.22 $p < 0,05$	1.42 ± 0.14 $p < 0,05$	2.27 ± 0.28 $p < 0,001$

Having evaluated the results, we conclude that the administration of saffron extract to male rats raised their sexual motivation and had positive influence on the serum testosterone level. Therefore, saffron extract application adds to maintaining of the reproductive functions of the body, which is biologically significant.

CONCLUSION

Saffron's unique medicinal properties largely owe to the diversity of ofbiologically active substances it contains (Kasumov et al., 2002; Abdullayev, 1993). The effects of saffron treatment observed in the course of this study could be explained by the direct impact of saffron and its components on the sex glands and the central nervous system.

There exists substantial literary evidence that the saffron extract is used to treat moderate depression (Akhondzadeh et al., 2004). Similar to the action of antidepressant drugs, saffron, one of saffron's major elements, inhibits serotonin re-up-

take and acts as a mild psychoactive drug (Georgiadou et al., 2012), thus contributing to the normalization of hypothalamic-pituitary-ovary system functions.

Our study showed that oral administration of the saffron extract at a dose of 50 mg/kg stabilized certain parameters of lipid metabolism, specifically, total lipids (TL), triglycerides (TG) and total cholesterol (TC). Henceforth, the administration of the saffron extract to animals receiving high-calorie diet promoted their weight loss, and reduced their blood levels of TL, TG and TC, compared to similar indicators in untreated animals (Gashimova et al., 2016), ultimately leading to lipid metabolism normalization.

In one of the tests (Verma et al., 1998), it was noted that a dose of 50 mg of saffron stigmas, dissolved in milk, administered twice a day, reduced the susceptibility of lipoproteins to oxidation, both in healthy control subjects and in patients with coronary heart disease. It is widely recognized, that lipoproteins are the transport forms of cholesterol. As cholesterol participates in the synthesis of vital hormones and all steroids, including testosterone and estradiol, as well as in the formation of cell membranes structure, its delivery to the body's peripheral tissues plays a key role (Colman et al., 2009). Saffron's ability to regulate lipid metabolism and reduce lipoproteins oxidizability may be one of the mechanisms behind the biological effects of saffron.

Taking into consideration that the imbalance of the neuroimmunoendocrine system lies at the base of premature aging, the effects of saffron on neuroendocrine relationships are of principal importance for the theory as much as the practice of the anti-aging medicine. Overall, the new findings of diverse pharmacological effects of the saffron extract open new horizons for the development of scientifically corroborated recommendations for application in practical medicine as a potential phyto-geroprotector.

The above-mentioned discoveries speak in favour of investigating the effects of saffron on the functioning of reproductive systems in both sexes, in experimental model, so as to understand the mechanisms that underlie pharmacological effects of saffron, and develop scientifically based recommendations for its application in anti-aging medicine and preventive geriatrics. Such an app-

roach would make the study particularly pertinent, as it is focused on the experimental research of the effects of saffron on the activity of the reproductive system.

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Erkək siçovulların qanında testosteronun səviyyəsinə və cinsi davranışa zəfəranın (*Crocus sativus* L. Iridaceae) təsiri

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Tədqiqat işinin əsas məqsədi Abşeronun Bilgəh kəndində becərilən endemic zəfəranın (*Crocus sativus* L. *Iridaceae*) dişicik ağzıçıqından alınmış ekstraktının normal erkək siçovulların qanında testosteronun səviyyəsinə və çütləşmə davranışlarının bəzi göstəricilərinə təsirinin öyrənilməsidir. Aparılmış tədqiqatlardan məlum olur ki, erkək siçovullara zəfəran ekstraktının verilməsi heyvanların qanında testosteronun səviyyəsini nəzarət qrupu ilə müqayisədə artırdığını göstərdi. Alınmış nəticələrə əsaslanaraq həmçinin demək olar ki, zəfəran ekstraktı cinsi davranışlara stimullaşdırıcı təsir göstərir.

Açar sözlər: Zəfəran ekstraktı, cinsi hormonlar, testosteron, cinsi davranış, erkək siçovullar, fitogeroprotektor

Влияние шафрана (*Crocus sativus* L. *Iridaceae*) на уровень тестостерона в крови и половое поведение у крыс-самцов

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Целью настоящего исследования было изучение влияния экстракта, полученного из рылец шафрана, произрастающего в Азербайджане, на уровень тестостерона в крови и некоторые параметры полового поведения нормальных самцов крыс. Полученные данные показали, что шафран повышает уровень тестостерона в крови по сравнению с контрольной группой, не получавших экстракт. Введение шафрана также приводило к выраженной стимуляции процептивных и рецептивных компонентов полового поведения у самцов крыс.

Ключевые слова: *Экстракт шафрана, половые гормоны, тестостерон, половое поведение, самцы крыс, фитогеропротектор*