

EFFECTS OF SAFFRON (*CROCUS SATIVUS L. IRIDACEAE*) ON BLOOD LEVELS OF SEX HORMONES, SEXUAL BEHAVIOR AND NUMBER OF OFFSPRING IN RATS

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This paper presents the results of the study of the effects of Azerbaijan-grown saffron (*Crocus sativus L. Iridaceae*) on the blood level of follicle-stimulating hormone (FSH) and a number of offspring in female rats, as well as on the blood level of testosterone and sexual behavior in male rats. The findings revealed a decrease in blood levels of FSH in 12-month-old female rats as compared to the control group (comprising animals of the same age that did not receive the saffron extract), as they approximated the registered levels of 6-month-old female rats. Saffron increased the blood level of testosterone as compared to untreated controls, which did not receive saffron. *Per os* administration of saffron also resulted in a notable increase in the number of pups from female rats which had received the saffron extract prior to pairing with the intact males, as well as manifested stimulation of proceptive and receptive components of sexual behavior in male rats.

Key words: saffron extract, female rats, follicle-stimulating hormone, male rats, testosterone, number of offspring, sexual behavior, phytoerotector

INTRODUCTION

Population aging is a matter of concern in most developed and developing countries [13, 10]. To this point, the objective of modern physiology, gerontology, and medicine is to extend the active period of life and maintain reproductive health. The reproductive system is one of the most important systems of the entire body, and age-related decline in reproductive functions is one of the manifestations of biological aging in humans and animals [3,14]. The activity of the reproductive system is related directly to age, and the levels of sex hormones reflect and determine the biological age of a person. As the body grows older, the

function of the sex glands gradually decreases until it fades completely. This is particularly manifest in women in the form of a complex multifaceted symptom (syndrome) called menopause. It is noteworthy that in the last 150 years women's life expectancy following the onset of menopause has significantly increased; currently, women spend approximately one-third of their lives in an estrogen-deficient state, which makes the medical and social status of middle- and senior-aged women a matter of acute concern [9].

Prevention and correction of premature aging are the key issues facing anti-aging 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 particular, in Europe and Central Asia [12].

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 the sexual behavior of experimental animals [4, 6, 11]. Kashani L. et al. in the study of the effects of the aqueous extract from the stigmas of saffron on serum levels 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 80 mg/kg significantly upregulated the serum levels of all studied hormones as well as a number of basic, secondary and tertiary follicles in treated rats [8]. Clinical studies also show the effectiveness of saffron in the treatment of premenstrual syndrome (PMS). For example, the double-blind and placebo-controlled trial on women aged 20-45 years, who have a 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 [1].

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

The objective of the research was to study the effects of the *Crocus sativus* L. Iridaceae

stigma extract on the blood level of follicle-stimulating hormone in female rats, a number of their offspring as well as on some parameters of sexual behavior, and the 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 a dry residue. The yield of the active extract as a viscous gumlike substance constituted 56% of the total mass of raw material.

The tests were conducted on 45 Wistar rats, kept in standard cages (10 animals per cage) at room temperature $22 \pm 2^\circ\text{C}$. All animals were fed ad libitum with standard laboratory chow, and had free 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.

For the purpose of the study, the effects of saffron on sex hormone levels, testosterone level in the blood of male rats, and follicle-stimulating hormone (FSH) level in the blood of female rats were determined. The present study also explored the effects of saffron on the number of offspring the female rats reproduced. Sexual behavior in male rats was studied via a standard 15-minute paired test [2] on the second day following the last administration of the saffron extract.

Before the tests on female rats began, the animals were kept separately for 21 days to avoid accidental pregnancy. Female rats suitable for experiments were selected in the following manner: for the duration of two weeks, vaginal smear samples were taken from animals daily,

for cytological evaluation of the estrous cycle. Females with a regular estrous cycle (4-5 days) were selected. Animals with an impaired estrous cycle were excluded from the study. All animals were in the diestrus phase at the start of the experiment.

The female rats were divided into four groups as follows: two control and two experimental groups, with 10 females in each subgroup. The experimental groups consisted of 12-month-old animals, which were subjected to daily morning saffron extract administration at a dose of 120 mg/kg for 14 days (1st experimental group) and 21 days (2nd experimental group). Control groups included 6-month-old (1st control) and 12-month-old (the 2nd control); animals were treated with saline in the same manner.

Male rats were divided into 3 groups: the 1st group included intact animals, the 2nd group (control) included animals that received saline, and the 3rd group (experimental) included animals that received the saffron extract at a dose of 120 mg/kg for 21 days. The saffron extract and the saline were administered to animals *per os* using a thin metal probe [14].

To study sexual behavior, the male rats were placed in the test chamber (50×35×25 cm) for 5 min 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 h. Each male was placed with 2 females. The females were returned to their cages after a 10-minute exposure. The components of sexual activity were recorded visually for 15 min. 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 coverages with/without intromission - the number of mounts (NM).

The follicle-stimulating hormone (FSH) blood test was carried out after finishing the treatment course. Upon completion of the experiments with saffron extract administration, the blood samples were taken from animals of the control and experimental groups. The blood testosterone levels were measured 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. The procedure was conducted in the morning (9-10 a.m.), and blood samples were obtained from the tail vein under light (1 min) diethyl ether anesthesia. The levels of FSH and testosterone were determined in the blood serum using hormonal test kits for enzyme immunoassay *in vitro* for mammals ("Pishtaz", Iran).

The group of intact males (5 animals aged 6 months) was introduced into the experiments to study the effects of the saffron extract on the number and body weight of the offspring of females aged 12 months, which were being treated with saffron extract for 14 and 21 days.

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

RESULTS AND DISCUSSION

The results of the experiments revealed significant differences in the blood levels of FSH between the control groups, reflecting age-related variation in hormonal production. In this case, the level of FSH was 0.7 ± 0.04 IU/L in the 1st controls vs 1.13 ± 0.05 IU/L in the 2nd controls. Referring to previously published data, a decline in sex-motivated behavior in Wistar rats is observed at the age of 18 months [4]. Observed natural signs signaling aging of reproductive systems in 15- to 18-month-old female rats are the following: short estrous cycles disappear, long ovulatory cycles prevail (more than 7 days), regular cycles gradually are transformed into irregular cycles and the syndrome of constant estrus develops, that ultimately turns into anestrus [3]. Premenopause and menopause are characterized by a significant and stable increase of FSH in the blood level [13]. An increase in FSH level in the rats of the 2nd control group may be due to the advent of the first signs of the reproductive system aging.

An analysis of data obtained in the course of the experiments with female rats showed (Figure 1) that 21-day treatment with saffron extract led to an almost 33% decrease in FSH level in the animals from the 2nd experimental group as compared to the same age rats of the 2nd control group ($p < 0.01$), and brought it closer to the level that was noted in the 1st control group, that included 6-month-old animals.

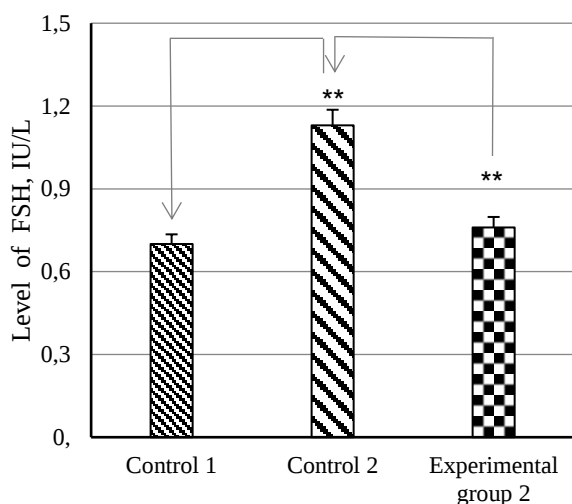


Figure 1. The levels of follicle-stimulating hormone (FSH) in the serum in the female rats of both control groups and the 2nd experimental group;

** $p < 0.01$

The results of this study clearly demonstrated that the saffron extract in a 21-day treatment regimen brought the FSH blood level of 12-month-old female rats to the reference values of younger age.

Given that FSH regulates the development, growth, pubertal maturation, and reproductive processes of the body [13], the obtained data allow surmising that the administration of saffron can help to slow down the age-related changes in the female reproductive system.

To this point, it appeared logical to study how saffron-induced changes in 12-month-old female rats would affect their fertility rate. Therefore, the next stage of the experiments was undertaken to determine the number of offspring in female rats, which had undergone the saffron extract treatment for 14 and 21 days prior to mating with intact males. The series of tests was performed on a group of intact males (5 animals,

6-month-old) that were paired with female rats from both experimental groups and the 2nd control group. The experiments were structured so as to make it possible to study the effect of the duration of the saffron extract treatment on a number of offspring in 12-month-old females.

The results revealed a significant increase in the number of offspring from female rats of both experimental groups compared to the age-matched females of the 2nd control group ($p < 0.01$, $p < 0.05$, respectively).

The seven-day difference in duration of saffron treatment did not affect the number of offspring in female rats, and no statistically significant difference between the groups was observed (see Fig. 2).

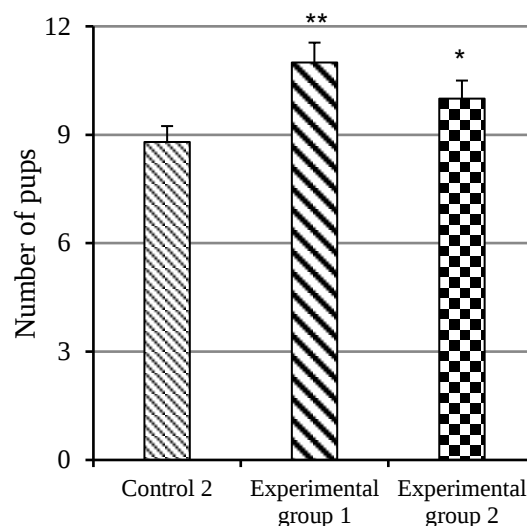


Figure 2. The number of pups in female rats of the 2nd control group and both experimental groups (before pairing with intact males).

* $p < 0.05$; ** $p < 0.01$

Analysis of the results of the study of saffron extract effect on the sexual behavior of male rats revealed positive dynamics in the proceptive and receptive sexual behavior. It was manifested by a decrease in LPS and an 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 ± 3.1 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 (Fig. 3).

The sexual behavior assessment shows that in male rats treated with saffron extract for 21 days, the proceptor behavior was activated by 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 making the conclusion that saffron extract had stimulatory effects on the sexual behavior of animals.

The male rats treated with saffron extract at a dose of 120 mg/kg displayed a significant decrease in the temporal parameters of sexual behavior and an increase in the qualitative parameters of sexual motivation (see Fig. 3).

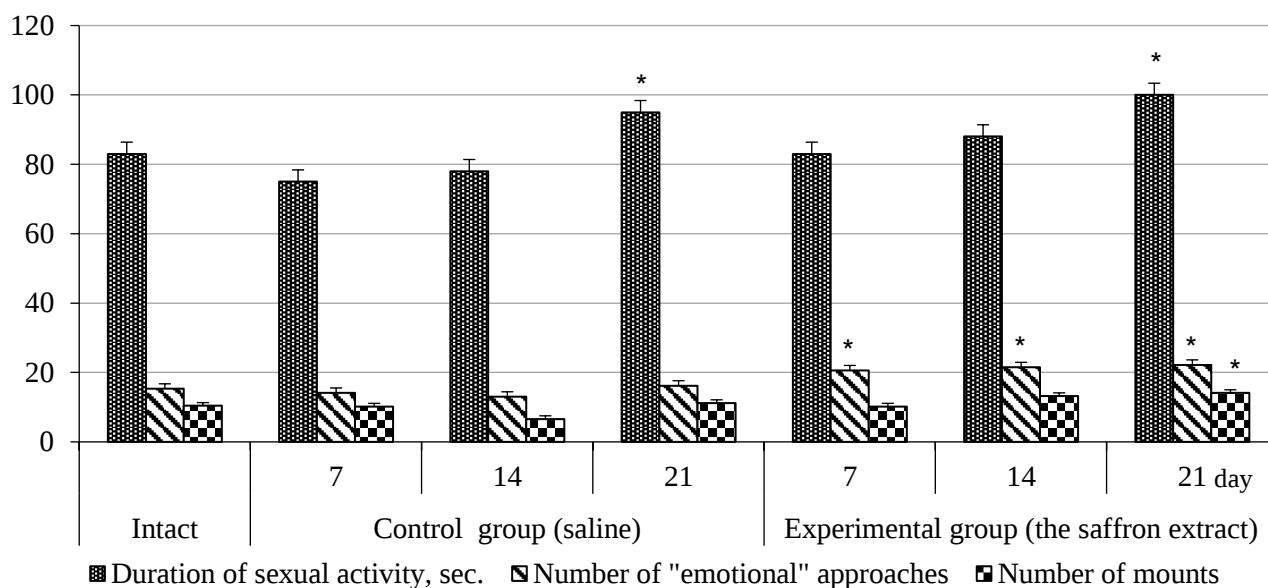


Figure 3. The effects of the saffron extract on the sexual behavior in male rats.

* $p < 0.05$

Consequently, it appeared reasonable to investigate the effects of saffron on male sex hormone content. For this purpose, blood samples were collected to register testosterone levels in male rats on days 7, 14, and 21 of saffron extract administration.

The results revealed, that the saffron extract application for 21 days manifested a statistically significant increase in the total testosterone levels 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 (Fig. 4). Consecutive changes in the total testosterone content occurred as follows: the testosterone concentration was 1.42 ± 0.14 IU/L on the 14th day of saffron extract treatment; it reached the point of 2.87 ± 0.28 IU/L ($p < 0.01$) on the 21st day of saffron extract administration. It is noteworthy that the difference in testosterone

levels on the 21st day of the treatment was statistically significant ($p < 0.01$) (Fig. 4).

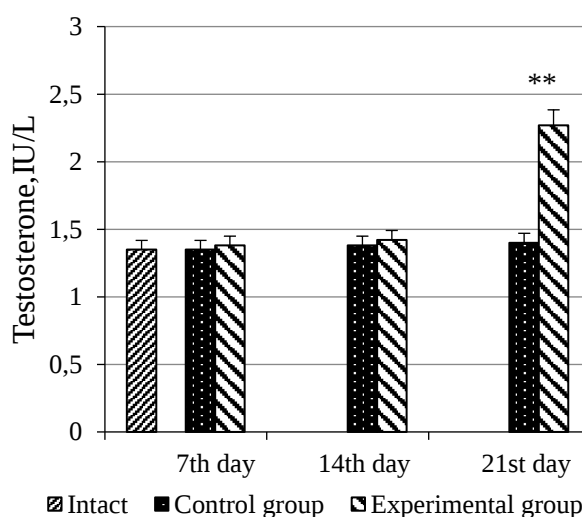


Figure 4. The serum testosterone levels in male rats under saffron extract treatment.

** $p < 0.01$.

Having evaluated the results, we conclude that the administration of saffron had an effect both on the levels of FSH in treated female rats, and a number of their offspring. In this case, saffron treatment significantly lowered the blood levels of FSH in 12-month-old female rats and increased the number of their offspring. Administration of the extract to male rats raised their sexual motivation and had a positive influence on the serum testosterone level.

Therefore, the application of saffron extract maintains reproductive functions of the body, which is biologically significant.

CONCLUSIONS

Saffron's unique medicinal properties are largely due to the diversity of biologically active substances it contains [9,15]. 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.

Substantial literature evidence that the saffron extract is used in treating moderate depression [6, 7]. Similar to the action of antidepressant drugs, saffron, one of saffron's major elements, inhibits serotonin re-uptake and acts as a mild psychoactive drug [6], 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 a high-calorie diet promoted their weight loss, and reduced their blood levels of TL, TG, and TC, compared to similar indicators in untreated animals [5], ultimately leading to lipid metabolism normalization.

In one of the tests [28], 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 membrane's structure, its delivery to the body's peripheral tissues plays a key role. Saffron's ability to regulate lipid metabolism and reduce lipoproteins oxidizability may be one of the mechanisms underlying 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. On a whole, 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.

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ВЛИЯНИЕ ШАФРАНА (*CROCUS SATIVUS* L. *IRIDACEAE*) НА УРОВЕНЬ ПОЛОВЫХ ГОРМОНОВ В КРОВИ, ПОЛОВОЕ ПОВЕДЕНИЕ И КОЛИЧЕСТВО ПОТОМСТВА У КРЫС

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В настоящей работе представлены результаты изучения влияния, выращенного в Азербайджане, шафрана (*Crocus sativus* L. *Iridaceae*) на уровень фолликулостимулирующего гормона (ФСГ) в крови и количество потомства у самок крыс, а также на уровень тестостерона в крови и половое поведение у самцов. Выявлено снижение уровня ФСГ в крови 12-месячных самок крыс по сравнению с контрольной группой, приближение к зарегистрированным уровням 6-месячных крыс и заметное увеличение числа детенышей от самок крыс, получавших экстракт шафрана до спаривания с интактными самцами. Пероральное введение шафрана также повышало уровень тестостерона в крови по сравнению с контрольной группой, не получавшей шафран, а также приводило к выраженной стимуляции процептивного и рецептивного компонентов полового поведения у самцов крыс.

Ключевые слова: экстракт шафрана, самки крыс, фолликулостимулирующий гормон, самцы крыс, тестостерон, количество потомства, половое поведение, фитогеропротектор

SİÇOVULLARIN QANINDA CİNSİ HORMONLARIN SƏVİYYƏSİNƏ, CİNSİ DAVRANIŞA VƏ NƏSİLLƏRİN SAYINA ZƏFƏRANIN (*CROCUS SATIVUS L. IRIDACEAE*) TƏSİRİ

Xanağa Fizuli oğlu Babayev, Pərvanə Ağababa qızı Şükürova, Afət Səday qızı İsmayılova, Rəvanə İqbal qızı Əliyeva, Gülnarə Zahir qızı Qasıмова

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Təqdim olunan məqalədə Abşeronun Bilgəh kəndində becərilən zəfəranın dişi heyvanların qanında follikulstimullaşdırıcı (FSH), erkək siçovulların qanında testosteronu hormonun səviyyəsinə, dişi heyvanlardan doğulan F1 nəsillərinin sayına və erkək siçovulların cütləşmə davranışlarının bəzi göstəricilərinə təsirinin nəticələri təqdim olunub. Müəyyən edilmişdir ki, zəfəran (*Crocus sativus L. Iridaceae*) ekstraktının təsiri nəticəsində yaşlı dişi heyvanların qanında follikulstimullaşdırıcı hormonun səviyyəsi aşağı düşərək cavan orqanizm üçün xarakterik olan səviyyəyə yaxınlaşır, erkəklərdə testosteronun konsentrasiyasına müsbət təsir göstərir. Dişi heyvanlara hamiləlikdən qabaq verilmiş zəfəran ekstraktı balaların sayına və çəki artımına müsbət təsir göstərir. Erkək siçovullara zəfəran ekstraktının verilməsi onların cinsi davranışlarına stimullaşdırıcı təsir göstərək, zaman parametrlərinin göstəricilərində etibarlı azalmasına, cinsi davranışlarının kəmiyyət parametrlərində isə artımına səbəb olur.

Açar sözlər: zəfəran ekstraktı, dişi siçovullar, follikulstimullaşdırıcı hormon, erkək siçovullar, testosteron, nəsillərin sayı, cinsi davranış, fitoheroprotektor

Çapa təqdim etmişdir: Venera Ramiz Xairova, b.ü.f.d., dosent.

Redaksiyaya daxil olma tarixi: 04.10.2021.

Təkrar işlənməyə göndərilmə tarixi: 15.10.2021.

Çapa qəbul edilmə tarixi: 18.04.2022.