**Original Article**

# Effects of Hydro-alcoholic Extract of Red Dried Stigmas of Crocus sativus L. Flowers (saffron) on the Levels of Pituitary-ovary Hormones and Folliculogenesis in Rats

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Abstract

**Background:** In the present study the effects of a hydro-alcoholic saffron (Crocus sativus) extract on pituitary-ovary axis and folliculogenesis were investigated.
The aim was to study the possible role of saffron in female fertility and thereby, show its usefulness in treating infertility and reproductive disorders in females.
**Materials and Methods:** The study consisted of 50 adult female Sprague dawley rats that were divided into five groups of tena control, sham and three experimental groups. The experimental groups received intreperitoneal injections of 1, 2 and 4 dg/kg body weight (B.W) extract, respectively over a ten day period. The control group was untreated and the sham group received only distilled water. After 10 days, blood samples were taken from all groups in order to measure the serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH) estradiol and progesterone hormones. Ovaries were removed, sectioned and examined by light microscope. The results were analyzed by ANOVA and TUKEY tests.
**Results:** Statistical analysis of the results showed a significance increase ($p \leq 0.05$) in the levels of FSH and estradiol in the experimental groups that received 2 and 4 dg/kg B.W extract with respect to the control group; while the level of LH hormone only rose in the experimental group that was administered the maximum dose (4 dg/kg). In addition, the results indicated that administration of 2 and 4 dg/kg extract had a significant effect on ovarian weight. Histological studies of the ovarian sections showed that administration of 2 and 4 dg/kg extracts enhanced folliculogenesis and increased the numbers of secondary follicles in the ovary.
**Conclusion:** According to the results of this and other studies, the hydro-alcoholic extract of saffron may enhance pituitary-ovary axis activities, boost the levels of FSH, LH and estradiol in addition to stimulate folliculogenesis in adult female rats.

***Keywords:*** Saffron, Gonadotropins, Estradiol, Progesterone, Rat

## Introduction

Both hormones and the nervous system have an elaborate control on the cyclic events of the female reproductive system. Numerous studies have been undertaken in order to understand the reproductive processes and treatment of its disorders, such as the use of fertility drugs, *in vitro* fertilization, surrogate pregnancies, and thermometers, to name a few (1). In recent decades, due to the undesirable side effects of chemical drugs, more emphasis has been placed on the use of traditional medicine, particularly plant therapy. Saffron is a perennial plant 10-30 cm in height with a round, fleshy, hardened bulb that is covered with thin brown layers. This plant contains lipids, minerals, mucilages and various carotenoids. In order to investigate the biological activities of saffron, a number of studies are being carried out. It is believed that the antioxidant and anti-tumour properties of saffron extract are due to the activities of its secondary metabolites and active derivatives such as: saffranal, crocin, crocetin and dimethylcrocetin (2). Crocin and dimethylcrocetin are found in saffron and saffranal is the main component of saffron oil (3). Pharmacological studies have shown that saffron extract and its active compounds have anticonvulsant (4), antidepressant (5), anti-inflammatory (6), and anti-tumour activities (7). It has been reported that saffron extract can affect learning behavior, improve memory and cause simplify oxygen diffusion in various tissues (8). In addition, according to recent findings, saffron is useful in the preven-

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tion of Parkinson's disease (9). If crocin is administered orally in either a single dose or multiple doses, it will not be absorbed, but rather it mostly will be lost through the digestive tract. Therefore, the digestive tract is an important place for crocin hydrolyses (10). Crocin (a glycosidic form of crostin) is absorbed as crocetin after being hydrolysed and is found free or conjugated with glucoronid (mono and diglucoronid) in plasma (11). Traditionally, people have believed that saffron is essential for the reproductive organs and, therefore, necessary for a healthy, complete pregnancy.

Thus far, there have been no research studies concerning the effects of a hydro-alcoholic extract of saffron on the female reproductive system and ovarian function. Because the main components of saffron such as crocetin and saffranal (11) have a wide range of various beneficial biological activities, often with no toxic side effects, it is possible that saffron extract may enhance ovarian function. In the present study, the possible effects of a hydro-alcoholic saffron extract on LH, FSH, estradiol and progesterone levels, and ovarian histological changes have been investigated. The results of this study can be useful in treating infertility in reproductive and endocrine centers.

## Materials and Methods

### *Animal groups*

Study was approved by Ethics Committee for animals at the Islamic Azad University, Kazeroun Branch.

In the present study, fifty adult female Sprague dawley rats, aged ten weeks, weighing about 70 ± 10 g each, were obtained from the animal house at Islamic Azad University, Kazeroun. They were kept at 22 ± 2°C on a 12 hours light/12 hours dark cycle, and fed adequate amounts of water and a standard dry diet. Animals were kept in polycarbonate cages with laced steel roofs. It is the size15×25×40 cm. The cage floors were covered with wood powder and cleaned three times each week. During the experiment, all cages were washed three times with water and detergent.

Rats were divided into five groups of ten as follows: control group (no intervention, other than dry food and water), and the sham group who received the same treatment as the control group, with the exception of 1 ml distilled water that was injected into each rat. Three experimental groups which received adequate food and water in addition to a saffron hydro-alcoholic extract in the following doses: experimental group I rats were administered the minimum dose of 1dg/kg B.W intraperitoneally for 10 days, experimental group II received a dose of 2dg/kg B.W for 10 days and experimental group III were given the maximum dose of 4dg/kg B.W for 10 days (Table 1).

*Table 1: Animal Groups*

| Groups | Food and water | Injection (ip) |
|---|---|---|
| **Control** | No limit | nothing |
| **Sham** | No limit | 1 ml distilled water |
| **Experimental group I** | No limit | 1 dg/kg B.W |
| **Experimental group II** | No limit | 2 dg/kg B.W |
| **Experimental group III** | No limit | 4 dg/kg B.W |

### *Preparation and administration of the hydro-alcoholic extract of dried red stigmas of Crocus sativus L. flowers (saffron)*

High quality saffron1 (100 g) was finely ground into a powder and subsequently placed in a glass flask with 50 ml of 96% alcohol. After 72 hours, the mixture was filtered twice and concentrated by evaporation at 40°C (8). The ratio of saffron to its extract was approximately 48%.

### *Synchronization of animals' reproductive cycles*

Prior to beginning the experiment, the reproductive cycles of the rats were synchronized by the following method. A few days before the extract was administered, 100µg estradiol valerate dissolved in 2 ml olive oil was injected intra- muscularly. All rats, after a 24 hour period, received intra-muscular injections of 50µg progesterone which had been dissolved in 2 ml olive oil. A few hours later, vaginal smears were prepared (12). Examination of vaginal smears showed that all the animals were in the estrous stage of strous cycle.

### *Adminstration of the extrac*t

Each day, for 10 days; 2, 4 and 8 g of the saffron extract were weighed, separately placed in separate beakers and readily dissolved in distilled water. Then, 20 ml distilled water was added to each beaker and mixed well. These mixtures were injected intraperitoneally with the use of insulin syringes into each of the experimental groups, respectively. After 10 days, the animals in each group were weighed, anesthetized by ether, and blood samples were taken from their hearts. Blood samples were centrifuged for 15 minutes at 4000 rpm and the separated serum samples were frozen at -20°C and kept for later testing. In addition, ovaries were carefully removed and fixed in a formalin solution at a pH of 7.0. Thin sections were prepared and, after staining with hematoxilin-eosin, ovarian sections were studied with a

light microscope. Concentrations of LH, FSH and estrogen and progesterone were measured by radio immunoassay (RIA). In order to determine the differences between experimental and control groups, the results were analyzed by the ANOVA, TUKEY, LSD and Dunnet tests. Results were considered significant at $p \leq 0.05$.

## Results

Results of hormone assesments among the various groups are shown in Tables 2 and 3. Statistical analyses of the results revealed an increase in ovarian weight in experimental groups that received 2 and 4 dg/kg at the end of the tenth day (Table 3). In addition, the concentration of LH showed a significant rise in experimental group II, which received the medium dose (4dg/kg). Furthermore, the serum levels of FSH and estradiol in experimental groups II and III increased significantly. No significant difference was observed in the concentrations of progesterone among the various groups (Table 2). Histological studies showed no important changes in ovarian tissues, such as: ovarian capsule, stroma tissue, cortex, ovarian medulla, follicles and corpus luteum, in any of the experimental groups. Statistical analyses of the data that resulted from follicle counting revealed no significant differences in primary follicles among the experimental and control groups, but the average number of secondary follicles in experimental groups II and III were significantly different (Figs 1-3). Also, the average number of graffian follicles and corpus luteum revealed no significant differences among the experimental and control groups (Table 3 and Figs 2, 3). There was no significant difference between experimental group 1 and controls in term of hormonal and histological changes.

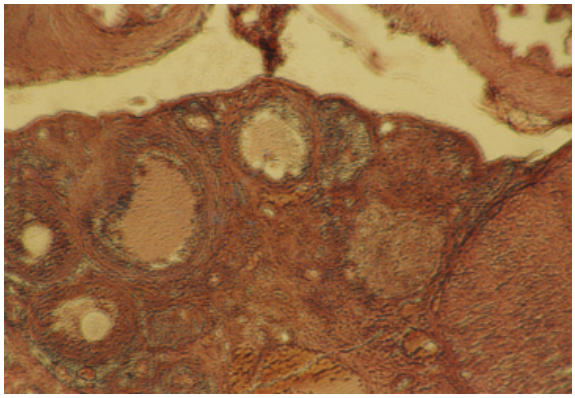

***Fig 1: Ovary tissue in control group (H&E staining; ×4)***

***Table 2: Mean ovarian weight and serum hormone levels in the experimental groups in contrast to the control and sham groups at the end of the experimental period***

| Groups | | Ovarian weight and hormone concentrations | | | | |
|---|---|---|---|---|---|---|
| | | Mean hormone level ± Mean criteria error (X ± SEM) | | | | |
| | | Ovarian weight (mg) | LH (mIU/mL) | FSH (mIU/mL) | Estradiol (pgr/ml) | Progesterone (ngr/ml) |
| Control | | 108 ± 3.96 | 0.13 ± 0.005 | 0.136 ± 0.005 | 40.63 ± 1.10 | 35.08 ± 1.72 |
| Sham | | 102 ± 4.8 | 0.12 ± 0.004 | 0.137 ± 0.008 | 40.25 ± 1.13 | 35.17 ± 2.26 |
| Test | 0.1 g/kg B.W | 93 ± 20.02 | 0.14 ± 0.005 | * 0.15± 0.005 | 38.88 ± 0.56 | 33 ± 2 |
| | 0.2 g/kg B.W | * 137 ± 5.83 | 0.13 ± 0.002 | * 0.19 ± 0.004 | * 49.14 ± 0.99 | 34.25 ± 1.66 |
| | 0.4 g/kg B.W | * 185 ± 8.33 | *0.16 ± .005 | * 0.18 ± 0.005 | * 51.11 ± 1.02 | 33.52 ± 1.9 |

****Indicates significant difference (p<0.05) between control and experimental groups.***
***B.W: Body weight***

***Table 3: Changes in means between primary, secondary, graffian follicles and corpus luteum in all groups***

| Groups | | Ovarian weight and hormone concentrations | | | |
|---|---|---|---|---|---|
| | | Mean hormone level ± Mean criteria error (X ± SEM) | | | |
| | | Primary follicle | Secondary follicle | Graffian follicle | Corpus luteum |
| Control | | 6.5 ± 0.29 | 1.09 ± 0.15 | 0.6 ± 0.09 | 3.8 ± 0.25 |
| Sham | | 6.3 ± 0.45 | 1.03 ± 0.15 | 0.68 ± 0.11 | 4.3 ± 0.22 |
| Test | 0.1 g/kg B.W | 6.8 ± 0.48 | 0.73 ± 0.12 | 0.58 ± 0. 08 | 3.8 ± 0.35 |
| | 0.2 g/kg B.W | 6.4 ± 0.3 | * 1.97 ± 0.35 | 0.7 ± 0.09 | 4.7 ± 0.28 |
| | 0.4 g/kg B.W | 7 ± 0.29 | * 1.03 ± 0.31 | 0.6 ± 0.1 | 3.7 ± 0.22 |

****Indicates significant difference (p<0.05) between control and experimental groups.***
***B.W: Body weight***

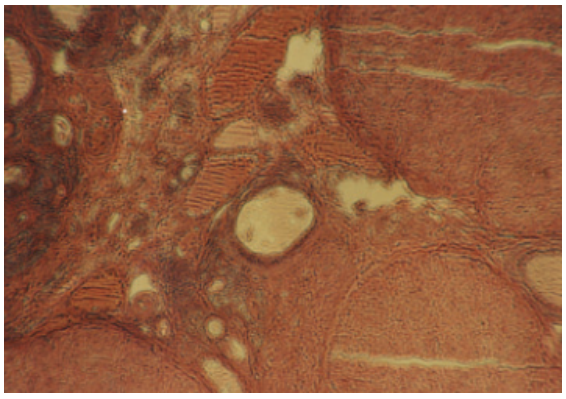

*Fig 2: Ovary tissue in experimental group II (H&E staining; ×4)*

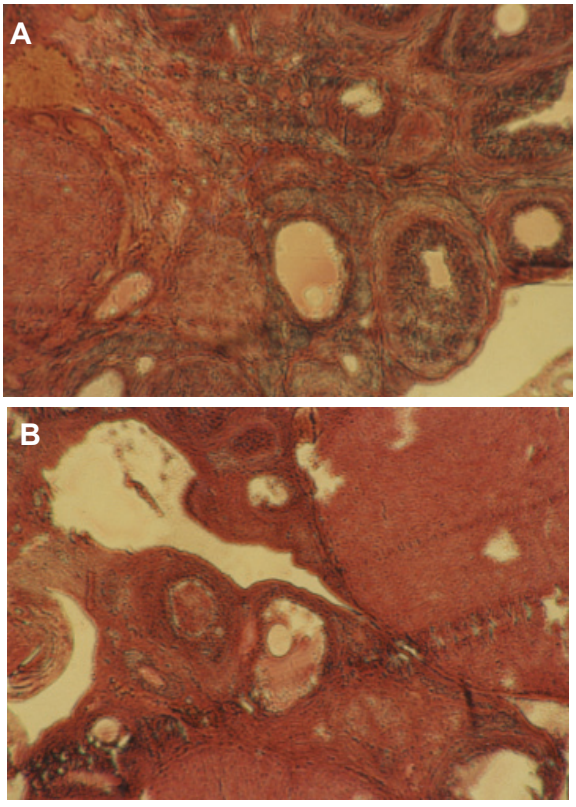


*Fig 3: Ovary tissue in experimental group III ( H&E staining; ×4)*

## Discussion

There is little information about the effect of saffron extracts on reproductive processes. In contrast to their numerous biological effects, the components of saffron have an unknown metabolism. Hence, understanding the precise mechanism of their functions requires numerous studies. In the present study, the effect of alcoholic extract of saffron on ovarian weight, LH, FSH, estradiol, and progesterone hormones, as well as the numbers of primary and secondary follicles, graffian and corpus luteum were investigated.

The results indicated that the administration of 2 and 4 dg/kg saffron extract caused a significant boost in ovarian weight. Previous studies have shown that the compounds present in saffron extracts enhance oxygen diffusion in tissues (8). Murasawa and his colleagues have shown that ovarian weight increases by rising in the numbers of small and medium follicles (secondary follicles). These follicles can be a considerable source of ovarian weight (13, 14). Thus, different compounds of saffron extracts can increase ovarian weight by enhancing oxygen diffusion to granulosa cells and decreasing atresia in small and medium follicles. This finding was confirmed by the significant rise in secondary follicles in the experimental groups II and III (Table 3).

According to table 2, the serum level of LH in the experimental group which received the maximum dose of 4 dg/kg increased significantly when compared to the control and sham groups. Similar effects were seen in the serum concentrations of FSH in experimental groups II and III. It is probable that GnRH and gonadotropic hormones are released by crocin activation of noradrenergic nerves in the locus ceruleus and with saffranal by stimulating the activities of serotonergic nerves, both of which are associated with the hypothalamus (5). Despite an FSH boost in the group that received a dose of 2dg/kg, the level of LH did not change significantly. This may indicate that the hypothalamus as well as the pituitary gland and ovaries are stimulated by the extract. Recent studies have revealed that crocin, crocetin and dimethyl crocetin can influence H1 histon, reduce the interaction between H1 histone and DNA, and increase gene transcription (15). Such influence can increase transcription of α and β subunits of FSH. On the other hand, recent studies have indicated that the positive effects of estradiol on gonadotropin secretion are alpha dependent and occur at the pituitary level (16). Considering the possible effect of crocetin on enhancing the activities of ovarian noradrenergic nerves (5), the role of these nerves in increasing FSH receptors in primary follicles and the rise in estradiol synthesis; one can attribute the increase in FSH concentration in experimental groups II and III to the positive feed-back effects of estradiol at the pituitary level. Recent studies have also shown that the reduced basal level of cyclic adenosin monophosphate response element binding protein (CREB) phosphorylation may sensitize the pituitary to GnRH, as there would be more CREB phosphorylation sites available to respond to the gonadotropin _ releasing hormone (GnRH) signal (17). A possible effect of crocin can cause an

increase in the sensitivities of gonadotrop cells to GnRH. Therefore, during gonadotrop stimulation by GnRH, these cells will respond vigorously to the stimulating agent, thus producing more gonadotropin (18). As shown in Table 1, the serum concentration of estradiol rose significantly in the groups administered the medium and maximum doses of extract. There was no significant difference in the concentration of progesterone among the various groups, while the serum level of LH significantly increased in the experimental group that received the maximum dose. During the experiment all animals were in the estrous phase, therefore the concentration boost of LH in experimental group III could not be responsible for the elevation of progesterone in this group (Table 2). Similarly, the lack of a significant difference in the numbers of corpus luteum among the various groups also confirmed this finding.

Histological studies of the ovarian sections showed that administration of saffron extract had no effect on the ovaries in the experimental groups. The number of secondary follicles in experimental groups that were administered 2 and 4 dg/kg extract increased significantly (Table 3), while the numbers of primary graffian follicles and corpus luteum were not significantly different among the various groups. Hence, this study has shown an enhanced folliculogenesis by saffron extract at the steps affected by gonadotropin, thereby enhancing the growth and differentiation of primary follicles and increasing secondary follicles. It has been reported that FSH stimulated gap junction formation and turnover in rat ovarian granulosa cells and increased connexin43 (Cx43) gene expression in these cells (19). Cx43 gene is expressed in granulosa cells and plays a crucial role in the development of germ cells. Postnatal folliculogenesis in Cx43-deficient ovaries do not proceed beyond the primary follicle stage (20). In addition, estradiol stimulates the proliferation of granulosa cells, protects against apoptosis, and modulates granulosa cell differentiation by enhancing the ability of FSH to induce expression of LH receptors (13). Therefore, in accordance with the increased concentrations of estradiol and FSH hormones in the experimental groups that received 2 and 4 dg/kg extract, the rise in the number of secondary follicles was not unexpected.

Recent studies have shown that high concentrations of carotenoids have the ability to stimulate Cx43 formation among cells (21). Thus, the presence of carotenoids in saffron extract can also promote differentiation of primary follicles through increasing the expression of Cx43. Furthermore, the probable effect of crocin which is present in saffron extract can be a factor in increased norepinephrine which in turn can affect increased FSH receptors in granulosa cells and enhance the growth and differentiation of primary follicles (22). For this reason, in the present study, the possible factors for promotion of folliculogenesis and elevation in the number of secondary follicles are a result of the probable effects of saffron extract on ovarian tissues to stimulate

cx43 gap junction formation and changes in the ratio of Bcl/Bax proteins (atresia inhibition of follicles). Also saffron extract caused the further stimulation of ovarian noradrenergic nerve activity and its effects on the levels of pituitary- ovary hormones and folliculogenesis increased.

## Conclusion

The findings of this study show that 2 and 4 dg/kg hydro-alcoholic saffron extract can promote the pituitary-ovary axis activities at all levels, cause an elevation in the serum concentrations of LH, FSH and estradiol hormones, as well as increase the mean numbers of secondary follicles and eventually ovarian weight. It is probable that these effects are the results of active compounds such as crocin, crocetin and saffranal, all of which are present in the extract. It can be suggested that one might use saffron to enhance fertility and treat infertility in females, although further studies are still required.

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There is no conflict of interest in this study.

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