



THE PROTECTIVE ROLE OF CROCIN IN THE FEMALE REPRODUCTIVE SYSTEM

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Abstract: Saffron is a highly valuable spice derived from the dried stigmas of *Crocus sativus* L., a plant species belonging to the Iridaceae family. Although saffron production is predominantly concentrated in Iran, significant quantities are also produced in countries such as Türkiye, India, France, Greece, Spain, and Morocco. The primary bioactive compounds in saffron include crocin, quercetin, and safranal. Additionally, saffron contains a range of essential nutrients, including vitamins, sugars, proteins, amino acids, vital minerals, flavonoids, and other chemical constituents. Saffron extract contains a variety of carotenoids, including crocin (crocetin di-gentiobiose ester), crocetin, crocetin di-glucose ester, and crocetin gentiobiose-glucose ester. Beyond its widespread use as a food additive, saffron extracts, which contain crocin, are utilized in traditional medicine as natural healing agents for the treatment of diseases. The therapeutic effects of crocin include antioxidant, hypolipidemic, anti-inflammatory, antifibrotic, and anticancer properties. The existing literature encompasses a substantial body of research that demonstrates the protective and therapeutic effects of crocin on the female reproductive system, employing contemporary scientific methodologies. This review offers a detailed summary of the use of crocin, a key component of saffron, which is also cultivated in the Western Black Sea region of Türkiye, within the context of traditional medicine. Furthermore, the review will present modern scientific evidence derived from both preclinical and clinical studies regarding the application of crocin in the treatment of disorders affecting the female reproductive system.


Keywords: Saffron, Crocin, *Crocus sativus*, Fertility, Female reproductive system

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1. Introduction

Infertility is a health issue with familial and societal impacts. It is noted that fertility in women is highest in their twenties and thirties, while in men it remains high until their forties. In healthy fertile couples, pregnancy typically occurs after an average of 8 months of regular intercourse (Knez, 2016). Infertility is characterized as the inability to conceive after one year of consistent, unprotected sexual intercourse (Vander Borgh and Wyns, 2018). Globally, fertility rates are declining due to various factors, primarily environmental ones, among other influences (Starc et al., 2019).

Sex hormone therapies (such as human chorionic gonadotropin, follicle stimulating hormone (FSH), etc.), tubal reconstructive surgery and in vitro fertilization (IVF) are conventional treatments for infertility. However, the interventions are associated with unavoidable side effects and potential negative consequences. For instance, hormone therapy may result in ovarian hyperstimulation syndrome (OHSS) or contribute to mental health concerns (Freeman et al., 2013; Rashidi et al., 2015). The prolonged, physically demanding, and financially burdensome nature of these treatments often drives individuals to explore complementary and adjunctive approaches (Öztürk et al., 2021). A study indicated that 65% of women utilized

complementary and supportive therapies as part of their infertility treatment (Çelik and Kırca, 2018). Another study found that 47.3% of 150 women undergoing infertility treatment reported using alternative medicine methods alongside conventional therapies (Kurt and Arslan, 2019).

Saffron is derived from the dried stigma of the saffron flower (*Crocus sativus* L.), a plant from the Iridaceae family, and is cultivated in countries such as Iran, Morocco, India, Greece, Türkiye, Spain, and France. This plant contains orange-colored pigments and is commonly used as a spice with coloring and aromatic properties in various cuisines, baked goods, confectionery products, and perfumes. In addition to its widespread use as a dietary supplement, saffron has historically been used as a therapeutic agent for a variety of ailments, including depression, mental disorders and cancer (Schmidt et al., 2007; Bathaie and Mousavi, 2010). Saffron is a source of carbohydrates, proteins, anthocyanins, as well as various vitamins and minerals (Petersen et al., 2008). Saffron also contains over 150 volatile aromatic compounds, and its value comes from these distinct phytochemical compounds (Deniaud et al., 2008). The majority of these compounds are carotenoids, including lycopene, zeaxanthin, vitamin A, and β -carotene. Saffron is



composed of three primary components: crocin, picocrocin, and safranal (Figure 1). Crocin, a carotenoid pigment, is responsible for the characteristic color of saffron, comprising more than 10% of its dry weight. Picocrocin contributes to the distinctive bitter aroma of saffron, while safranal, the primary volatile oil compound, is chiefly responsible for its unique flavor profile.

Crocin exhibits antioxidant, hypolipidemic, anti-inflammatory, antifibrotic, and anticancer properties, and there are studies that suggest its potential use in infertility research. This review will summarize current scientific findings from studies investigating the effects of crocin, a key component of the saffron plant, on the female genital system.

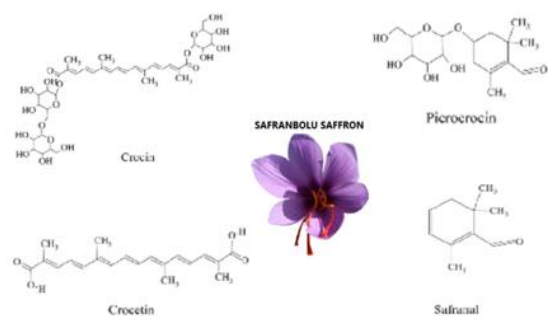


Figure 1. The main structural components of saffron are crocin, picocrocin, crocetin and safranal, which are also instrumental in determining the quality and aroma of the substance (Giaccio, 2004).

2. Effects of Crocin on the Female Genital System

Oogenesis and follicle development in the female genital system are regulated hormonally in the ovary. The follicular structure consists of an oocyte and the surrounding granulosa and theca cells. Within the follicle, the growth of the oocyte and the development of granulosa and theca cells occur in a coordinated manner. This balance influences the fertilization potential of the ovum after ovulation and its subsequent embryonic development (Matur and Solmaz, 2010).

In the literature, a study was found to examine the effects of crocin, a bioactive compound found in saffron, on the hormonal regulation of the female reproductive system. It is noteworthy that this study focused significantly on its effect on ovarian function and oocyte development. In a study conducted by Maleki et al., the impact of crocin and saffron extract on murine oocytes were examined through the collection of cumulus-oocyte complexes from ovarian tissues. The retrieved oocyte complexes were subsequently cultured in the presence of aqueous saffron extract and crocin. The saffron extract was introduced into the culture medium at concentrations of 5, 20 and 40 µg/ml, while crocin was administered at concentrations of 50, 100 and 400 µg/ml. The research yielded findings that indicated the significant enhancement of in vitro oocyte

maturation (IVM) and IVF rates by aqueous saffron extract and crocin. This finding suggests that these substances may have the potential to modulate reproductive processes (Maleki et al., 2014).

Chen et al. (2019) conducted a comparable study in which porcine oocytes aspirated from the ovaries of female pigs were cultured in a medium supplemented with 0, 300 µg/ml, 400 µg/ml, or 500 µg/ml of crocin. Following IVM, a range of parameters were evaluated, encompassing IVM rates, levels of reactive oxygen substances (ROS) and glutathione (GSH), and the kinetics of major antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Furthermore, the protein levels of apoptosis-regulating genes, like Bcl-2, Bcl-2-associated X protein (BAX) and caspase-3, were analysed together with the subsequent embryonic cleavage rates. The results demonstrated that oocytes cultured with 300 µg/ml and 400 µg/ml of crocin exhibited a significant upregulation in SOD and CAT activity, while those treated with 300 µg/ml, 400 µg/ml, and 500 µg/ml of crocin displayed a notable increase in GPx levels and IVM rates. The highest IVM rate was recorded in the 400 µg/ml crocin-treated group. Furthermore, a significant downregulation of caspase-3 expression was shown in the 400 µg/ml and 500 µg/ml groups alongside a substantial alteration in the Bcl-2/BAX mRNA expression ratio in these treatment groups. These findings suggest that crocin exerts a dose-dependent influence on IVM, oxidative stress regulation and apoptotic gene expression, potentially enhancing in vitro developmental competence (Chen et al., 2019).

A research was carried out by Zohrabi et al. in Wistar albino rats to investigate the effects of crocin on ovarian function and hormone regulation. In this experiment, crocin was administered intraperitoneally (IP) at doses ranging from 100 to 200 mg/kg, administered every other day, for a period of 30 days. Following the treatment, the serum hormone levels were quantified from blood samples and ovarian tissues were subjected to histopathological analysis. The findings indicated that the administration of 200 mg/kg crocin led to a remarkable decrease in the number of atretic follicles. Furthermore, treatment with 100–200 mg/kg crocin led to a significant decrease in estradiol (E₂) and progesterone concentrations in comparison with the control group, while FSH and luteinizing hormone (LH) levels remained unaltered. These results suggest that crocin may influence ovarian follicular dynamics and steroidogenesis without altering gonadotropin levels (Zohrabi et al., 2018).

A research paper was published by Nili-Ahmadabadi et al., which evaluated the impact of saffron aqueous extract and crocin on FSH, LH, progesterone and E₂ levels in pregnant rats. The saffron aqueous extract was administered via oral gavage at doses of 500, 1000, and 2000 mg/kg, while crocin was administered at doses of 150, 300, and 600 mg/kg. Reproductive hormone levels were assessed during the early, mid-, and late stages of pregnancy. Female rats were mated, and the presence of vaginal plugs

was designated as gestational day 0. The animals were then assigned to experimental groups, with the study comprising 21 groups, each consisting of six rats. While control groups received oral saline, the experimental groups were administered saffron aqueous extract or crocin at the specified doses. On days 9, 13 and 22 of gestation, the rats were sacrificed and blood samples, ovaries, uteri and fetuses were sampled for analysis. On day 9 of gestation (early pregnancy), no statistically significant effect on FSH levels was observed between the groups. However, on days 13 (mid-pregnancy) and 22 (late pregnancy), FSH levels in the group receiving 2000 mg/kg saffron aqueous extract were significantly elevated compared to the control group. Conversely, the administration of 600 mg/kg of crocin led to a substantial increase in FSH levels exclusively during the late stages of pregnancy, with no significant alterations observed in the earlier stages. Additionally, a significant reduction in serum progesterone levels was observed across all stages of pregnancy in the groups receiving saffron aqueous extract at all doses. However, in the crocin-treated groups, a significant decrease in progesterone levels was detected only in late pregnancy. In the initial phase of pregnancy, a substantial decline in serum E₂ levels was observed among subjects administered 2000 mg/kg of saffron aqueous extract, in contrast to the control group. During mid-pregnancy, E₂ levels remained comparable to the control group, whereas in late pregnancy, E₂ levels were again significantly lower in the saffron-treated group. Furthermore, saffron aqueous extract administration resulted in a dose-dependent elevation in serum LH levels during early pregnancy, whereas no significant alterations were observed in the mid- or late-gestational stages. Conversely, crocin treatment led to a significant increase in serum LH levels exclusively during late pregnancy. The study also demonstrated that high doses of saffron and crocin were associated with a reduction in fetal count. In the high-dose groups, increased incidences of small embryo formation, embryonic degeneration, placental resorption, and fetal resorption were observed, along with occasional occurrences of decidual bleeding (Nili-Ahmadabadi et al., 2022). The findings indicate that saffron and crocin exert dose-dependent effects on reproductive hormone levels and fetal development during pregnancy.

In a research by Sadoughi et al., the protective effect of crocin was investigated in a letrozole-induced rat model of polycystic ovarian syndrome (PCOS). Crocin was administered IP to rats for 28 days, after which serum levels of LH, FSH, testosterone, and E₂ hormones were measured, and histological examination of the ovaries was conducted. The results indicated that crocin significantly reduced LH, testosterone, and E₂ levels in the PCOS rats, while FSH levels were significantly increased. Histopathological analysis showed a significant increase in the number of preantral and antral follicles, as well as corpora lutea in the treated group. These findings suggest that crocin may be effective in addressing hormonal

imbalances and ovarian cysts associated with PCOS in rats (Sadoughi, 2017).

In another study, PCOS was induced in rats by administering letrozole via oral gavage for 21 days, followed by crocin treatment at a dose of 50 mg/kg via IP for a period of six weeks. The results of this study showed that rats treated with letrozole had elevated levels of testosterone, FSH, and LH and reduced levels of E₂. Crocin treatment restored normal hormonal parameters and eliminated cystic formations in the ovarian cortex. These findings suggest that crocin may have significant therapeutic potential in the treatment of PCOS (Elham et al, 2019).

In a clinical trial by Rahimi et al., the study population comprised women suffering from PCOS. The study sample was partitioned into two groups (n = 25 each). The first group was administered crocin (15 mg, twice daily), while the second group received a placebo. After 12 weeks, a comprehensive assessment was conducted to evaluate the changes in the patients' lipid profiles, fasting blood glucose levels, fasting insulin, as well as inflammatory markers such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α). These assessments were conducted before and after the administration of the treatment. The results demonstrated that the crocin-treated group exhibited significant improvements in lipid profile and fasting glucose levels in comparison to the placebo group. Furthermore, the administration of crocin resulted in a substantial decrease in serum levels of the inflammatory cytokines IL-6 and TNF- α . These results indicate that crocin may serve as a promising therapeutic agent for the treatment of PCOS (Rahimi et al., 2022). The results of the animal and clinical studies indicate the possible therapeutic value of crocin in the management of PCOS.

Some drugs used in cancer treatment cause adverse side effects on ovarian follicles and folliculogenesis (Spears et al., 2019). There is a paucity of research on the use of crocin to treat side effects arising from cancer therapies in the female genital system (Wang et al., 2022). Khanmohammadi et al. showed in a study in which crocin was given together with Cyclophosphamide (CP), which is frequently used in cancer therapies, that the administration of crocin together with CP in 6-8 week old female mice caused an increase in the number of follicles in all developmental stages and a significant decrease in mast cells. In the same study, it was shown that crocin positively changed the oxidative stress parameters due to CP administration and caused a significant decrease in malondialdehyde (MDA) level and a significant increase in total antioxidant capacity (TAC) and SOD levels. These favourable changes in redox balance were also reflected in hormonal parameters and significant increases in LH, FSH and E₂ levels were shown in subjects given crocin together with CP (Khanmohammadi et al. 2021).

In other study conducted by Shams et al. (2024), the potential protective effects of crocin against CP-induced ovarian damage were examined. The study included 24 female Wistar albino rats weighing 160-180 grams, which

were assigned to four groups. The control group received only saline, while the second group received 30 mg/kg CP. The third and fourth groups were administered 30 mg/kg CP combined with 100 mg/kg or 200 mg/kg crocin, respectively. Treatments were administered IP three times per week. In the CP treated group, the number of primordial follicles and the diameter of primary follicles were reduced compared to the control group. However, both crocin-treated groups exhibited an increase in these parameters relative to the CP group. Furthermore, the number of atretic follicles was markedly elevated in the CP group when compared to the control group, whereas it exhibited a profound and statistically significant reduction in the 200 mg/kg crocin group in comparison to the CP group (Shams et al., 2024). In conclusion, the study by Khanmohammadi et al. and Shams et al. indicate that crocin may exert protective effects on ovarian follicles and enhance redox balance in females exposed to CP, potentially mitigating the detrimental side effects of cancer therapies on ovarian function.

As reported by Chen et al. (2021), Gestational hypertension (pre-eclampsia) was induced in female pregnant Sprague-Dawley rats by administering nitro-arginine methyl ester hydrochloride (L-NAME) at a dose of 50 mg/kg/day by oral gavage on days 14-19 of pregnancy, and rats treated with crocin at a dose of 50 mg/kg, 100 mg/kg and 200 mg/kg by oral gavage on days 10-19 of pregnancy were sacrificed on day 20 of pregnancy and placental tissue and serum samples were collected. Enzyme Linked Immunosorbent Assay (ELISA) was performed to determine whether crocin treatment could ameliorate abnormal changes in placental growth factor (PlGF) and soluble fms-like tyrosine kinase (sFlt-1). Treatment with 200 mg crocin decreased the circulating level of sFlt-1 but increased the circulating level of PlGF and significantly decreased the serum sFlt-1/PlGF ratio in hypertensive rats. In L-NAME-induced hypertensive rats, crocin treatment at a dose of 200 mg was found to improve the clinical signs of systolic high blood pressure and proteinuria. Both placental and serum levels of TNF- α , IL-6 and IL-1 β (interleukin-1 beta) cytokines were detected by ELISA quantitative real-time polymerase chain reaction (RT-PCR). TNF- α , IL-1 β and IL-6 inflammatory cytokine levels were significantly reduced in hypertensive rats treated with 200 mg crocin. Placental MDA, hydrogen peroxide (H₂O₂), SOD, CAT, GPx and GSH antioxidant parameters were measured in rats in which pre-eclampsia was induced. While MDA and H₂O₂ levels were very low, SOD, CAT, GPx and GSH parameters were found to be very high in rats treated with a dose of 200 mg of crocin. Levels of heme oxygenase-1 (HO-1) and nuclear factor-erythroid 2-like 2 (Nrf-2) were analysed by Western blot in rats with a pre-eclampsia model, and both HO-1 and Nrf-2 levels were significantly raised in the group of hypertensive rats treated with a dose of 200 mg of crocin. In the assessment of fetal weight and fetal survival rate, it was also observed that fetal weight and fetal survival rate were significantly elevated in crocin-treated hypertensive rats (Chen et al.,

2021).

In an experimental study, Liu et al. (2017) sought to ascertain the potential therapeutic properties of crocin on endometriosis-associated infertility and inflammation in a mouse model. Endometriosis was induced in 7-week-old female BALB/c donor mice through IP administration of estradiol benzoate. One week post-injection, uterine tissues were harvested, and the endometrial layer was meticulously isolated and suspended in sterile saline solution. The collected endometrial tissue was then fragmented into pieces smaller than 1 mm, homogenized, and introduced into the peritoneal cavity of 30 recipient mice via laparoscopy to establish endometriotic lesions. Following the induction of endometriosis, the recipient mice were randomly allocated into three groups (n=10 per group). Group 1 was designated as the control group and received no intervention. Group 2, the sham group, was administered IP saline injections. Group 3 was treated with crocin at a dose of 25 mg/kg IP for 20 consecutive days. On the 21st day of the study, the animals were euthanised. Their uterine tissues and serum samples were then examined for further histological and biochemical analysis. The results demonstrated a statistically significant size and weight reduction of endometriotic lesions in the crocin-treated group compared to both the control and sham groups. Histopathological assessments supported these findings. Further immunohistochemical analysis demonstrated that administration of crocin led to a substantial reduction in the levels of vascular endothelial growth factor (VEGF) and proliferating cell nuclear antigen (PCNA). Furthermore, a marked reduction in serum levels of pro-inflammatory cytokines, including TNF- α , IL-6, interferon-gamma (IFN- γ) and VEGF, was observed in the crocin-treated group compared with the control and sham groups. These findings suggest that crocin may exert therapeutic effects by attenuating inflammation and suppressing endometriotic lesion development in an experimental endometriosis model (Liu et al., 2017).

A substantial body of studies has been conducted using various experimental models, which collectively indicate that crocin exerts a beneficial effect on the ovaries by modulating hormonal balance and ameliorating symptoms associated with conditions such as endometriosis, PCOS and gestational hypertension. Furthermore, the incorporation of crocin into oocyte culture media has been demonstrated to significantly enhance IVM and fertilization IVF outcomes. Crocin has been demonstrated to exert a protective effect against ovarian toxicities induced by cancer treatment, to alleviate oxidative stress, and to have anti-apoptotic and anti-inflammatory properties. Crocin administration at different doses has been found to regulate hormonal levels, particularly by altering the concentrations of LH, FSH, E₂ and progesterone during pregnancy.

3. Effects of Saffron Compounds on the Estrus Cycle

Rodents, particularly rats, are frequently utilized in reproductive and infertility research due to the similarity of their estrous cycle to the human menstrual cycle. These cycles are commonly referred to as the estrous cycle (Auta et al., 2016). In female rats, puberty typically begins after the fourth postnatal week, which is marked by a surge in LH (Ekambaram et al., 2017). The estrous cycle in rodents is a dynamic process, during which ovulation can be monitored, and fluctuations in the levels of E₂ and progesterone secreted by the ovaries mirror the cyclical changes in various ovarian cell types, which become prominent and recede throughout the cycle. This cycle typically recurs every 4-5 days, unless disrupted by conditions such as pregnancy, pseudopregnancy, or anestrus. The cycle is divided into four distinct phases: proestrus, estrus, metestrus, and diestrus (Byers et al., 2012; Cora et al., 2015). The proestrus phase lasts approximately 12 hours, estrus lasts between 12 to 24 hours, metestrus lasts 6-8 hours, and diestrus lasts 52-60 hours (Petroianu et al., 2005). During the proestrus and estrus phases, follicular development occurs in the ovaries, ovulation typically occurs around the midpoint of estrus, and corpus luteum formation follows ovulation. The diestrus phase serves as a resting phase for the reproductive system (Hubscher et al., 2005).

Vaginal cytology is a non-invasive and relatively cost-effective method commonly employed to assess the stages of the estrous cycle (Sahoo et al., 2014). This technique is typically performed using two methods. In the vaginal smear technique, a cotton swab moistened with saline at room temperature is inserted into the vaginal opening and

gently rotated to collect cells from the vaginal wall. These cells are then spread onto a microscope slide, air-dried, fixed, and stained (Barret et al., 2018). In the vaginal lavage technique, a sterile pipette is carefully inserted into the vaginal canal for several millimeters. A volume of 100 µl of phosphate-buffered saline, distilled water or isotonic serum is slowly administered into the vagina and subsequently aspirated. This procedure should be repeated 4-5 times using the sterile latex balloon. It is important to position the rubber pipette at the entry of the vaginal canal and not to enter the vaginal opening. The cell suspension, including a few drops of liquid, is then placed on a microscope slide, air dried, and properly dyed and analyzed (Auta et al., 2016).

In vaginal cytology, the determination of the estrous phase after staining is based on the distribution and density of various cell types. During the proestrus phase, the presence of small and large round nucleated cells, which are homogeneous in appearance and size, is characteristic of this stage. These cells may be observed individually or in clusters. There may also be a small number of anucleated, keratinized epithelium cells. Early proestrus may also show few neutrophils. In the estrus phase, a high number of anucleate keratinized epithelial cells is observed. The cytoplasm of these cells appears granular, and the cells exhibit irregular shapes, with very few nucleated epithelial cells occasionally seen. The metestrus phase is marked by the presence of numerous leukocytes and a few large, granule-free, anucleate keratinized epithelial cells, as well as some nucleated epithelial cells in the late metestrus phase. In the diestrus phase, prominent leukocytes, nucleated epithelial cells, and rare keratinized cells may be detected (Figure 2, Table 1) (McLean et al., 2012; Auta et al., 2016; Barret et al., 2018).

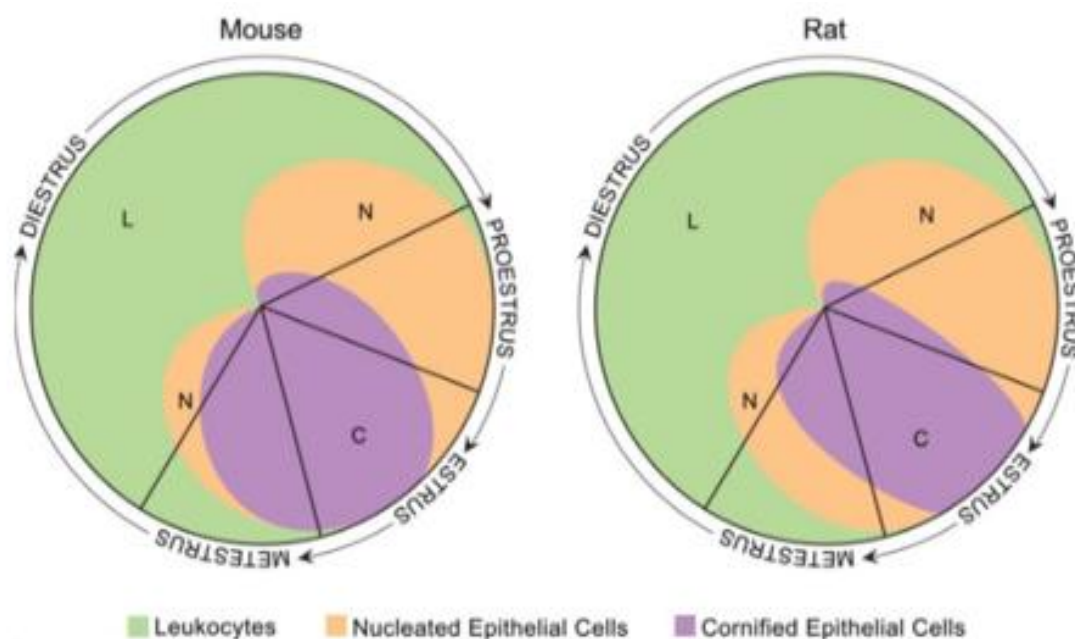


Figure 2. The fundamental principle for determining the stage of the estrous cycle is to know the predominant cell type for each stage (Ajayi and Akhigbe, 2020).

Table 1. Phases of the estrous cycle based on the density of vaginal smear cell types (Cora et al., 2015)

Stage	Neutrophils	Small Nucleated Epithelial Cells	Large Nucleated Epithelial Cells	Anucleated Keratinized Epithelial Cells	Relative Cell Density
Proestrus	0 to +	++ to +++	0 to +	0 to +	Low to moderate
Estrus					
Rat	0 to +	0 to ++	0 to ++	++ to +++	Moderate to high
Mouse	0 to +	0 to +	0 to +	++ to +++	Moderate to high
Metestrus					
Rat	+ to +++	+ to ++	+ to ++	+ to +++	Moderate to high
Mouse	+ to +++	0 to +	0 to +	++ to +++	Moderate to high
Diestrus	++ to +++	+ to ++	+ to ++	0 to +	Low to moderate

0 = none; + = few; ++ = moderate; +++ = high.

Cellat et al. (2024) examined the protective effects of safranal, a compound derived from saffron, against letrozole-induced PCOS in Wistar albino rats. At the start of the study, estrous cycles were monitored in 50 rats, with 44 of them showing a normal cycle duration of 4 to 5 days. From these, 32 female rats were selected at random and allocated to one of four groups, with each group consisting of eight rats. Group 1 received 1.2 ml/kg of 1% carboxymethylcellulose, group 2 received 1 mg/kg of letrozole, group 3 received safranal at a dose of 200 mg/kg, and group 4 received a combination of letrozole (1 mg/kg) and safranal (200 mg/kg) for 21 days via oral gavage. Throughout the study, vaginal lavage was performed to monitor the estrous cycle and assess its length. Irregular estrous cycles were monitored in 7 rats in the letrozole group, while regular estrous cycles were monitored in 8 rats in control group, 8 rats in safranal group, and 6 rats in letrozole + safranal group. The results indicated that while safranal alone did not alter the estrous cycle length, it effectively reversed the prolonged estrous cycle induced by letrozole (Cellat et al., 2024).

In their study, Bashir et al. (2022) investigated the impact of saffron extract on the onset of temporary infertility and the estrous cycle in Wistar albino rats. Female rats with a regular estrous cycle were randomly assigned to either the control group (12 rats) or the experimental group (12 rats), which received 1 g/kg of saffron extract for 12 days. Vaginal smear samples were collected from days 13 to 43, and the Giemsa staining protocol was used for analysis. Monitoring of the estrous cycle through Giemsa staining revealed that the saffron-treated group exhibited disruptions in their estrous cycles. In addition, the number of offspring born to mated rats between days 44 and 60 was remarkably lower in the group treated with saffron

versus the control group. These results suggest disruption of the estrous cycle by saffron extract, which may have a negative effect on fertility (Bashir et al., 2022).

Hu et al. (2018) induced PCOS in female mice offspring by administering 250 µg/kg dihydrotestosterone (DHT) subcutaneously on days 16-18 of pregnancy. This study evaluated the impact of crocetin, a component of saffron, on the length of the estrous cycle in 36 female offspring with PCOS. The study's control group comprised 18 female offspring without induced PCOS. Of the 36 PCOS-induced offspring, 18 were treated with 40 mg/kg of crocetin for a period of 4 weeks. The results indicated that in the PCOS group, the duration of diestrus was extended, while the durations of other estrous cycle phases remained normal. Conversely, the administration of crocetin to the PCOS treated group restored the diestrus to its normal duration (Hu et al., 2018).

The authors of this review comprehensively report on the modulatory effects of crocin, a pharmacologically active compound derived from saffron, on the regulation of the estrous cycle in female Wistar albino rats between 8 and 12 weeks of age. In the study, estrous cycle monitoring and cycle length were determined after 20 days of treatment with crocin at a dose of 100 mg/kg/day by IP injection. In our study, vaginal lavage samples were collected continuously for 10 days. After drying and fixation, H&E and toluidine blue staining were performed. It was observed that crocin, when administered alone, did not alter the estrous cycle length, as confirmed by H&E and toluidine blue staining of the vaginal lavage samples. The samples were analyzed using a light microscope, and the estrous phase was identified. Photographs of the crocin and control groups, captured at 20X magnification, are presented in Figure 3.

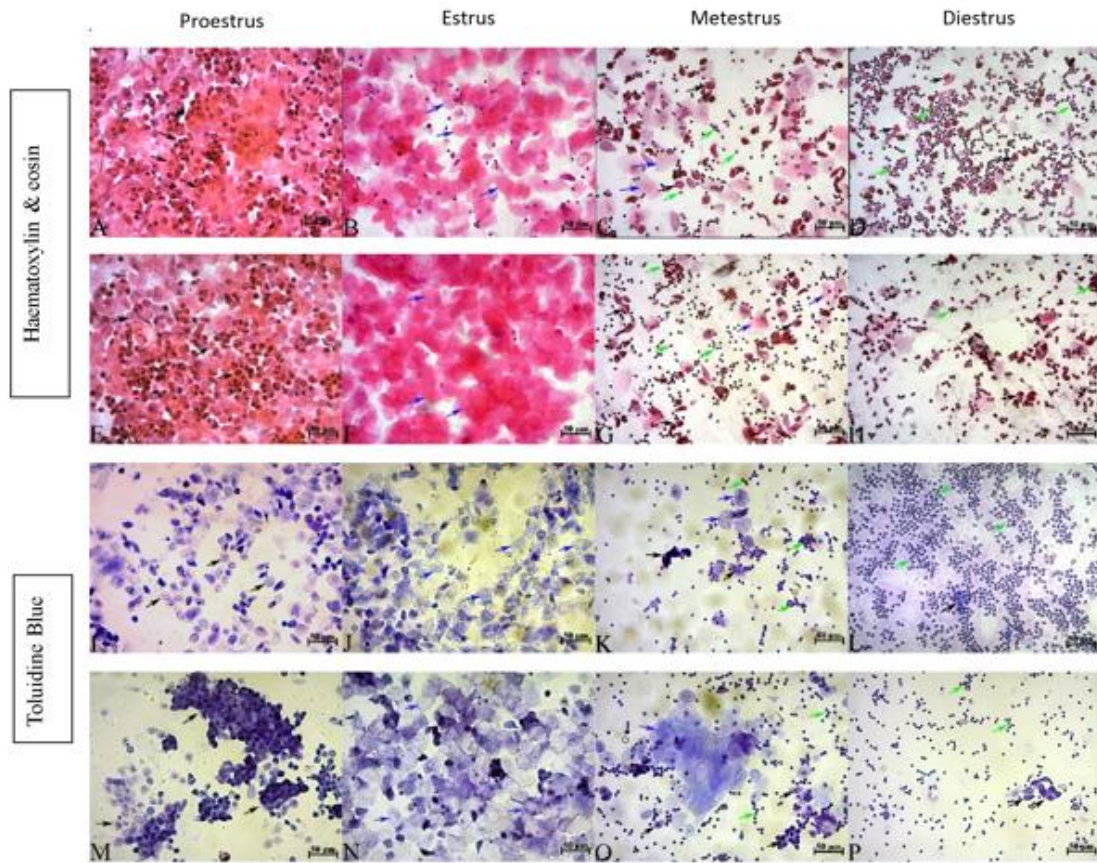


Figure 3. A, B, C, D, I, J, K, L; control, E, F, G, H, M, N, O, P; crocin. Black arrow: Nucleated Epithelial Cells, blue arrow: cornified epithelial cells, green arrow: neutrophils. Scale bar: 50 μ m. (Ramazan ŞİMŞEK Archive).

4. Discussion

The World Health Organization (WHO) asserts that traditional medicine, encompassing practices derived from plants, minerals, and animals, is utilized either as a standalone modality or in conjunction with other therapeutic approaches for the management of diseases and the promotion of health and well-being (Hoenders et al., 2024). According to the WHO, approximately 80 percent of the global population uses traditional medicine for their health care needs. (Kamboj, 2008). In many developing countries, traditional medicine is regarded as a complementary aspect of modern medical practice, with numerous natural remedies having been used for centuries in Asia. A scientific framework is needed to integrate such remedies into modern medical care (Agarwal et al., 2007). Numerous studies have demonstrated the successful use of conventional treatments together with traditional therapies, and have provided evidence of their benefit through modern approaches.

Environmental chemicals, genetic factors, and the side effects of cancer treatments can induce various histopathological changes and hormonal regulation disorders in the female reproductive organs, which may impact the estrous cycle and lead to infertility (Pan et al., 2024) Environmental pollutants and chemicals have the

potential to adversely affect folliculogenesis, oogenesis, female reproductive hormones, and the regulation of oxidative stress, thereby playing a significant role in the pathogenesis of female infertility (Yao et al., 2023).

This review focuses on the potent antioxidant, anti-apoptotic, and anti-inflammatory properties of saffron extract and its key component, crocin, and their effects on hormone regulation and the female reproductive system in experimental and clinical studies. Specifically, it examines the effects of crocin on the female reproductive system, including its influence on estrous cycle length, oocyte maturation and antioxidant activity in embryo cultures. It also sheds light on crocin's potential to mitigate the side effects of cancer treatments, diabetes, gestational hypertension, endometriosis and PCOS (Table 2). However, the teratogenic impact of crocin on embryos and developing foetuses has not yet been scientifically investigated in detail. Therefore, further in vivo and in vitro research is necessary to ensure its safety for human use and to gain a more sophisticated understanding of its potential effects on fertility and fetal development.

Table 2. Effects of crocin on the female reproductive system

Study type	Inducer	Inducer type	Crocin treatment dose	Duration	Effect of crocin	Referenses
<i>In vitro</i> / Mouse Oosit	No	No	50, 100 and 400 µg/mL Crocin	6 days	IVM ↑ IVF ↑	Maleki et al., 2014
<i>In vitro</i> / Swine Oosit	No	No	0, 300, 400 veya 500 µg/mL Crocin	22 hours	IVM ↑ IVF ↑ SOD ↑ CAT ↑ GSH ↑ GPx ↑ Bcl-2/BAX ↑ Caspase-3 ↓	Chen et al., 2019
<i>In vivo</i> / Female Pregnant Rat	No	No	150, 300 and 600 mg/kg Crocin (Oral)	22 Day	FSH ↑ LH ↑ progesterone ↓ Number of fetuses ↓	Nili- Ahmadabadi et al., 2022
<i>In vivo</i> / Female Rat	No	No	100-200 mg/kg/ every other day Crocin (IP)	30 days	Atretic follicles ↓ E ₂ ↓ Progesterone ↓	Zohrabi et al., 2018
<i>In vivo</i> / Female Rat	Letrazole	PCOS	50 and 100 mg/kg Crocin (IP)	28 days	FSH ↑ LH ↓ Testosterone ↓ E ₂ ↓ Atretic follicles ↓ Cystic follicles ↓ Developing Follicles ↑	Sadoughi et al, 2017
<i>In vivo</i> / Female Rat	Letrazole	PCOS	50 mg/kg/day Crocin (IP)	46 Days	FSH ↓ LH ↓ Testosterone ↓ E ₂ ↑ Atretic follicles ↓ Cystic follicles ↓	Elham et al., 2019
<i>In vivo</i> / Women	Patients wiht PCOS	No	15 mg / day Crocin (Oral)	90 days	IL-6 ↓ TNF-α ↓	Rahimi et al., 2022
<i>In vivo</i> / Female Mice	Cyclophosphamide	Anticancer Medicine	200 mg/kg/day Crocin (IP)	21 days	MDA TAC ↑ SOD ↑ LH ↑ FSH ↑ E ₂ ↑ Number of follicles ↑ Mast cells ↓	Khanmohammadi et al., 2021
<i>In vivo</i> / Female Rat	Cyclophosphamide	Anticancer Medicine	100, and 200 mg/kg,Crocin (IP)		Atretic follicles ↓ Primordial follicles ↑ Primary follicles ↑	Shams et al., 2024
<i>In vivo</i> / Female Rat	L-NAME	Gestational Hypertension	50, 100, and 200 mg/kg/day,Cro cin (Oral)	10 days	sFlt-1/PlGF ↓ IL-1β ↓ IL-6 ↓, TNF-α ↓ MDA ↓ H ₂ O ₂ ↓ SOD ↑ CAT ↑ GPx ↑ GSH ↑ Nrf-2 ↑ HO-1 ↑ Fetal survival rates ↑ Fetal weight ↑	Chen et al., 2020
<i>In vivo</i> / Female Mice	Estradiol benzoate	Endometriosis	25 mg/kg Crocin (IP)	21 days	VEGF ↓ PCNA ↓ TNF-α ↓ IL- 6 ↓ IFN-γ ↓	Liu et al. 2017
<i>In vivo</i> / Female Rat	No	No	1g/Kg Saffron extract (Oral)	80 Days	Irregular estrous cycle	Bashir et al., 2022
<i>In vivo</i> / Female Mice	DHT	PCOS	40 mg/kg Crocin (Oral)	30 Days	Cured prolonged diestrus phase	Hu et al.,2018
<i>In vivo</i> / Female Rat	Letrazole	PCOS	200 mg/kg Safranlal	21 days	Cured Irregular estrous cycles	Cellat et al., 2024

↓= Statistically significant decrease, ↑= Statistically significant increase, Bcl-2/BAX= B-cell lymphoma 2 / Bcl-2 associated X protein, CAT= catalase, DHT= dihydrotestosterone, E₂= estradiol, FSH= follicle-stimulating hormone, GPx= glutathione peroxidase, GSH= glutathione, HO-1= heme oxygenase-1, H₂O₂= hydrogen peroxide, IFN-γ= interferon gamma, IL-1β= interleukin-1 beta, IL-6= interleukin-6, IVF= in vitro fertilisation, IVM= in vitro oocyte maturation, L-NAME= Nω-Nitro-L-arginine methyl ester, LH= luteinizing hormone, MDA= malondialdehyde, Nrf-2= nuclear factor erythroid 2-related factor 2, PCNA= proliferating cell nuclear antigen, PCOS= polycystic ovary syndrome, SOD= superoxide dismutase, sFlt-1/PlGF= soluble fms-like tyrosine kinase-1 / placental growth factor, TAC= total antioxidant capacity, TNF-α= tumor necrosis factor-alpha, VEGF= vascular endothelial growth factor.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	R.Ş	M.A.F	M.K
C	40	30	30
D	50	25	25
S	50	25	25
DCP	70	10	20
DAI	70	10	20
L	80	10	10
W	65	10	25
CR	60	20	20
SR	70	15	15

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of interest

The authors declare that they have no conflicts of interest related to this study.

Limitations of the study

The heterogeneity of the included studies, particularly in terms of species (humans, rats, and mice), as well as variations in crocin dosage, duration of administration, and routes of delivery, may limit the external validity and generalizability of the review's findings.

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