



# Protective Effect of Crocin on Ovarian Ischemia-Reperfusion Injury in Rats

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## Abstract

**Background & Aims:** Ischemia-reperfusion (I-R) injury of the ovary may lead to ovarian injury. In this study, we investigated the protective effect of the crocin on ovarian I-R injury.

**Materials & Methods:** Thirty-six male Sprague-Dawley rats were divided into 6 groups: sham surgery, ischemia, I-R, I-R+normal saline (NS), I-R+low dose crocin (20 mg/kg crocin), and I-R+ high dose crocin (80 mg/kg crocin). Neurological function, biochemical and histological evaluation was done 72 hours after ischemia.

**Results:** The plasma levels of malondialdehyde (MDA) and Total Antioxidative Capacity (TAC) in the ischemia, I-R, and I-R+ NS groups increased and decreased significantly compared to the crocin groups, respectively ( $p<0.01$ ,  $p<0.05$ , respectively). Catalase activity in the high dose crocin group was higher than the ischemia, I-R, and I-R+ NS groups ( $p<0.01$ ). The mean scores of edema, congestion, hemorrhage, and follicular degeneration were significantly lower than in the crocin groups than in the ischemia, I-R, and I-R+NS groups ( $p<0.05$ ).

**Conclusion:** Findings suggest that crocin may protect ovary from ischemia-reperfusion injury.

**Keywords:** Crocin, Ischemia, Reperfusion, Ovary, Rat

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## Introduction

Ovarian torsion is the rotation of the ovary around the suspensory ligament containing the ovarian artery (1), and occurs more frequently in women of childbearing age (2). Ovarian torsion may severely reduce or cut off the blood flow to the ovary (ischemia) and ovarian detorsion (during emergency surgery)

causes blood flow (reperfusion). Previous studies showed that ischemia-reperfusion (I-R) injury causes oxidative stress and inflammation in a number of tissues, including the spinal cord (3), brain, heart, kidney, testis (4), renal graft (5), and ovary (6).

In addition to the development of surgical techniques (7), the administration of synthetic and

natural medicines with anti-inflammatory and antioxidant properties, including losartan (8), iloprost (9), sildenafil, and tadalafil (10) are used to reduce ovarian damage after I-R.

Saffron is effective in reducing I-R injury (11). Crocin is one of the effective components of saffron as a carotenoid (12). Numerous studies showed that crocin has anti-inflammatory (13), and antioxidant properties (14). It has a protective effect on hepatic (15), gastric (16), and cerebral I-R injury (17) and acts as a neuroprotective agent (18).

A previous study showed that crocin improves oocytes development during *in vitro* (19). To the best of our knowledge, although crocin has antioxidant and anti-inflammatory features, its effect on ovarian I-R injury has not yet been investigated. In the present study, we examined whether crocin administration can protect rat ovaries from I-R injury.

## Materials and Methods

### Experimental Design:

The animals (male rats, Sprague-Dawley, 200-240 g, n=36) were randomly divided into six groups: Sham surgery, ischemia, I-R, I-R + normal saline (NS), I-R + Low dose crocin (20 mg/kg crocin), and I-R + high dose crocin (80 mg/kg crocin). This study was approved by the ethical committee of Urmia University of Medical Sciences. The animals were kept under a 12-h light cycle (Ethical No: IR.UMSU.REC.1395.591).

### Surgery Procedure:

Rats were anesthetized with ketamine-xylazine (90 and 10 mg/kg, respectively, i.p.), and anesthesia was maintained with continuous or repeated administration of it. Then, under sterile conditions, a longitudinal incision (2.5 cm) was performed in the median line of the lower abdomen.

In the sham surgery group (without induction of I-R injuries), the left ovary was observed, and then the abdominal wall was closed in 3-0 silk sutures.

The I-R procedure was performed as follows: left ovary was rotated 360° in a clockwise direction by the vascular clips and fixed on the abdominal wall with silk suture material (20). At the end of a 3-hour period of

torsion (ischemia), the vascular clips were removed, and a 3-hour period of detorsion (reperfusion) was continued (21).

Thirty minutes before the induction of ischemia, the animals in crocin and NS groups received a single dose of crocin (20 and 80 mg/kg; i.p., Sigma -Aldrich) and NS (1ml; i.p.), respectively.

The estrous stage of each rat was determined by a vaginal smear and cell types were examined under a microscope (staining Papanicolaou) (22).

### Blood Sampling:

At the end of detorsion period, all the animals were anesthetized. The blood samples from each rat were obtained by a direct cardiac puncture, centrifuged at 1500 g for 15 min at +4 °C, and the plasma samples were stored at -80°C until the time of assay for plasma levels of malondialdehyde (MDA), Total Antioxidative Capacity (TAC), and catalase (23).

### Biochemical Measurements:

In accordance with the kit guides, the plasma levels of catalase, MDA, TAC were performed (Zellbio Veltlingweg 29, 89075 Ulm, Germany). Briefly, MDA of plasma was treated with the thiobarbituric, and the pink color was evaluated using spectrophotometer (532 nm). The level of TAC was determined based on the peroxide-peroxidase activity, and changes in the color from pink to yellow were measured using a spectrophotometer (450 nm). In addition, the level of catalase was determined by a spectrophotometer (240 nm) (23).

### Histological Evaluation:

In the experimental groups, the left ovary was removed. After performing the tissue preparation steps (fixed in 10% formalin, embedded in paraffin, cut in 5 µm) the samples were stained with hematoxylin-eosin (H&E). All the sections were examined to determine the ovary tissue damage (interstitial edema, congestion or vascular dilation, hemorrhage, and degenerated follicles), and were ranked as follows 0: none; 1: less than 33%; 2: 33-66%; 3: >66%). The follicles that contained an oocyte with a pyknotic nucleus and shrunken ooplasm were considered to be degenerated follicles (22).

### Statistical Analysis:

Statistical analysis was done by one-way ANOVA, and followed by Tukey's post hoc test. Tissue damage scores were analyzed by the Kruskal-Wallis, and followed by Mann-Whitney test. Data were expressed as means  $\pm$  standard error and statistical significance was defined as  $p < 0.05$ .

### Results

All animals survived the test. In the macroscopic study, all ovaries (in the experimental groups) had a hemorrhagic appearance.

The plasma level of MDA increased significantly in ischemia, I-R, and I-R+ NS groups, compared to the crocin and sham groups ( $p < 0.01$ ). This data shows that 20 and 80 mg/kg doses of crocin reduced the plasma level of MDA to  $9.81 \pm 1.41$ , and  $8.12 \pm 0.94$ , respectively. There was no significant difference between the crocin groups in plasma concentrations of MDA ( $p > 0.05$ ) (Fig. 1).

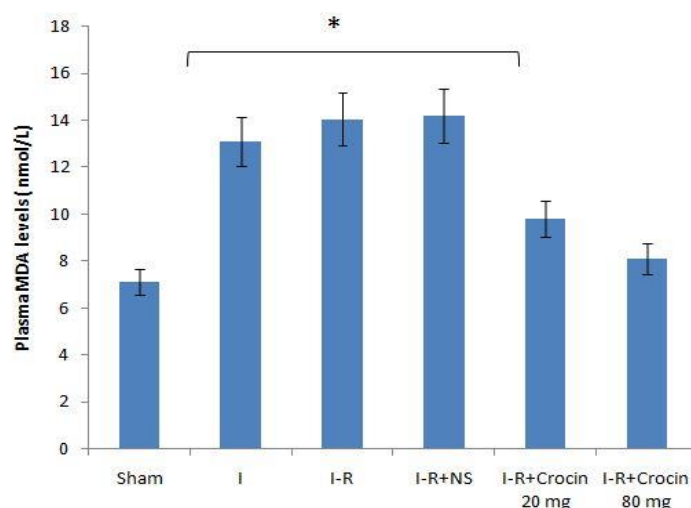
Finding from the ischemia, I-R, and I-R+NS groups showed a significant decrease in plasma level of TAC, when compared with crocin groups ( $p < 0.05$ ). In addition, plasma level of TAC increased significantly in crocin (80 mg) than in crocin (20 mg) ( $p < 0.01$ ) (Fig. 2).

Catalase activity was significantly higher in the crocin (80 mg) group than in the ischemia, I-R, and I-R+NS groups ( $p < 0.01$ ). The effect of a 20 mg/kg dose of crocin was not significantly increased in plasma level of catalase in comparison with the ischemia, I-R, and I-R+NS groups ( $p > 0.05$ ) (Fig. 3).

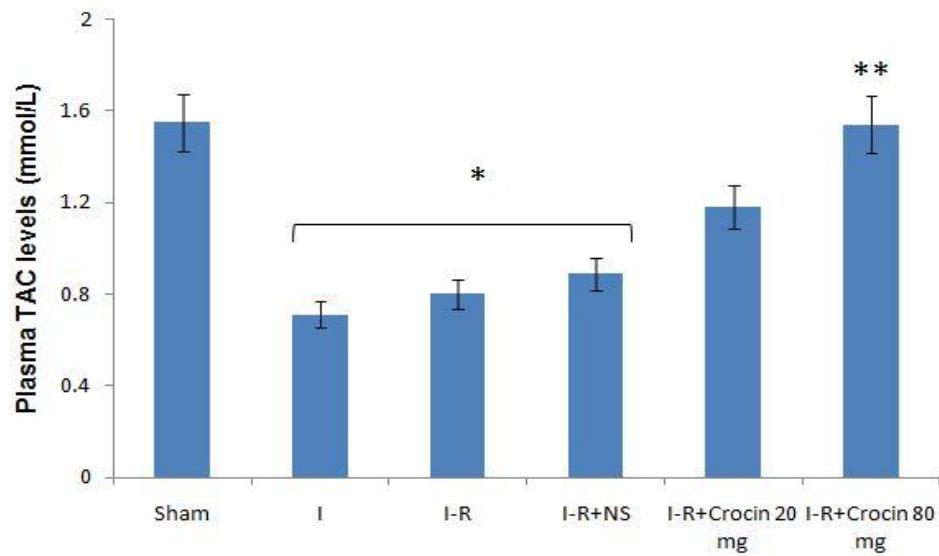
The light microscopy showed a degree of edema, congestion, hemorrhage, and degenerated follicles in the experimental groups. In the sham surgery group, the ovaries had a histologically normal appearance without hemorrhage, and congestion (Fig. 4).

The mean scores of edema, congestion, and hemorrhage were significantly lower in the crocin groups than in the ischemia, I-R, and I-R+NS groups ( $p < 0.01$ ). In addition, the mean scores of follicular degeneration were significantly lower in the crocin groups than in the ischemia, I-R, I-R+NS groups ( $p < 0.05$ ). Besides, the mean score of follicular degeneration was significantly lower in the high crocin group than in the low crocin group ( $p < 0.05$ ).

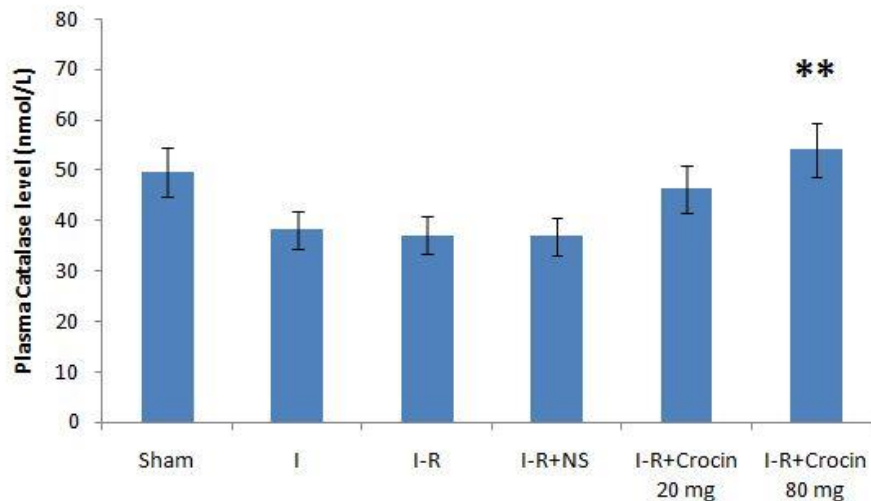
However, approximately 33%, 53%, and 62% of follicular??? were lost in ischemia, I-R, and I-R+NS groups, respectively. This amount was only 16% and 25% in high and low crocin groups, respectively (Table 1).



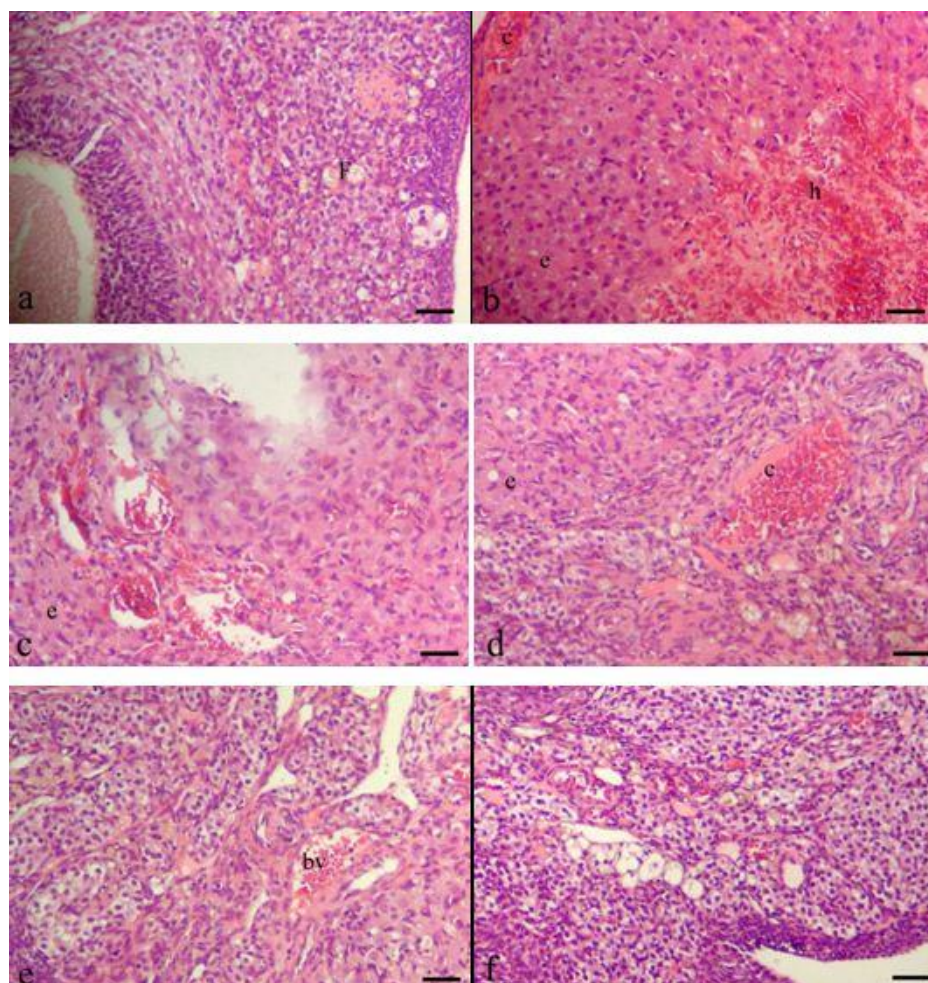
**Fig. 1.** The mean plasma level of Malondialdehyde (MDA) determined at 72 hours after ovarian ischemia reperfusion injury. \* demonstrated a significant difference ischemia, I-R, and I-R+NS groups in comparison with crocin groups ( $p < 0.01$ ).



**Fig. 2.** The mean plasma level of Total Antioxidative Capacity (TAC) determined at 72 hours after ovarian ischemia reperfusion injury. \* demonstrated a significant difference ischemia, I-R, and I-R+NS groups in comparison with crocin groups ( $p < 0.05$ ). \*\* demonstrated a significant difference crocin (80 mg/kg) group in comparison with crocin (20 mg/kg) group ( $p < 0.01$ ).



**Fig. 3.** The mean plasma level of Catalase determined at 72 hours after ovarian ischemia reperfusion injury. \*\* demonstrated a significant difference crocin (80 mg/kg) group in comparison with ischemia, I-R, and I-R+NS groups ( $p < 0.05$ ).



**Fig. 4.** Light microphotographs of ovary at 72 hours after ischemia reperfusion injury. sham surgery (a), ischemia (b), I-R (c), I-R+NS (d), I-R+Crocin 20 mg (e), I-R+Crocin 80 mg (f). f: follicles, C:congestion, e:edema, h:hemorrhage, fd:follicular degeneration, bv:blood vessel (H-E staining, Scale bar =100  $\mu$ m).

**Table 1.** Histopathological scores, 72 hours after ovarian ischemia reperfusion injury

Groups	Edema	Vascular Congestion	Hemorrhage	Follicular Degeneration
Sham Surgery	0.33±0.17	0.16±0.09	0.16±0.07	0.11±0.08
Ischemia	2.37±0.46	2.12±0.31	2.25±0.34	0.63±0.19
I-R	2.97±0.25	1.83±0.24	2.1±0.28	0.93±0.32
I-R+ NS	2.19±3.4	1.88±0.31	2.27±0.24	0.89±0.31
I-R+ Crocin (20 mg)	1.33±0.3*	1.24±0.16*	1.5±0.37*	0.25±0.11*
I-R+ Crocin (80 mg)	0.53±0.19*	1.1±0.15*	1.21±0.34*	0.16±0.07*, #

\*Difference between crocin 80 mg group and crocin 20 mg group (P<0.05).

\*Difference between crocin groups and ischemia, I-R, I-R+NS groups (P<0.01).

## Discussion

Our results showed that crocin had a protective effect on ovarian I-R injury 72 h after ischemia. The present study is the first report describing the protective effect of crocin on I-R of ovary in rats.

Similar to the previous study (24), our results show that in the I-R groups, the biochemical analysis showed that TAC and CAT levels were significantly higher and MDA levels were significantly lower in crocin groups (especially in the crocin 80 mg group) compared to the I-R groups. Previous study showed that crocin plays a protective effect on gastric mucosal I-R injury (25). So, crocin decreased MDA, and increased the mRNA expression of superoxide dismutase (SOD) and glutathione peroxidase (Gpx) (25). In addition, our findings showed that MDA levels were higher in I-R groups compared to the ischemia group. In the reperfusion stage, free oxygen radicals react with lipids and lipid peroxides are formed (24). Sefidgar et al. (2019) showed that administration of crocin increased the plasma level of TAC in diabetic rats (26). On the other hand, the enzyme catalase has a great effect on tissue protection against reactive oxygen species (ROS) (27). It seems that antioxidant properties of crocin (28) may protect the ovaries from I-R injury by reducing free radicals.

Our findings revealed that crocin therapy (especially 80 mg) compared to NS protects ovarian tissue from I-R injury. The ovarian torsion blocks the ovarian vessels and prevents the return of venous blood, which in turn causes edema and internal hemorrhage (22). Reperfusion injury increased many factors including inflammation and neutrophil accumulation (29). The administration of crocin significantly decreased edema, hemorrhaging, vascular congestion, and follicular degeneration. A previous study observed that crocin can inhibit inflammation in the I-R injury (28).

We found that ischemia for 3 h led to histopathological changes in ovaries similar to the findings of Tunc et al. (2016) (24). Following long-term ischemia, the improper microvascular function is observed in the reperfusion stage (30). On the

reperfusion stage and after infiltrating neutrophils, reactive oxygen species (ROS) are produced in large quantities (31). However, crocin causes ROS inhibition. So that the ROS block is considered a therapeutic approach (32). In addition, the previous study showed that ROS plays a key role in myocardial healing by clearance of dead cells (31).

In the present study, the mean number of degenerated follicles in the crocin groups was lower than the ischemia, I-R, I-R+NS groups. A previous study showed that honokiol, which has antioxidant and anti-inflammatory effects, reduces cell degeneration (24). Administration of crocin can maintain the normal shape and the microscopic architecture of testicular tissue in diabetic rats (26). Crocin can also be considered as an anti-aging agent by inhibiting ROS production and advanced glycation end product expression (33). Thus, it was suggested that antioxidant and anti-inflammatory properties of crocin decrease I-R injury, and increase the survival of follicles in ovarian tissue.

One of the limitations of this study was short-term. Since it is possible to increase the damage of tissue by increasing the time of reperfusion, we believe that it is necessary to study the long-term effect of crocin on the I-R injury of ovarian.

Our findings are in agreement with a previous study that crocin with a dose of 80 mg/kg has more protective effect than a dose of 20 mg/kg (18). So that the mean scores of edema, congestion, and hemorrhage were significantly lower in the crocin (80 mg/kg) group than in the crocin (20 mg/kg) group. Crocin may attenuate cellular apoptosis by reducing oxidative stress and inflammation (18), and reduce brain damage after ischemia (34).

## Conclusion

According to the histological and biochemical results of this study, it is suggested that crocin may have a protective effect on ovarian ischemia-reperfusion injuries. While the anti-inflammatory and antioxidant activity of crocin is known, the effect of crocin on ovarian tissue may be multifactorial. Therefore, further

studies are needed to investigate the molecular mechanism of crocin on I-R of the ovary.

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