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## ORIGINAL ARTICLE

### Effect of Systemic Administration of Curcumin on Ischemia-Reperfusion Injury in Ovaries: An Animal Model Study

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#### Keywords:

Ischemia-reperfusion;  
Curcumin;  
Intraperitoneal;  
Ovary.

#### Abstract

**Objective-** Ovarian torsion must be diagnosed and treated as much early as possible. The aim of the present study was to investigate effects of intraperitoneal administration of curcumin on ischemia-reperfusion injury in ovaries.

**Design-** Experimental Study.

**Animals-** Twenty-four healthy female Wistar rats.

**Procedures-** Twenty-four healthy female Wistar rats weighing approximately 260g were randomized into four experimental groups (n = 6): Group Sham: The rats underwent only laparotomy. Group I: A 3- hour ischemia only. Group I/R: A 3-hour ischemia and a 3-hour reperfusion. Group I/R/C: A 3-hour ischemia, a 3-hour reperfusion and 1 mg/kg intraperitoneal administration of curcumin 2.5 hours after induction of ischemia.

**Results-** Curcumin treated animals showed significantly ameliorated development of ischemia and reperfusion tissue injury compared to those of other groups ( $p < 0.05$ ). The significant higher values of SOD, GPO and GST were observed in I/R/C animals compared to those of other groups ( $p < 0.05$ ). The damage indicators (MDA) was significantly lower in I/R/C animal compared to those of other groups ( $p < 0.05$ ).

**Conclusion and clinical relevance-** Intraperitoneal administration of curcumin could be helpful in minimizing ischemia-reperfusion injury in ovarian tissue exposed to ischemia.

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## 1. Introduction

There are various conditions like long mesovarium and adnexal venous congestion that could result in torsion of ovary and subsequently obstruction of the ovarian vessels. This causes a life-threatening reduction in tissue blood flow and permanent tissue damage.<sup>1</sup> Therefore, ovarian torsion must be diagnosed and treated as much early as possible to preserve ovarian functions and prevent future infertility.<sup>2</sup> Upon detection of ovarian torsion, detorsion of the twisted adnexa and evaluation the tissue reperfusion is proposed to prevent future infertility even in case of cyanotic tissues.<sup>3</sup> This ovarian torsion-detorsion process is named as ischemia- reperfusion injury.<sup>4</sup>

Reperfusion of the ischemic tissue leads to much more serious damage to the tissue than the damage caused by ischemia.<sup>5</sup> Reperfusion-related damage in the cell is created by many factors, mostly including oxygen-derived free radicals, which are rapidly generated in the tissue as a result of reperfusion due to physiological or pathological alterations, oxidative damage takes place with changes in favor of the oxidation process.<sup>6</sup> Prompt diagnosis to reduce ischemic and reperfusion injury, and its consequents are still inevitable with this approach. Therefore, studies on preventing reperfusion injury seem very important.<sup>7</sup>

A proposed pathogenesis of tissue injury during reperfusion is accumulation of the activated neutrophils that release reactive oxygen species.<sup>8</sup> Lipid peroxidation in the cell is the most deleterious effects of free radicals that end up reduction in the membrane potential and subsequently, cell injury. Malondialdehyde (MDA), one of the end products of lipid peroxidation, also results in serious cell damage through induction of polymerization and cross linking in membrane components.<sup>9</sup> In spite of the fact that generation of free oxygen radicals occurs continuously in cells, the presence of endogenous antioxidant defense systems preserves tissues from the harmful effects of free oxygen radicals.<sup>10</sup> Various agents, anti-inflammatory and antioxidant free radical scavengers have been reported with promising beneficial effects on prevention of ischemic/reperfusion injuries in tissues.<sup>11</sup>

Curcumin is the main phenolic pigment extracted from turmeric, the powdered rhizome of *Curcuma longa*, along with demethoxy curcumin and bisdemethoxy curcumin.<sup>12</sup> Extensive research indicates that curcumin possesses potent antioxidant, anti-inflammatory, properties, and it also inhibits lipid peroxidation and scavenges superoxide anion, singlet oxygen, nitric oxide, and hydroxyl radicals.<sup>13</sup> The present study was different from the other studies in the literature for using curcumin on ischemia/reperfusion injury. Aimed to study peritoneal effects of curcumin on

ischemia/reperfusion injury, a study was designed to determine if curcumin could protect against ischemia/reperfusion induced ovarian damage.

## 2. Materials and Methods

### *Study design and animals*

Two weeks before and during the experiments, the animals were housed in individual plastic cages with an ambient temperature of  $(23\pm 3)^{\circ}\text{C}$ , stable air humidity and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water. All measurements were made by two blinded observers unaware of the analyzed groups.

The present study was designed and modified based on a method described by Oral *et al.* (2010).<sup>9</sup> Thirty five healthy female Wistar rats weighing approximately 260g were randomized into four experimental groups (n = 6): Group Sham: The rats underwent only laparotomy. Group I: A 3-hour ischemia only. Group I/R: A 3-hour ischemia and a 3-hour reperfusion. Group I/R/C: A 3-hour ischemia, a 3-hour reperfusion and 1 mg/kg intraperitoneal administration of curcumin 2.5 hours after induction of ischemia. The right ovaries were transferred to a 10% formaldehyde solution for histopathological assessments and the left ovaries were cleaned of surrounding soft tissues and then stored in a freezer at  $-80^{\circ}\text{C}$  for biochemical assessments.

### *Surgical procedure*

Animals were anesthetized by intraperitoneal administration of ketamine-xylazine (ketamine 5%, 90 mg/kg and xylazine 2%, 5 mg/kg). The procedure was carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain.<sup>14</sup> The ethical Committee of the Urmia University of Medical Sciences approved all the experiments. A longitudinal midline incision was made in the lower abdomen and the uterine horns and adnexa were exposed. For induction of ischemia, a vascular clamp was applied on vessels of the ovaries in rats. After a 3-hour period of ischemia, both ovaries were surgically dissected out for histopathological and biochemical assessments. For induction of ischemia/reperfusion, both ovaries underwent ischemia the same way and at the end of a 3-hour period, the vascular clamps were chosen, removed and a 3-hour reperfusion was continued. Then, the ovaries were dissected out for histopathological and biochemical assessments.

## Biochemistry

The tissue samples of ovaries were kept at  $-80^{\circ}\text{C}$  for 3 days, and then enzyme activities were determined in rat ovary tissues. The ovary tissues were ground with liquid nitrogen in a mortar. One half gram was weighed for each group and then treated with 4.5 mL of an appropriate buffer. This mixture was homogenized on ice with use of an ultra-turrax homogenizer (IKA, Werke, Germany) for 15 minutes. Homogenates were filtered and centrifuged by using a refrigerator centrifuge at  $4^{\circ}\text{C}$ . Then the supernatants were used to determine the enzymatic activities. All assays were carried out at room temperature.

### Superoxide dismutase (SOD) analysis

Superoxide dismutase estimation was based on the generation of superoxide radicals produced by xanthine and the xanthine oxidase system, which reacts with nitroblue tetrazolium to form formazan dye.<sup>15</sup> Superoxide dismutase activity was then measured at 560 nm by the degree of inhibition of this reaction and is expressed as millimoles per minute per milligram of tissue.

### Malondialdehyde (MDA) analysis

Concentrations of ovarian lipid peroxidation were determined by estimating MDA using the thiobarbituric acid test.<sup>16</sup> The rat ovaries were rinsed with cold saline. The corpus mucosa was scraped, weighed, and homogenized in 10 ml of 100 g/l KCl. The homogenate (0.5 ml) was added to a solution containing 2-thiobarbiturate (1.5 ml of 8 g/l), acetic acid (1.5 ml of 200 g/l), sodium lauryl sulfate (0.2 ml of 80 g/l), and distilled water (0.3 ml). The mixture was incubated at  $98^{\circ}\text{C}$  for 1 hr. n-butanol:pyridine 5 ml (ratio:15:1) was then added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4000 rpm. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane.

### Glutathione peroxidase (GPO) analysis

GPO activity was determined according to the method of Lawrence and Burk.<sup>17</sup> After tissue homogenization, supernatant was used for GPO measurement. Following the addition of  $\text{KH}_2\text{PO}_4$ , EDTA, GSH, B-NADPH,  $\text{NaN}_3$ , and GR, the mixture was incubated. As soon  $\text{H}_2\text{O}_2$  was added the chronometer was turned on and the absorbance at 340 nm was recorded for 5 min every 15 sec.

## Glutathione S-transferase (GST) activity

GST activity was determined by Habig and Jakoby.<sup>18</sup> Enzyme activity was determined in a 4-ml cuvette containing 30 mM GSH, 30 mM 1-chloro-2,6-dinitrobenzene, 0.1 M PBS (pH: 6.5), and tissue homogenate at 340 nm using a spectrophotometer.

## Histopathology

Ovaries were fixed in 10% buffered formalin for 24 hours. The tissue samples were then processed and embedded in paraffin. A 5- $\mu\text{m}$  semi-thin section was paraffin-embedded. The samples were then dewaxed, rehydrated and stained routinely with hematoxylin and eosin. The sections were then observed under a light photomicroscope. For semi thin sections, ovaries were fixed in 2.5% buffered glutaraldehyde nad post fixed in 2%  $\text{OsO}_4$  for 2 h, dehydrated through an ethanol series and embedded in epon. Semi thin transverse sections (5  $\mu\text{m}$ ) were next stained with toluidine blue and examined under a light microscope.

## Statistical Analysis

Experimental results were expressed as means  $\pm$  SD. Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA). Model assumptions were evaluated by examining the residual plot. Results were analyzed using repeated measures and a factorial ANOVA with two between-subject factors. Bonferroni test for pairwise comparisons was used to examine the effect of time and treatments. The differences were considered significant when  $p < 0.05$ .

## 3. Results

### Biochemistry

#### Superoxide dismutase (SOD) analysis

The value of SOD activity was  $69.5 \pm 0.57$  mmol/min/mg tissue in the Sham group. The values of SOD were decreased to  $35.8 \pm 0.22$  and  $57.2 \pm 0.21$  mmol/min/mg tissue in I and I/R groups, respectively. However, intraperitoneal administration of 1 mg/kg of curcumin inverted the trend and increased the activity of SOD in the ovarian tissue in I/R/C group. The value of SOD activity in I/R/C group was significantly higher than those of the other experimental groups ( $p < 0.05$ ) (Figure 1).

### Malondialdehyde (MDA) analysis

The results of the present study showed that concentration of MDA in Sham group was  $5.7 \pm 0.19$   $\mu\text{mol/g}$  protein in ovarian tissue. The MDA level I/R group was significantly increased to  $11.6 \pm 0.23$   $\mu\text{mol/g}$  protein ( $p < 0.01$ ). Intraperitoneal administration of curcumin significantly decreased level of MDA in ovarian tissues of I/R/C animals ( $p < 0.05$ ) (Figure 1).

### Myeloperoxidase (MPO) analysis

The level of MPO was significantly increased in I and I/R groups ( $p < 0.05$ ). Intraperitoneal administration of curcumin reversed the trend and significantly decreased level of MPO in ovarian tissues of I/R/C animals ( $p < 0.05$ ) (Figure 1).

### Glutathione S-transferase (GST) activity

The GST activities in ovarian tissue in the SSG and I/R animals were  $21.6 \pm 1.21$  and  $14.7 \pm 1.38$  u/g protein, respectively. Intraperitoneal administration of curcumin significantly increased level of GST in ovarian tissues of I/R /NC animals ( $p < 0.05$ ) (Figure 1).

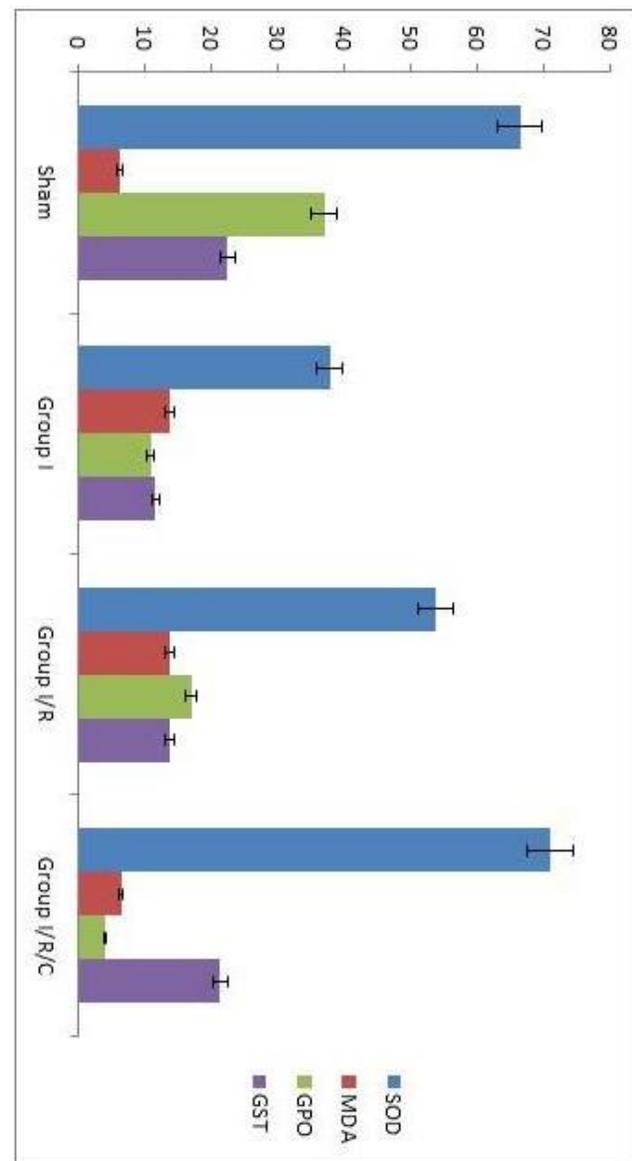
### Histopathology

The histologic design of the ovarian tissue in the SSG animals was normal. Ovarian tissues in the ischemia group showed condensed hemorrhage and severe vascular congestion along with degenerative and necrotic changes in many of the cells. The tissues in the I/R group showed histopathological changes of condensed hemorrhage, infiltration of inflammatory cells along with degenerative and apoptotic cells. Polymorphonuclear leukocytes (neutrophils) were dominant cell types. In I/R/C group general histologic and cellular structures of the tissues were not normal in appearance, however, mild vascular congestion and edema were observed. In I/R/C group only a slightly mild hemorrhage was around ovarian follicles. The general histologic structure of the ovarian tissue in this group was normal and no important pathologic findings in the structural level were observed except for only a slightly mild inflammation, vascular congestion and edema (Figure 2).

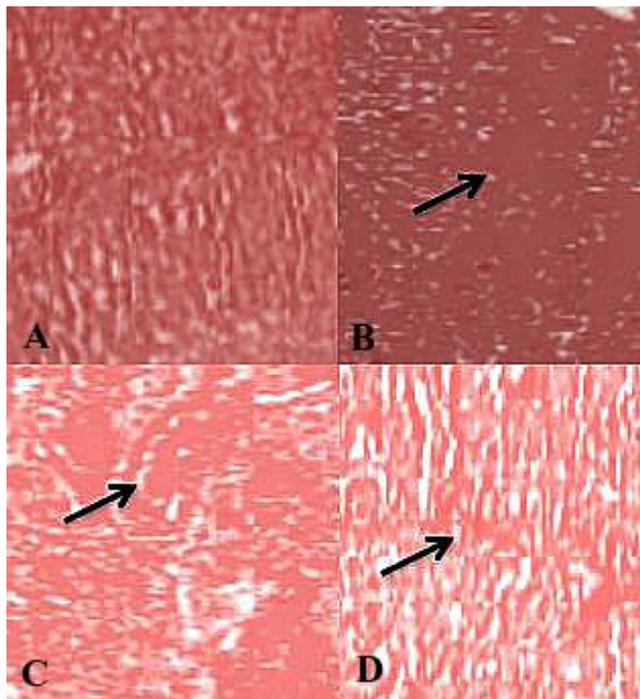
## 4. Discussion

Biochemical and histopathological evaluations were done in sham, ischemia, ischemia-reperfusion-controlled plus IP

administration of curcumin groups. Biochemically, the activities of SOD, MDA, GPO and GST were assessed in the ovarian tissues of the animals of the all experimental groups. In the present study, levels of SOD in ovarian tissue were assessed and compared in all the experimental groups. SOD is an antioxidant enzyme that catalyzes the conversion of superoxide free radical into hydrogen peroxide and molecular oxygen. SOD and endogenous antioxidant enzymes neutralize free radicals and protect tissues from the harmful effects of free radicals and active oxygen species.<sup>19</sup> Our results showed that in the I/R/C animals, SOD was increased compared to that in I and I/R groups and interaperitoneal administration of curcumin, secured ovarian tissue against ischemia-reperfusion injury.



**Figure 1.** Barograph shows comparison of the activities of SOD, MDA, GPO and GST in the ovarian tissues of the animals of the all experimental groups.



**Figure 2.** Histologic micrographs of the ovarian tissue in Sham (A), I (B), I/R (C), I/R/C (D), groups. Micrograph B shows condensed hemorrhage and severe vascular congestion (arrow), and severe edema (arrowhead). Micrograph C shows condensed hemorrhage and vascular congestion (arrow), and edema (arrowhead). Micrograph D shows slightly mild vascular congestion and edema (arrows). Scale bar: 200  $\mu$ m

MDA is a lipid peroxidation product and occurs as a result of the peroxidation of fatty acids that contain three or more double bonds. MDA causes cross-linking of membrane components and leads to negative consequences like changes in ion permeability and enzyme activity via affecting the ion exchange through the cell membranes.<sup>20,21</sup> MDA levels in the present study were found to be much lower in the I/R/C animals compared to those in other experimental groups. This could protect the tissues against ischemia-reperfusion injury in curcumin treated animals.

GPO activity is significantly reduced in tissues undergoing oxidative stress-related conditions like ischemia-reperfusion injury.<sup>22</sup> GPO detoxifies the hydrogen peroxide radical that forms in the cell by converting it to water and prevents the formation of more toxic products from hydrogen peroxide radical.<sup>23</sup> In the present study a significant decrease in GPO activity was observed in ovarian tissues of I/R/C animals.

GST binds foreign substances to the -SH group of cysteine in glutathione, neutralizes the electrophilic regions and protects the cells from the harmful effects of foreign substance regions.<sup>24</sup> Activity of GST has been reported to be suppressed in oxidative tissue injury induced by ischemia.<sup>24</sup> Consistently, our findings showed that GST

activity in ovarian tissue of curcumin treated animals was significantly lower than those in I and I/R groups.

Ischemia, ischemia-reperfusion and intraperitoneal curcumin applied to tissues were analyzed histopathologically. Results showed that oxidative stress level followed a parallelism with the tissue damage. Edema, vascular congestion, hemorrhages, and leukocyte infiltration have been used as histopathological parameters in the evaluation of the condition of the cell.<sup>25</sup> Edema, vascular congestion, hemorrhage, and leukocyte infiltration in the I/R/C animals were milder than in the I/R group.

There are many studies in the literature about the improvement of ischemia reperfusion injury. Studies demonstrated that the agents with antioxidant or anti-inflammatory activities may be beneficial in reducing ovarian ischemia reperfusion injury. Also, studies revealed the beneficial effect of controlled reperfusion in the prevention of ovarian tissue damage. Although there are many studies in the literature; ischemia/reperfusion damage continues to be a serious problem clinically. Essentially, early diagnosis and treatment of ovarian torsion plays an important role to provide urgent protection against life-threatening complications from ischemia and to prevent future infertility.<sup>26</sup>

Curcumin has been reported as a useful agent both for the prevention and treatment of I/R injury in many organs.<sup>27</sup> These protective effects are mainly believed to be based on inhibitory actions of curcumin on disease-mediated induction of inflammatory transcription factors, protein kinases, adhesion molecules, oxidative stress and inflammation.<sup>27</sup> The administration of curcumin has reported to reduce the generation of reactive oxygen species (ROS), monocyte adhesion, phosphorylation of c-Jun N-terminal kinase (JNK), p38 MAP kinase, and signal transducer and activator of transcription (STAT)-3 in TNF- $\alpha$ -stimulated cells.<sup>27</sup> The comparison between the oxidative status and antioxidative status is clear enough to suggest that the administration of curcumin, as reported previously, leads to a decrease in the oxidative stress and an increase in the antioxidation.<sup>27</sup>

Substances are administered by a wide variety of routes. A key factor determining the route selected is whether the agent is being administered for a local or systemic (either enteral or parenteral) effect. Parenteral administration methods typically produce the highest bioavailability of substances because these methods avoid the first-pass effect of hepatic metabolism, which occurs commonly with orally administered chemicals and therapeutics.<sup>28</sup> Systemic administration seems more effective and available where oral administration of an agent may cause difficulties. It is clear that transperitoneal absorption of the agent is far

faster than oral administration.<sup>28</sup> It seems time saving is very important in emergency conditions like ovarian torsion.

In conclusion, histopathological results obtained from all the experimental groups were consistent with the results of the biochemical analyses indicating that intraperitoneal administration of curcumin could be helpful in minimizing ischemia-reperfusion injury in ovarian tissue exposed to ischemia.

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## Conflict of Interests

None.

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چکیده

تأثیر تجویز سیستمیک کورکومین بر روی جراحات ایسکمی/خون‌رسانی مجدد در تخمدان‌ها: مطالعه مدل حیوانی

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**هدف-** چرخش تخمدان می‌بایست در زودترین زمان ممکن تشخیص داده شده و درمان شود. هدف از مطالعه حاضر بررسی تأثیر تجویز سیستمیک کورکومین بر روی جراحات ایسکمی/خون‌رسانی مجدد در تخمدان‌ها در موش صحرایی بود.

**طرح-** مطالعه تجربی.

**حیوانات-** ۲۴ موش صحرایی ماده ویستار سالم.

**روش کار-** در این مطالعه ۲۴ قطعه رت بالغ ماده ویستار سالم به وزن تقریبی ۲۶۰ گرم به‌طور تصادفی به ۴ گروه ۶ تایی تقسیم‌بندی شدند. گروه ۱ (گروه شم): در این گروه برش خط وسط ایجاد شده و تخمدان‌ها دستکاری شده و محل برش بسته شد. گروه ۲ (گروه ایسکمی): در این گروه به مدت ۳ ساعت ایسکمی تخمدانی القا شد. ۲۰ میکرو لیتر روغن سویا (حلال) به‌طور داخل صفاقی تزریق شد. گروه ۳ (گروه ایسکمی/خون‌رسانی مجدد): در این گروه ۳ ساعت ایسکمی و ۳ ساعت خون‌رسانی مجدد ایجاد شد. نیم ساعت مانده به پایان ایسکمی ۲۰ میکرو لیتر روغن سویا (حلال) به‌طور داخل صفاقی تزریق شد. گروه ۴ (گروه ایسکمی/خون‌رسانی مجدد/کورکومین): در این گروه ۳ ساعت ایسکمی و ۳ ساعت خون‌رسانی مجدد ایجاد شد و نیم ساعت مانده به پایان ایسکمی ۲۰ میکرو لیتر کورکومین به‌طور داخل صفاقی تزریق شد.

**نتایج-** درمان با کورکومین به‌طور معناداری سبب بهبود ایسکمی نسبت به سایر گروه‌ها شد ( $p < 0.05$ ). حیوانات درمان شده با کورکومین به شکل معناداری مقادیر بالاتری از SOD، GPO و GST را در مقایسه با سایر گروه‌ها نشان دادند ( $p < 0.05$ ). شاخص آسیب MDA، به شکل معناداری در گروه درمان شده با کورکومین نسبت به سایر گروه‌ها پایین‌تر بود ( $p < 0.05$ ).

**نتیجه‌گیری و کاربرد بالینی-** تجویز داخل صفاقی کورکومین می‌تواند در به حداقل رسانیدن جراحات ایسکمی/خون‌رسانی مجدد در تخمدان‌ها در موش صحرایی در معرض ایسکمی مفید باشد.

**واژه‌های کلیدی:** ایسکمی/خون‌رسانی مجدد، کورکومین، داخل صفاقی، تخمدان