

EXPERIMENTAL STUDY

The ameliorative effect of lutein on ovarian ischemia-reperfusion injury in rats

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ABSTRACT

OBJECTIVES: The aim of our study is to investigate biochemical and histopathological effects of lutein on the ovarian ischemia-reperfusion (I/R) injury in rats.

BACKGROUND: Reactive oxygen species and cytokines have a very important role in the pathogenesis of I/R injury. Lutein and its derivatives may show an anti-inflammatory effect in relation to the decrease in inflammatory cytokines and increase in antioxidant enzymes.

METHODS: Wistar albino female rats were randomly divided into three groups before surgery as follows: I/R group (IRG; n = 6), 1 mg/kg lutein + I/R group (LIRG; n = 6), and a healthy control group scheduled for a sham operation (SG; n = 6). The condition of ovarian ischemia was created by vascular clips. After two hours, the ovary was reperused. Then, cyclooxygenase-1, cyclooxygenase-2, malondialdehyde and total glutathione levels were examined in ovary tissues of rats.

RESULTS: As the results of our study demonstrated, in ovarian tissues of animals after I/R, there was an increase in the levels of malondialdehyde and cyclooxygenase-2, while total glutathione and cyclooxygenase-1 were decreased. At the same time, it has been observed however that these ratios are reversed in the LIRG group ($p < 0.05$).

CONCLUSION: Lutein ameliorates the I/R-induced ovarian injury in rats by its antioxidative and anti-inflammatory activities (Fig. 2, Ref. 39). Text in PDF www.elis.sk.

KEY WORDS: lutein, ischemia-reperfusion, rat, ovary.

Introduction

Ovarian torsion is a common gynecological emergency occurring mostly in women at reproductive age, especially in first three decades (1). Surgical detorsion might be considered to restore the ovarian blood supply and reperuse the ovarian tissue (2). The damage that occurs in the tissue after its reperfusion may sometimes be greater than that caused by ischemia. This process is known as ischemia-reperfusion (I/R) injury (3).

I/R injury happens because of proinflammatory polymorphonuclear leukocytes (PMNLs), neutrophils and thrombocytes which are carried by the blood to the ischemic tissue after reperfusion. The release of reactive oxygen species (ROS) and cytokines are very important factors in I/R injury (4, 5). ROS damage the tissue especially through lipid peroxidation. They affect cellular membrane lipids with lipid peroxidation and lead to the formation of malondialdehyde (MDA), which is a toxic product. MDA can

damage both membrane structure and functions of the cell (6). Some antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) or nonezymatic compounds such as glutathione (GSH), ascorbic acid (AA), and α -tocopherol protect the tissue from oxidative injury (7). The balance of ROS and antioxidants identifies the severity of oxidative stress (8).

Prostaglandins (PGs) play a very important role in the I/R-related inflammation. Cyclooxygenase (COX) is the main enzyme in the synthesis of these PGs (9). Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) are the most common isoenzymes. The purpose of COX-1 is to protect the normal cell activity and cytoprotective activity in tissues. On the other hand, COX-2 rapidly increases in pathological conditions and induces the synthesis of proinflammatory PGs (10).

Lutein is a carotenoid synthesized by plants, bacteria, and algae. It is not synthesized in the body (11). High plasma carotenoid concentrations have been associated with a reduced risk of developing chronic disease (12). Studies have shown that diets high in lutein may be associated with a reduced risk of age-related macular degeneration (13). Also, lutein inhibits proliferation of prostate carcinoma cells in rats (14). However, the effect of lutein on ovarian I/R injury has not been yet reported in literature. Therefore, the target of our study is to investigate the biochemical and histopathological effects of lutein on the ovarian I/R injury in a rat study.

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Materials and methods

Animals

Experimental animals were obtained from Ataturk University, Medical Experimental Application and Research Center. A total of eighteen Wistar albino female rats weighing 237–243 grams were randomly selected to be used in the experiment. Animals were housed and fed at normal room temperature (22 °C) prior to the experiment. The study was conducted at Ataturk University, Experimental Studies and Research Center, Erzurum. The experimental procedure was approved by the Committee for Animal Research of Ataturk University, Erzurum. This study was carried out in accordance with international guidelines on the ethical use of animals (Ethics Committee Number: (04.04.2018/1800109522).

Experimental groups

Rats were randomly divided into three groups before experiment as follows: I/R group (IRG; n = 6), 1 mg/kg lutein + I/R group (LIRG; n = 6), and a healthy control group scheduled for a sham operation (SG; n = 6).

Surgical and pharmacological procedures

All surgical procedures were performed under sterile conditions in a proper laboratory setting. All rats were administered 25 mg/kg intraperitoneal (i.p.) thiopental sodium anesthesia and then the ovaries were visualized by a 2–2.5 incision in the lower abdomen under anesthesia. Vascular clips were applied on the lower parts of the right ovaries of the rats in IRG and LIRG groups. Then, 2-hour ischemia followed by 2-hour reperfusion was performed. The thiopental sodium used in the experiment was provided from Ibrahim Etem Ulagay (Istanbul, Turkey). Lutein, supplied from Solgar (USA), was given perorally at a dose of 1 mg/kg to LIRG group 1 h before the application of reperfusion in the LIRG group. At the same time, the same volume of saline (0.9 % NaCl) was applied to IRG and SG rat groups by oral route. Ovaries in the SG group were not subjected to these processes. After these processes, rats were euthanized, and their right ovaries were removed for biochemical and histopathological examination. The results were compared among the groups.

Biochemical analysis of MDA and total GSH (tGSH)

MDA measurements were based on the method used by Ohkawa et al. involving spectrophotometrical measurement at 532 nm of absorbance of the pink-colored complex formed by thiobarbituric acid and MDA (15). The results were expressed as micromole/mg protein.

tGSH levels in ovarian tissues were measured according to the method defined by Sedlak J and Lindsay RH (16). DTNB (5,5'-dithiobis [2-nitrobenzoic acid]) disulfide is chromogenic in the medium, and DTNB is reduced easily by sulfhydryl groups. The yellow color produced during the reduction is measured by spectrophotometry at 412 nm. The results were expressed as $\mu\text{mol/mg}$ protein.

Measurement of COX activity

Measurements were performed according to the method of Kulmacz and Lands (17). Firstly, the analysis buffer and arachidonic acid solution were prepared. The other substances used were COX standard, colorimetric substrate, DuP697 (COX-2 inhibitor), and SC-560 (COX-1 inhibitor), which are available in commercial kits. Then, absorbances at a wavelength of 590 nm were read. The activity of COX in the tissue was expressed as nmol/min/mg protein (U/mg protein).

Histopathological analysis

Histological examination was performed at the pathology department of Erzincan University Hospital. After removal of the ovaries, samples were fixed in 10% formaldehyde, dehydrated in ethanol, and embedded in paraffin. The tissues were cut into 4–6- μm -thick sections, which were deparaffinized with xylene and rehydrated with alcohol and water. Sections were stained with hematoxylin and eosin (H&E) and examined under a microscope (Olympus pX53, Olympus Optical Co., Tokyo, Japan) with a digital camera system (Olympus UTVO.5XG-3, Olympus Optical Co., Tokyo, Japan) by a pathologist blinded to the study. Ovarian damage, including follicular cell degeneration, vascular congestion, hemorrhage, and inflammation (neutrophil infiltration), was evaluated histologically.

Statistical analysis

The software SPSS 16.0 was employed for the statistical analysis (SPSS Inc., Chicago, IL). Descriptive statistical results such as mean and standard deviation were obtained. Differences among the three groups were evaluated with Tukey analysis. The value of $p < 0.05$ was considered statistically significant.

Results

The MDA level in the ovarian tissue was $9.0 \pm 0.3 \mu\text{mol/mg}$ protein in SG group and $31.5 \pm 3.9 \mu\text{mol/mg}$ protein in IRG group.

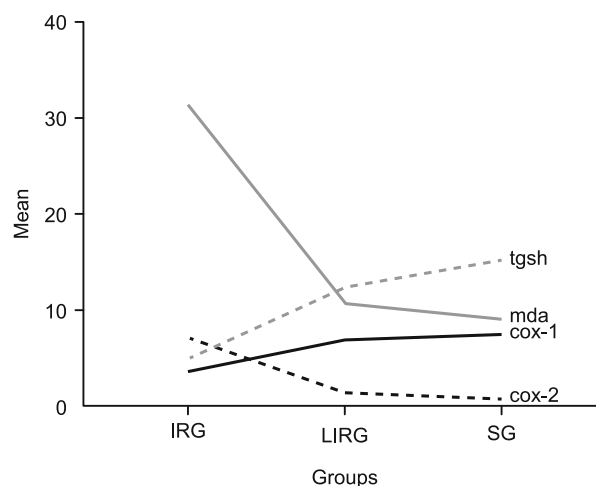


Fig. 1. COX-1, COX-2, MDA, and tGSH levels between groups.

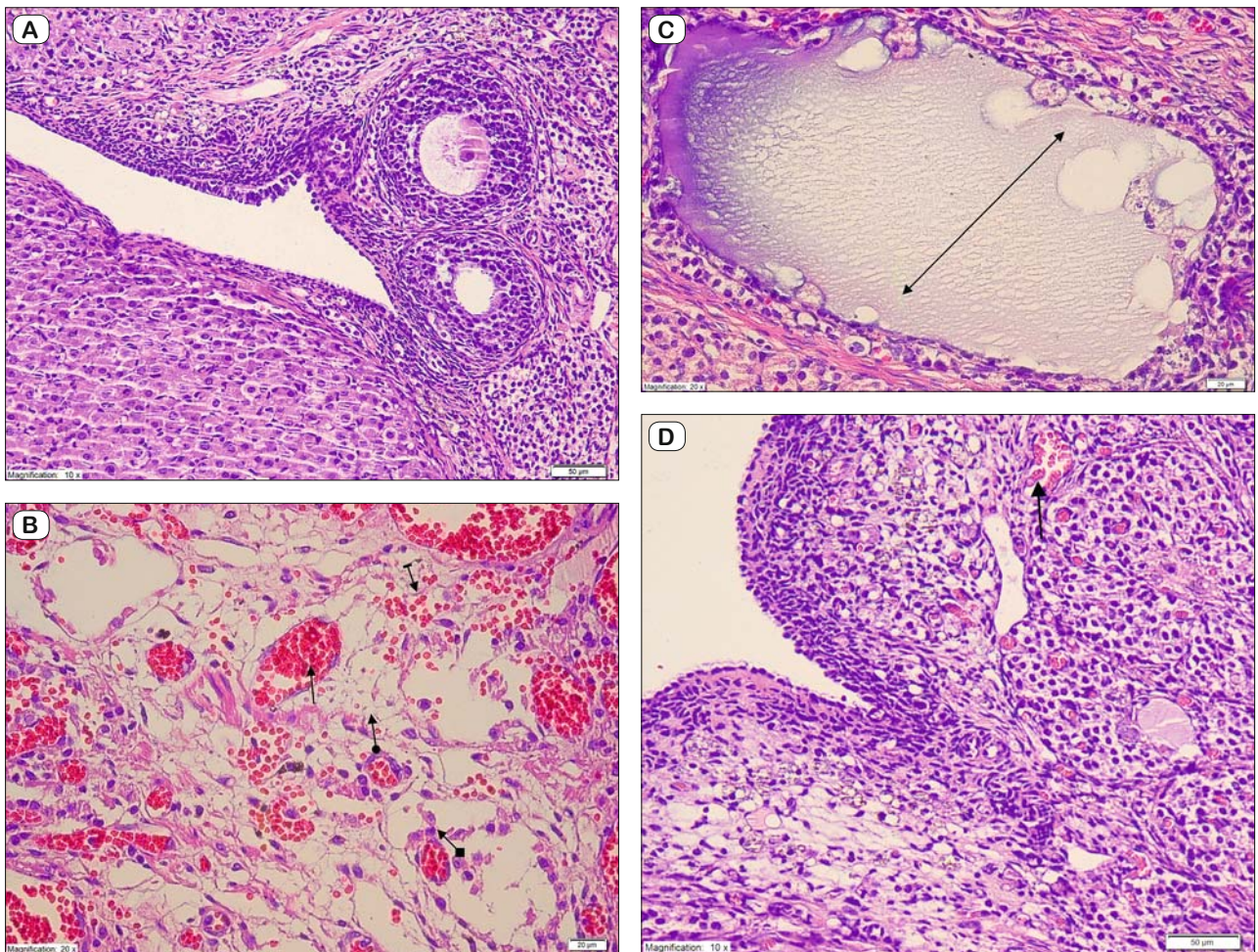


Fig. 2. Hematoxylin & eosin (H&E) sections from a single rat ovary of each group. (A) Sections of ovarian tissue showing secondary follicle in the healthy control group with sham operation (SG) (H&E X200). (B) Ovarian tissue in the ischemia-reperfusion (IRG) group showing polymorphonuclear leukocytes (PMNLs; arrow with square at the edge), dilated congested blood vessel (arrow), hemorrhage (arrow with line at the edge) and edema (arrow with circle at the edge) (H&E X400). (C) Ovarian tissue in the IRG group showing degenerated secondary follicle (double-headed arrow) (H&E X400). (D) Ovarian tissue with almost normal appearance except for dilated congested blood vessels (arrow) in the 1 mg/kg lutein+ischemia-reperfusion group (LIRG) (H&E X200).

When compared to the SG group, the increase in MDA level in IRG group was significant ($p < 0.05$). The dose of 1 mg/kg lutein reduced the level of MDA ($10.8 \pm 1.4 \mu\text{mol/mg protein}$) significantly compared to the IRG group ($p < 0.05$). On the other hand, the I/R process caused a significant decrease in tGSH level in the ovarian tissue of rats compared to the SG group ($p < 0.05$). It was measured as $15.1 \pm 2.3 \mu\text{mol/mg protein}$ in SG group and $5.0 \pm 0.5 \mu\text{mol/mg protein}$ in IRG group. The dose of 1 mg/kg lutein significantly ameliorated the tGSH level ($12.3 \pm 2.4 \mu\text{mol/mg protein}$) in LIRG group when compared to IRG group ($p < 0.05$) (Fig. 1).

As seen in Figure 1, the COX-1 activity was suppressed in IRG group compared to SG group ($3.7 \pm 0.3 \text{ U/mg protein}$ vs $7.3 \pm 0.3 \text{ U/mg protein}$) and this was significant ($p < 0.05$). But a significant increase ($6.9 \pm 0.2 \text{ U/mg protein}$) was found in LIRG compared to the IRG group ($p < 0.05$). On the contrary, COX-2 activity was significantly increased in IRG group when compared

to SG group ($7.0 \pm 0.5 \text{ U/mg protein}$ vs. $0.7 \pm 0.0 \text{ U/mg protein}$) while the dose of 1 mg/kg lutein significantly decreased COX-2 activity ($1.4 \pm 0.2 \text{ U/mg protein}$; $p < 0.05$).

The results of our study demonstrated that in ovarian tissues of animals administered with I/R, there was an increase in the levels of MDA and COX-2, and decrease in those of tGSH and COX-1, while these ratios were observed to be reversed in the LIRG group.

In histopathologic evaluation, normal histopathological appearance and secondary follicle were observed in the ovarian tissue of the SG group (Fig. 2A). Also, PMNLs, dilated congested blood vessels, hemorrhage, and edema were markedly seen in the ovarian tissue in IRG group (Fig. 2B). Degenerated secondary follicles were seen in the ovarian tissue in the IRG group (Fig. 2C). On the other hand, the dose of 1 mg/kg lutein corrected the pathological signs, only except for dilated congested blood vessels caused by

I/R. Ovarian tissue with almost normal morphology in the LIRG group is shown in Figure 2D.

Discussion

Lutein is one of 600 known naturally occurring carotenoids (18). The mechanism by which carotenoids affect the risk of developing chronic disease is not clear but the risk reduction typically depends on anti-inflammatory and antioxidant effects (12, 19). Lutein ameliorated acute inflammation in rats by inhibiting the production of nitrites, MDA, PGE₂, tumor necrosis factor alpha (TNF- α), and interleukin 6 (IL-6) cytokines. The anti-inflammatory mechanism of lutein might be related to the decrease in inflammatory cytokines and increase in antioxidant enzymes (SOD, CAT, GPx, glutathione S transferase, glutathione reductase), which would result in the reduction of inducible nitric oxide synthase (iNOS), COX-2 and MDA and subsequent inflammatory responses (20).

There are numerous experimental drug studies about lutein. In these published studies, anti-inflammatory and antioxidative effects of lutein were investigated in several organs such as eye, prostate, kidney, liver, lung, and colon in animal models (7, 13, 14, 20–25). There are no data about the effect of lutein on I/R-related ovarian injury.

In the beginning of reperfusion following ischemia, approximately 70 % of the oxygen provided to the tissue is oxidized to superoxide by xanthine oxidase (XO) (26). XO leads to the formation of excessive amounts of ROS (27). These ROS lead to the formation of toxic products such as aldehyde and MDA by oxidizing cell membrane lipids (28). GSH is one of the most important molecules of antioxidant capacity of the body that protect the tissues from ROS (29). In our study, I/R injury increased MDA levels and decreased the levels of tGSH in rat ovaries. Nevertheless, lutein significantly reduced MDA levels while significantly increasing the tGSH levels in rat ovaries injured by I/R.

Our results were in agreement with previous studies. For example, Cheng et al showed that lutein protects against skeletal muscle I/R injury by downregulating oxidative stress and inflammatory mechanisms (30). In another study, lutein was reported to have a beneficial effect against testicular I/R injury in rats (31). Additionally, the protective effect of lutein was demonstrated in small intestine and cardiac muscle (32, 33).

PGs synthesis is carried out via the COX pathway which contains two main isoenzymes named COX-1 and COX-2. COX-1 is involved in the synthesis of cytoprotective PGs, while COX-2 is induced by proinflammatory agents in pathological conditions (34, 35). Ibrahim et al. reported that I/R injury increased the COX-2 activity in hepatic tissue, while COX-2 activity was found to be lower than that of COX-1 in healthy hepatic tissue in rats (36). With its anti-inflammatory property, we found that lutein decreased the COX-2 activity as well as increased the COX-1 activity in ovarian tissue injured by I/R. Also, we found the activity of COX-2 to be increased in IRG group compared to the healthy control group, while the activity of COX-1 was suppressed (Fig. 1).

Histopathological signs of hemorrhage, dilated congested blood vessels, PMNL infiltration, and degenerated follicles in

the ovarian tissue of the IRG group were also observed in this study. Same histopathological changes in rat ovaries related to I/R injury were shown in previous studies (10, 37, 38). Lutein also ameliorated these histopathological changes in the ovaries of the rats from LIRG group in this study and when compared to the IRG group, we observed normal appearance except for dilated congested blood vessels.

Finally, Aksak Karamese et al reported that betacarotene, which is a kind of carotenoids, exerts protective effects on experimentally induced ovarian I/R injury. In this rat study, biochemical results (MDA, GSH) and histopathological evaluation after betacarotene administration were similar to those in our study (39).

There are some limitations to our study. Firstly, prior to this study, there have been no data about the effect of lutein on I/R-related ovarian injury. Secondly, as the aim of the study was to investigate if lutein had beneficial effects on ovarian I/R injury, only a single and average dose of 1 mg/kg was examined. It would be better to compare different doses of lutein to find the mean effective dose. Thirdly, I/R-related damage was demonstrated by histopathological changes in ovaries. This damage should be evaluated by infarction size or apoptosis in further studies. Fourthly, the results of experimental studies on animals should not be extrapolated to humans. We think that most accurate information will be reached in the future should more studies on this subject be done.

We detected the ameliorative effect of lutein on ovarian I/R injury in rats. Because of this, lutein may be suggested to patients before detorsion surgery of ovaries. Nevertheless, further clinical studies are required to reach more accurate results.

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