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Original Article

The Effect of Resveratrol on Ischemia–Reperfusion Induced Oxidative Rat Ovary Injury: A Biochemical, Histopathological, and Genetic Evaluation

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ABSTRACT

Objective: The cellular protective effects of resveratrol and co-enzyme Q10 supplementation have been reported in different models. However, there is insufficient data on the effects of resveratrol supplementation on oxidative DNA damage and co-enzyme Q10 levels in ovarian ischemia–reperfusion injury. The current study aims to investigate how resveratrol supplementation affects oxidative DNA damage, co-enzyme Q10 levels, inflammatory and proinflammatory indicators, and growth factors in ovarian ischemia–reperfusion injury.

Methods: Thirty albino Wistar female rats were divided into 5 groups; control, ovarian ischemia–reperfusion, and ischemia–reperfusion + treatment (25, 50, and 100 mg/kg resveratrol). Hematoxylin–eosin stain was used to evaluate histopathological results. The levels of 8-oxo-2′-deoxy guanosine and co-enzyme Q10 were obtained by enzyme-linked immunosorbent assay. Following RNA isolation, the expression of tumor necrosis factor-α , interleukin-1β, transforming growth factor-β3, hepatocyte growth factor, epidermal growth factor, and vascular endothelial growth factor was assessed.

Results: Regarding the histopathological evaluation score, 8-oxo-2′-deoxyguanosine levels, and co-enzyme Q10 levels, there was no statistically significant difference between the ischemia–reperfusion group and the groups that received resveratrol treatment. Tumor necrosis factor-α, interleukin-1β, transforming growth factor-β3, hepatocyte growth factor, epidermal growth factor, and vascular endothelial growth factor expression levels were significantly decreased in the resveratrol -treated groups compared to the ischemia–reperfusion group.

Conclusion: The results imply that the positive effects of resveratrol on inflammatory-proinflammatory cytokine expression may be a major contributor to the anti-oxidant, anti-inflammatory, anti-proliferative, anti-carcinogenic, and therapeutic effects of resveratrol documented in the literature.

Keywords: DNA damage, ischemia, reperfusion, resveratrol, ubiquinone

INTRODUCTION

Adnexal torsion affects females of all ages, from newborns to those in menopause, but it is most common in women of reproductive age, including pregnant women. Consequently, early detection and treatment are critical for preserving the affected ovary and, thus, fertility. Adnexal torsion has 2 pathological periods: the ischemia period, which begins with torsion and ends with detorsion, and the reperfusion period, which begins with detorsion and is characterized by blood re-circulation and formation of reactive oxygen species (ROS).

Ischemia–reperfusion (IR) injury is result of the first period "ischemia," and is characterized by a shift in metabolism from aerobic to anaerobic.[1] When oxygenation is restored

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during the reperfusion period, anaerobic metabolism metabolites are oxidized, released into the circulation, and are responsible for distant organ damage.[1] The destructive effects of the reperfusion procedure are wider and more severe than the ischemic injury, and are caused by many factors, most of which are ROS that are produced as a result of reperfusion. Migration of leukocytes into ischemic tissue after reperfusion causes the tissue damage to continue and spread.[1]

DNA, lipids, and proteins are among the cellular targets for oxidative modification by ROS. DNA is the most notable of the molecules exposed to oxidative modification because it reveals biomarkers that confirm the exposure.[2] The resulting high levels of oxidative DNA damage indicate a high level of oxidative stress. Despite the discovery of numerous base- and sugar-induced DNA damage mechanisms, most studies have focused on guanine modification.[2] Simply put, the levels of 8-oxo-7,8-dihyd roguanine (8-oxo-G) and 8-oxo-7,8-dihydro-2′-deox yguanosine (8-oxo-dG) indicate that oxidative stress or much-needed information about the functioning of DNA repair mechanisms can be provided.

Co-enzyme Q10 (CoQ10) is a vitamin-like benzoquinone compound found in all cell types and their membranes and plays a vital role in the electron transport system.[3] As a strong lipophilic antioxidant, CoQ10 plays a role in providing lipoproteins and membrane stability and in facilitating energy conversion and ATP production. Reduced levels have been associated with inflammatory and neurodegenerative diseases such as cardiovascular diseases, muscle diseases, bone diseases, cancers, diabetes, as reported in studies investigating its role in the anti-oxidative system, endothelial function, its negative effect on lymphocytes and monocytes, its effects on cell signaling and gene expression, and its positive effects on atherosclerosis and inflammation.[5,6] It is also suggested that its supplementation may improve the symptoms of mitochondrial diseases and lessen the impact of aging.[3]

Tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), transforming growth factor-β3 (TGF-β3), hepatocyte growth factor (HGF), epidermal growth factor (EGF), and vascular endothelial growth factor-α (VEGF-α) are very important growth factors and cytokines for an organism. Numerous cell types, such as keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets, generate them. Tumor necrosis factor-α and IL-1β are rapidly produced by different types of cells in response to inflammatory and apoptotic signals. Transforming growth factor-β3, HGF, EGF, and VEGF-α have important functions in cellular growth, differentiation, proliferation, inflammatory response, angiogenesis, and inflammation.[4-12]

Resveratrol (RSV) is a compound produced by plants in response to stressful environmental circumstances or pathogenic attack.[6-10,12,13] Inflammation, atherosclerosis, and carcinogenesis are pathogenic processes that may be prevented by RSV, according to increasing data.[13] The protective effects of RSV are attributed to its antioxidant and antiaggregant properties, its anti-estrogenic and anticyclooxygenase activity, and its modulatory activity in lipid and lipoprotein metabolism. Moreover, RSV has been shown to slow proliferation in various human malignant cell lines.[13] There have also been reports that RSV causes apoptosis.[13]

Despite a comprehensive review of the literature, there is insufficient evidence on oxidative DNA damage and CoQ10 levels in ovarian IR damage with RSV supplementation. Thus, this study aimed to determine the effects of RSV supplementation on oxidative DNA damage, CoQ10 levels, and the expression of TNF-α, IL-1, TGF-β3, HGF, EGF, and VEGF-α in ovarian IR injury.

MAIN POINTS

- Resveratrol treatment significantly suppressed tumor necrosis factor-α, interleukin-1β, transforming growth factor-β3, hepatocyte growth factor, epidermal growth factor, and vascular endothelial growth factor-α in the ischemia–reperfusion model.
- Our study confirms the literature studies demonstrating the anticancer, antiproliferative, and anti-inflammatory effects of resveratrol, as shown by the statistically significant decrease of cytokines and growth factors.
- Although there were positive changes in the groups treated with resveratrol after ischemia–reperfusion in terms of 8-oxo-dG, co-enzyme Q10 levels and histopathological evaluation, the changes did not gain statistical significance.

METHODS

Chemical Agents and Kits

The RSV used in this study was supplied by Santa Cruz Biotechnology Laboratory (Santa Cruz Biotechnology, Dallas, TX, USA). Thiopental sodium was supplied by Abbott (Pentothal Sodium, Istanbul, Türkiye), xylazine was purchased from Bioveta (XylazinBio, Ankara, Türkiye), and all the other chemicals were supplied by Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) and Merck (Merck KGaA, Darmstadt, Germany). Resveratrol was prepared for use by dissolving it in 1% dimethyl sulfoxide (DMSO).

Animals

Thirty albino Wistar female rats weighing between 250 and 265 g were used for the experiment. The animals were maintained in plastic breeding cages both throughout the experiment, and they were given unlimited access to water and basal food to establish standard chow. In addition, a 12-hour light/dark cycle, a temperature of 21 ± 1°C, and a relative humidity of approximately 60% were maintained to produce controlled experimental conditions.

Experimental Procedure

The 30 female albino rats were equally divided into: a healthy control (HC) group, an IR group without RSV, and 3 IR + RSV groups in which RSV was used intraperitoneally in 3 different doses (25, 50, 100 mg/kg, respectively) dissolved in 1% DMSO solution (RSV-1, RSV-2, and RSV-3).

All surgical procedures were performed in sterile surroundings in appropriate lab settings. The animals were anesthetized using 25 mg/kg of thiopental sodium i.p. and breathing xylazine at appropriate intervals, then a 2-cm midline vertical incision was made.

In the HC group (abdominal incision without IR), only laparotomy and left oophorectomy were performed; no ovarian torsion was performed.

The left ovaries of the rats in groups 2, 3, 4, and 5 were torsioned 360 degrees clockwise, involving the tube and ovarian arteries, and fixed to the abdominal wall with a 5/0 silk suture. Three hours later, the torsion was reversed. Intraperitoneal RSV (25, 50, or 100 mg/kg, respectively, for groups 3, 4, and 5) + DMSO was administered 30 minutes before detorsion (the 150th minute of ischemia). After 3-hour reperfusion in groups 2, 3, 4, and 5, all the rats were euthanized, then their left ovaries were excised and blood samples were taken for biochemical and histopathologic examination. Tissues taken from each sacrificed rat and washed with 0.9% saline were digested with a homogenizer (IKA Ultra-Turrax T25 basic homogenizer, Staufen, Germany). To obtain serum, blood samples (~5 mL) were centrifuged at 2500 rpm for 15 minutes (Eppendorf 5430R, Hamburg, Germany). For the biochemical and polymerase chain reaction (PCR) analyses, the tissue homogenization samples and serum samples were kept at –80°C degrees.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was isolated from the tissue homogenization samples using the GeneAll® Hybrid-RTM total RNA purification kit (Cat No: 305-101, Songpa-gu, Seoul, South Korea). A High-capacity cDNA Reverse Transcription Kit was used to synthesize complementary DNA (cDNA) from the acquired RNA (Applied Biosystems, Foster City, CA, USA). This kit was used for both total RNA purification and cDNA synthesis.

The cDNA concentration was measured using a µDrop™ Plate fitted into a Multiskan Sky Microplate Spectrophotometer (Thermo Scientific, Waltham, MA, USA). Reverse transcription and quantitative real-time PCR (qRT-PCR) were carried out using the QuantStudio 5 Real-Time PCR equipment (Thermo Fisher Scientific, Waltham, MA, USA), as well as SYBR Green-based expression analysis in QuantiTect. Reverse transcription and quantitative real-time PCR was performed using Primer Assays (Qiagen, Hilden, Germany). The total volume of all the samples was calculated to be 20 µL. This amount contained 5 µL of cDNA, 12.5 µL of the master mix, and 2.5 µL of the primer solution. Table 1 shows the catalog and NCBI reference sequence numbers of the primers used in this study.

The *ACTB1* gene was used for normalization. To determine the gene expression, PCR cycling was used under the following conditions: 95°C for 15 minutes, 94°C for 15 seconds (40 cycles), 55°C for 30 seconds (40 cycles), and 72°C for 30 seconds (40 cycles). Moreover, the melting curve analysis was performed to understand whether the

Table 1. The Catalog and NCBI Reference Sequence Numbers of the Primers Used

Genes	Catalog Name	Symbol	Catalog No.	Accession Codes
β-actin	Rn_Actb_1_SG	*Actb1*	QT00193473	NM_031144.3
TNF-α	Rn_Tnf_1_SG	*Tnf*	QT00178717	NM_012675.3
EGF	Rn_Egf_1_SG	*Egf*	QT00176463	NM_012842.1
VEGF-α	Rn_RGD:619991_1_SG	*Vegfa*	QT00198954	NM_001110333.1
IL-1β	Rn_Il1b_1_SG	*Il1b*	QT00181657	NM_031512.2
HGF	Rn_Hgf_1_SG	*Hgf*	QT00177289	NM_017017.2
TGF-β3	Rn_Tgfb3_1_SG	*Tgfb3*	QT00177065	NM_013174.2

EGF, epidermal growth factor; IL-1β, interleukin-1β; HGF, hepatocyte growth factor; TGF-β3, transforming growth factor-β3; TNF-α, tumor necrosis factor-α; VEGF-α, vascular endothelial growth factor-α.

generation of the amplification products occurred immediately after this process. The cycling conditions of the melting curve analysis were 95°C for 15 seconds, 60°C for 1 minute, and 95°C for 15 seconds. After the qRT-PCR process was performed, the data were evaluated using the 2-ΔΔCT method.

Enzyme-Linked Immunosorbent Assay Procedure

Next, 8-oxodG and CoQ10 were evaluated with a Multiskan Sky Microplate Spectrophotometer (Thermo Scientific, Waltham, MA, USA) using the sandwich enzyme-linked immunosorbent assay (ELISA) method using SinoGeneClon rat 8-Oxo-2′-deoxy Guanosine ELISA kit (Cat. No: SG-21168) and SinoGeneClon rat CoQ10 ELISA kit (cat. no.: SG-21187) (SinoGeneClon Biotech, Hangzhou, China), respectively. The manufacturer's recommended procedures for these kits were followed during the study.

Histopathological Examination

Tissue samples were collected and processed promptly for histological examination. The tissues were preserved in a 10% formaldehyde solution for 48 hours (pH: 7). The tissues were dehydrated with ethanol after being rinsed with water. The tissues were then immersed in xylene to increase transparency before being imbedded in paraffin. The paraffin-blocked tissues were sliced into 4-mm thick slices and stained with hematoxylin–eosin using a microtome (Leica RM2235; Leica Instruments, Nussloch, Germany). A pathologist blinded to the 5 study groups performed the semi-quantitative histological examination. The evaluation was carried out with the aid of a light microscope (Olympus BX53, Tokyo, Japan) as well as a digital camera and imaging system (CellSens Standard, Olympus, Tokyo, Japan). At least 5 regions were evaluated and the degree of histopathological damage—congestion, edema, hemorrhage, and leukocyte infiltration (inflammation) parameters—was determined. Each parameter was scored as follows: 0: normal tissue (no damage), 1: slight damage (lesion rate <33%), 2: moderate damage (lesion rate between 33% and 66%), and 3: serious damage (lesion rate >66%). The total histopathological scores were calculated by summing the scores of each parameter.

This study was approved by the local animal ethics committee of Atatürk University, Erzurum, Türkiye (Ethics Committee No.: 7/108, dated Aug 24, 2017).

Statistical Analysis

For the statistical analysis, IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA) was utilized. The categorical variables were given as n (%), while the continuous variables' descriptive statistics were given as mean ± SD or the median (minimum–maximum) value, taking into consideration their distribution. The categorical variables were analyzed using the chi-square test. The Kruskal–Wallis test was used to assess abnormal distribution, the ANOVA test for normal distribution, and Dunn's test for comparing several groups. For the correlation test, Spearman correlation analysis was performed. For all tests, the statistical threshold of significance was set at 0.05.

RESULTS

In the statistical evaluation, it was determined that there was a significant difference between the groups in terms of total histopathological score ($P < .001$). When the groups were evaluated separately, a statistically significant difference was observed between the HC and IR groups ($P < .001$) and between the HC and RSV-1 groups ($P = .037$). However, no significant difference was detected between the HC and RSV-2 groups ($P=.573$), the HC and RSV-3 groups ($P=.056$), the IR and RSV-1 groups ($P=1.000$), the IR and RSV-2 groups ($P=.112$), and the IR and RSV-3 groups ($P=.954$). The histopathological evaluation results are shown in Figure 1. Examples of the histopathological evaluation of the groups are presented in Figures 2, 3, 4, 5, and 6.

In terms of the CoQ10 levels, there was no statistically significant difference between the groups ($P=.712$). The level of CoQ10 was lower in the IR group than the HC group, but it was higher in the RSV-1, RSV-2, and RSV-3 groups than the IR group as the RSV dose increased (Figure 7). However, the increase in the CoQ10 level in the RSV groups was not statistically significant.

In terms of 8-oxo-dG, there was no statistically significant difference between the groups ($P = .613$). The 8-oxo-dG level increased in the IR group in comparison to the HC group; it decreased in the RSV-1, RSV-2, and RSV-3 groups in comparison to the IR group. However, this reduction was not statistically significant. The mean and SD values for the 8-oxo-dG parameter given in each group are presented in Figure 7.

Regarding the evaluation of the expression levels of TNF-α, IL-1β, TGF-β3, HGF, EGF, and VEGF-α parameters, a significant difference was found between the groups. Expression fold values for the TNF-α, IL-1β, TGF-β3, HGF, EGF, and VEGF-α parameters were given as mean ± SD. $2^{-\Delta\Delta CT}$ values in the HC group were calculated as 1.00 ± 0.12, 1.00 ± 0.06, 1.00 ± 0.09, 1.01 ± 0.12, 1.00 ± 0.07, and 1.01 ± 0.16, respectively. The differences in the statistically

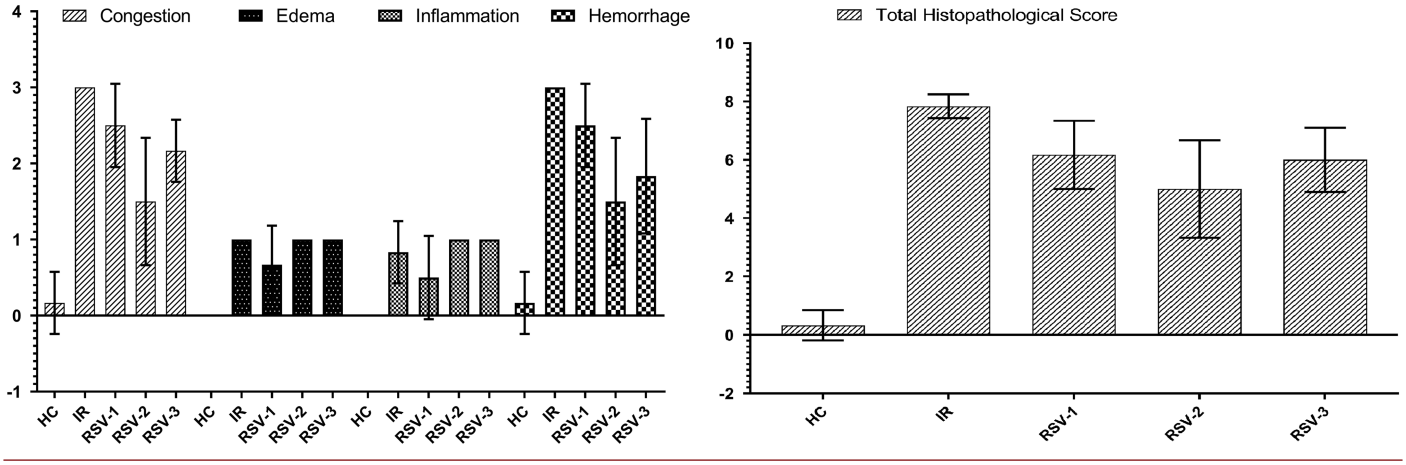

Figure 1. Histopathological evaluation results. Congestion, edema, inflammation and hemorrhage scores (left) and total histopathological score (right) in groups. HC, healthy control group; IR, ischemia–reperfusion group; RSV-1, ischemia–reperfusion + resveratrol 25 mg/kg group; RSV-2, ischemia–reperfusion + resveratrol 50 mg/kg group; RSV-3, ischemia–reperfusion + resveratrol 100 mg/kg group.

significant values between the HC and IR groups and the RSV treatment groups for the expression levels of the TNF-α, IL-1β, TGF-β3, HGF, EGF, and VEGF-α are presented in Table 2. It was found that there was a difference between the HC and IR groups for each parameter. Between the IR group and the RSV-1 group, there was a statistically significant difference in terms of the IL-1β, TGF- β3, HGF, and VEGF-α parameters. Between the IR group and the RSV-2 group, there was a statistically significant difference in terms of the IL-1β, TGF-β3, HGF, EGF, and VEGF-α parameters. It was also observed that there was a statistically significant difference for each parameter between the IR group and the RSV-3 group. The mean ± SD values of the expression of TNF-α, IL-1β, TGF-β3, HGF, EGF, and VEGF-α of the groups are shown in Figure 8.

The correlation test results showed that there was a statistically significant correlation between the total histopathological score and TNF-α (P=.005, rs=0.686), IL-1β (P = .001, rs=0.769), TGF-β3 (P < .001, rs=0.884), HGF (P=.017, rs=0.605), EGF (P=.001, rs=0.761), and VEGF-α (P = .003, rs=0.712). No correlation was found between the total histopathological score and CoQ10 (P=.330, rs=-0.184) or 8-oxo-dG (P=.383, rs=0.183). Furthermore, no association was found between CoQ10 or 8-oxo-dG and the other parameters.

DISCUSSION

In our study, which examined the effects of RSV in an ovarian IR injury model, we found that there were positive changes in total histopathological evaluation, CoQ10

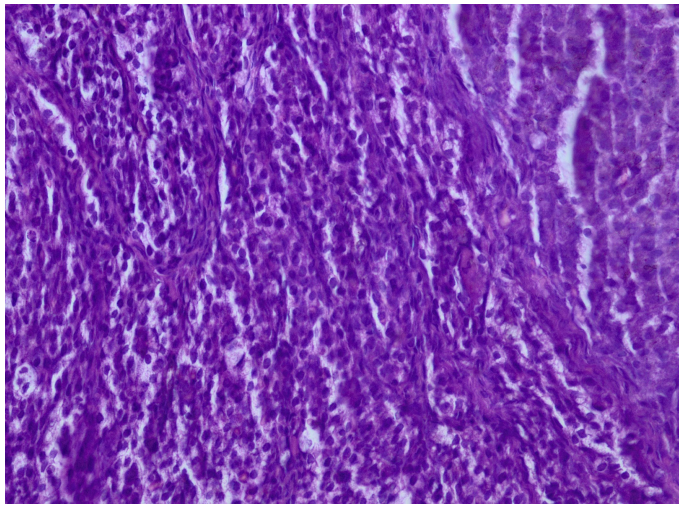

Figure 2. Hematoxylin–eosin-stained ovary tissue belonging to the healthy control group, significant edema, inflammation, hemorrhage, and congestion are not observed, ×400.

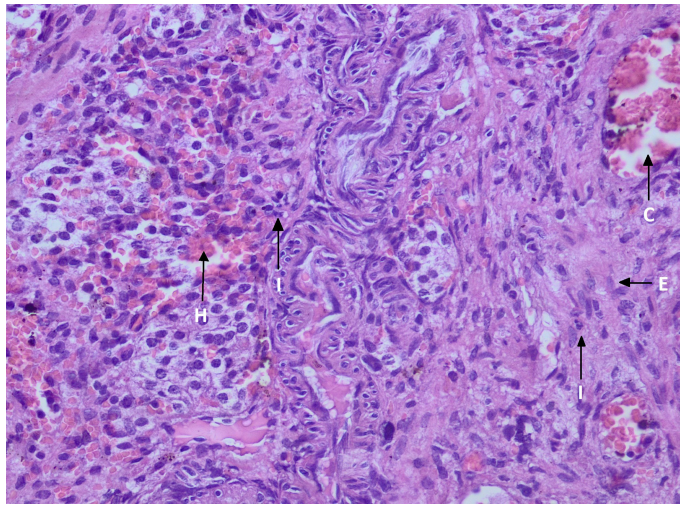

Figure 3. Hematoxylin–eosin-stained ovary tissue belonging to the ischemia–reperfusion group. E, edema (+); I, inflammation (+); H, hemorrhage (+++); C, congestion (+++), ×400.

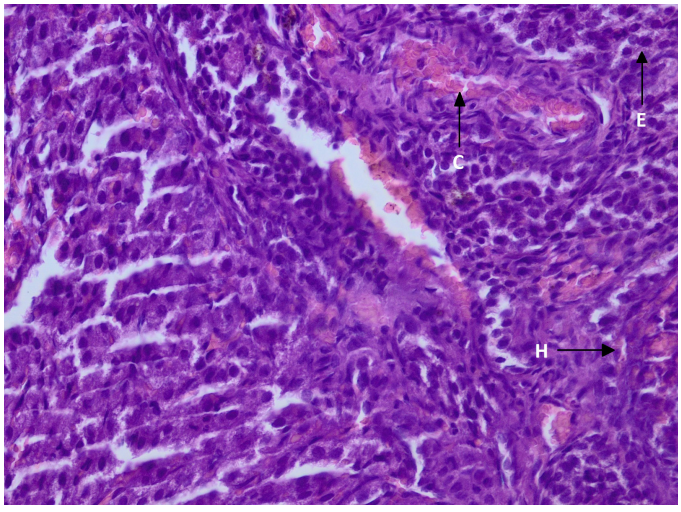

Figure 4. Hematoxylin–eosin-stained ovary tissue belonging to the ischemia–reperfusion + resveratrol-1 (25 mg/kg) group. E, edema (+); I, inflammation (-); H, hemorrhage (++); C, congestion (++), ×400.

and 8-oxo-dG levels, although no statistically significant difference was detected. In addition, when the expression coefficients of the TNF-α, IL-1β, TGF-β3, HGF, EGF, and VEGF-α parameters were evaluated, there was a significant difference between the groups. Finally, in the correlation test, it was observed that the total histopathological evaluation score had a significant correlation with the parameters whose genetic expression was evaluated.

While the protective effects of RSV in many tissues have been studied using the IR model, only a few studies have investigated the effects of RSV in experimental ovarian IR injury. Hasçalık et al[14] evaluated rat ovaries after IR injury (3 hours ischemia, 3 hours reperfusion, 10 mg/kg RSV) and detected acute infiltration with polymorphonuclear leukocyte, vascular dilatation, and edema; as a result of the histopathological analysis, better preservation of ovarian morphology was reported in rats treated with RSV. Ertürk et al[15] (2 hours ischemia, 2 hours reperfusion, 10 mg/kg RSV) evaluated the effects of RSV and sildenafil in a rat ovarian IR injury model; they reported that the histopathological damage score was significantly lower in the sildenafil, RSV, and sildenafil + RSV groups in comparison to the IR group. Using an rat ovary IR injury model (2 hours ischemia, 2 hours reperfusion, 20 mg/kg RSV), Kılıç et al[16] examined the protective effect of *N*-acetyl cysteine and RSV; that study reported that RSV statistically significantly reduced the histopathological damage score and provided significant protection in terms of congestion, hemorrhage, leukocyte infiltration, and edema parameters. In our study, it was observed that the histopathological damage score in the IR group increased significantly in comparison to the HC group, and it was lower in the RSV treatment groups in comparison to the IR group, but the difference was not statistically significant. Methodological differences (differences in ischemia and reperfusion times and RSV doses, surgical technique differences) in the few studies examining the protective effect of RSV in ovarian IR may explain the different results obtained in our study.

Many studies have reported that CoQ10 supplementation in different tissues reduces inflammation, apoptosis, and necrosis in IR injury. However, very few studies have examined the endogenous/plasma CoQ10 level in IR injury, and according to our literature review, no study

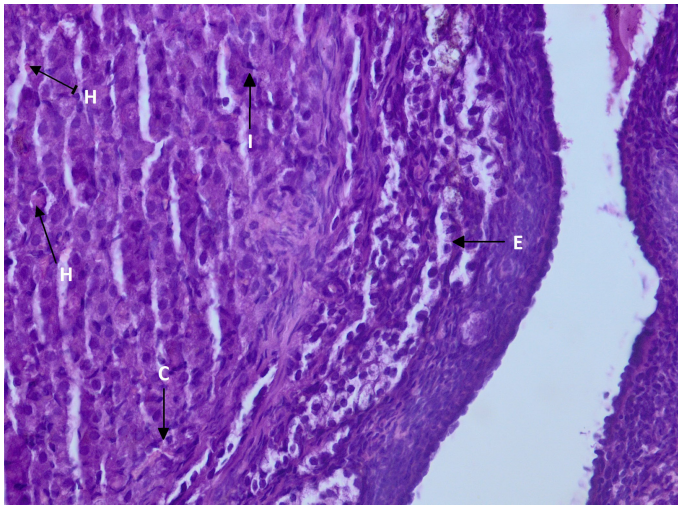

Figure 5. Hematoxylin–eosin-stained ovary tissue belonging to the ischemia–reperfusion + resveratrol-2 (50 mg/kg) group. E, edema (+); I, inflammation (+); H, hemorrhage (+); C, congestion (+), ×400.

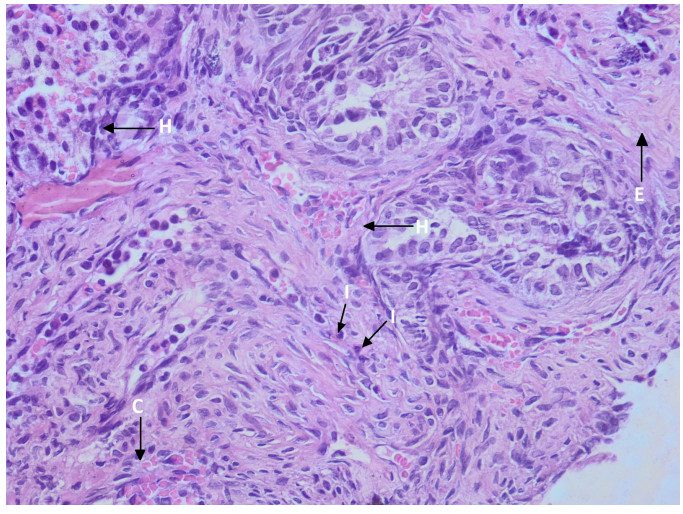

Figure 6. Hematoxylin–eosin-stained ovary tissue belonging to the ischemia–reperfusion + resveratrol-3 (100 mg/kg) group. E, edema (+); I, inflammation (+); H, hemorrhage (++); C, congestion (++), ×400.

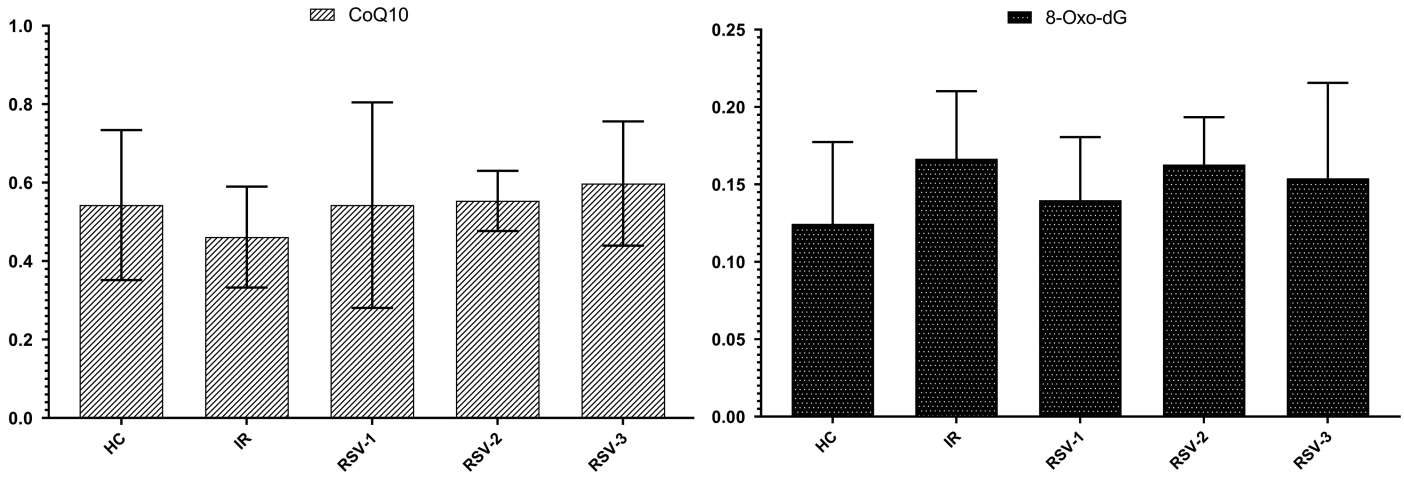

Figure 7. Co-enzyme Q10 (left, ng/mL) and 8-oxo-dG (right, ng/mL) levels (mean ± SD) in groups. HC, healthy control group; IR, ischemia-reperfusion group; RSV-1, ischemia-reperfusion + resveratrol 25 mg/kg group; RSV-2, ischemia-reperfusion + resveratrol 50 mg/kg group; RSV-3, ischemia-reperfusion + resveratrol 100 mg/kg group.

has evaluated this component in ovarian IR injury. Huang et al[17] reported that circulating CoQ10 concentrations were significantly decreased in ST-elevation myocardial infarction patients treated with primary coronary balloon angioplasty in comparison to the HC group. Nagase et al[18] showed that the level of the oxidized form of coenzyme in CoQ10 increases in patients with post-cardiac arrest syndrome (PCAS) in comparison to HCs. Cocchi et al[19] evaluated CoQ10 levels in PCAS patients and found that those levels were lower in patients who died and those with poor neurological progress. However, in a study evaluating the relationship between iatrogenic IR damage and CoQ10 during balloon angioplasty without myocardial infarction, no change was found in CoQ10 levels.[20] This result was attributed to the short duration of myocardial ischemia during balloon angioplasty, and it was stated that reperfusion after short-term acute ischemia did not change the concentration of lipophilic antioxidants; it only reduced the level of water-soluble antioxidants.[20] Although no comparison can be made to ovarian IR, in our study, the CoQ10 levels decreased in the IR group and increased moderately in the RSV groups, but this increase was not statistically significant.

The chemopreventive effect of RSV is probably due to its ability to prevent DNA damage and increase DNA repair.[21] It has been shown that, at a dose of 100 mg/kg/day, started 2 days before the procedure, RSV provides protection against radiation-induced chromosomal aberrations in mice.[22] It has been reported that RSV treatment (50 to 100 mg/kg/day for 7 and 14 days) reduces the 8-oxo-dG levels in urine by reducing the oxidative DNA damage induced by cisplatin.[23] Consistent with these findings, Aydın et al[21] showed that RSV treatment applied in the sepsis model created in rats significantly reduced DNA damage in liver and kidney tissues. In our study, the

Table 2. Statistical Significance Values Between the HC, IR Groups, and the RSV Treatment Groups for the Expression of the Parameters

Groups		TNF-α	IL-1β	TGF- β3	HGF	EGF	VEGF-α
HC	IR	<.001	<.001	<.001	<.001	.001	<.001
	RSV-1	<.001	.008	.012	.997	<.001	.700
	RSV-2	.001	.221	.983	1.000	.855	.201
	RSV-3	.523	.881	.979	1.000	.980	.772
IR	HC	<.001	<.001	<.001	<.001	.001	<.001
	RSV-1	.999	.001	<.001	<.001	.981	<.001
	RSV-2	.139	<.001	<.001	<.001	.003	.002
	RSV-3	<.001	<.001	<.001	<.001	.001	<.001

EGF, epidermal growth factor; HC, healthy control group; HGF, hepatocyte growth factor; IL, interleukin; IR, ischemia–reperfusion group; RSV-1, ischemia–reperfusion + resveratrol 25 mg/kg group; RSV-2, ischemia–reperfusion + resveratrol 50 mg/kg group; RSV-3, ischemia–reperfusion + resveratrol 100 mg/kg group; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

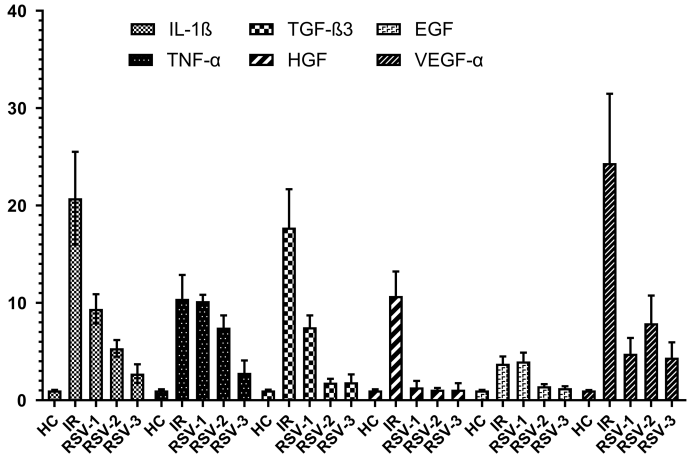

Figure 8. Cytokine and growth factors evaluated by gene expression. Expression coefficients of TNF-α, IL-1β, TGF-β3, HGF, EGF, and VEGF-α in groups are presented. TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; TGF-β, transforming growth factor-β3; HGF, hepatocyte growth factor; EGF, epidermal growth factor; VEGF-α, vascular endothelial growth factor-α; HC, healthy control group; IR, ischemia-reperfusion group; RSV-1, ischemia-reperfusion + resveratrol 25 mg/kg group; RSV-2, ischemia-reperfusion + resveratrol 50 mg/kg group; RSV-3, ischemia-reperfusion + resveratrol 100 mg/kg group.

8-oxo-dG level increased in the IR group in comparison to the HC group; it decreased in the treatment groups (RSV 25, 50, and 100 mg/kg) in comparison the IR group. However, this decrease was not statistically significant. The lack of statistical significance of this decrease was attributed to the short duration of the timeframe of the model used in our study; a longer amount of time would cause significant damage while a shorter amount of time would prevent a significant level of damage. As a matter of fact, according to our literature review, to the best of our knowledge, our study is the first to evaluate the 8-oxo-dG parameter in relation to IR injury.

Previous studies have reported that RSV can inhibit the synthesis and release of many pro-inflammatory mediators, such as nitric oxide and a wide variety of cytokines (IL-1β, IL-6, IL-12, and TNF-α).[24] Moreover, RSV modifies eicosanoid production and suppresses cyclooxygenase-1 or cyclooxygenase-2, which are important enzymes involved in the synthesis of pro-inflammatory mediators.[25] The mechanisms responsible for this phenomenon have not been fully elucidated. However, it is probably related to the modulation of nuclear factor-kB and activator protein-1 as transcription factors.[24]

Very few studies in the literature have examined the effects of RSV on various growth factors. According to the study conducted by Arablou et al.[26] resveratrol reduces the expression of TGF-β in endometrial stromal cells. Abd-Elgawad et al[5] investigated the ability of piceatannol (a polyphenolic RSV analog) to alleviate liver fibrosis and protect hepatocytes from damage. In liver fibrosis induced by injection of thioacetamide into adult male mice, RSV has been shown to significantly decrease the expression of MDA, CK18, TGF-21, and α-SMA and increase the levels of HGF and IL-10.[5] In the study conducted by Hsieh et al.[27] RSV inhibited HGF expression in prostate stromal cells. Similarly, Arablou et al[7] reported that RSV decreased the expression of insulin-like growth factor-1 and HGF in the stromal cells of women with endometriosis in comparison to women without endometriosis.

The effects of RSV in cell lines induced by EGF or VEGF have been investigated by a relatively large number of studies. However, few studies have examined the level or expression of EGF and VEGF in response to RSV supplementation. According to the findings of Arablou et al.[26] by lowering the expression of VEGF, TGF-β, and MMP-9 in endometrial stromal cells, resveratrol may slow the progression of endometriosis. In a study conducted in vitro by Chatterjee et al.[9] VEGF expression in cervical cancer cell lines was suppressed by RSV supplementation. Similarly, Chen et al[10] reported decreased VEGF expression in a diabetic retinopathy model. It has also been reported that the combination of RSV and thymoquinone in breast cancer cell lines reduces VEGF expression.[6] On the contrary, Feng et al[28] reports that RSV protects against myocardial infarction. In a study performed on ischemic skin flaps reported that RSV had a protective effect by increasing VEGF levels.[12] In another study examining wound healing in an animal model, Christovam et al[11] reported that calorie restriction and/or RSV supplementation increased VEGF levels. Bahramrezaie et al[8] conducted a randomized triple-blind study in polycystic ovarian patients in vivo and reported that RSV increased VEGF expression. According to the study of Kawabata et al.[29] RSV substantially and dose-dependently inhibited osteoblast migration stimulated by EGF. In contrast, Liu et al[30] reported that after 15 days of heat stress, resveratrol lowered protein expression of HSP70, HSP90, and NF-B in the jejunal villi and increased EGF expression.

Although a few studies have reported conflicting results, which is thought to be due to the use of different models, methods, and approaches (such as in vitro/in vivo, animal/human, level/expression), most of the literature has reported that RSV has a suppressive effect on growth factors, such as TGF-β3, HGF, EGF, and VEGF-α. Our study also supports previous studies reporting the anti-cancer and anti-proliferative effects of RSV by evaluating all of these growth factors together. Moreover, it is striking that

both TNF-α and IL-1β, which were selected as inflammation markers, and growth factors, such as TGF-β3, HGF, EGF, and VEGF-α, are significantly correlated with the histopathological score, which was not statistically significant with RSV treatment.

In conclusion, despite positive changes in the CoQ10 and 8-oxo-dG levels and the total histopathological score, the effects of RSV treatment on these parameters were not statistically significant. However, RSV suppressed the expression of inflammatory/proinflammatory cytokines and growth factors (TNF-α, IL-1β, TGF-β3, HGF, EGF, and VEGF-α) in ovarian IR injury and that impact was found to be statistically significant. Moreover, considering the correlation between the histopathological score and these parameters, it was concluded that the suppression of these parameters may play a primary role in the anti-oxidant, anti-inflammatory, anti-proliferative, anti-carcinogenic, and potential therapeutic effects of RSV reported in the literature.

Ethics Committee Approval: This study was approved by the local animal ethics committee of Atatürk University, Erzurum, Türkiye (Ethics Committee No.: 7/108, dated Aug 24, 2017).

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