Investigation of the Effects of Curcumin and Gallic Acid in the Liver and Kidneys of Rat Ovarian Transplant Recipients

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Abstract

Objectives: **Recent studies have shown that oxidative stress, inflammation, and apoptosis play substantial roles in the pathogenesis of tissue damage and distant organ damage during ovarian transplantation. Because of their potential antioxidant and anti-inflammatory properties, investigating natural compounds like curcumin and gallic acid is crucial. This study investigated the effects of curcumin, gallic acid, and their combination on liver and kidney tissues in a rat model of ovarian transplantation.**

Materials and Methods: **For this study, we used 42 adult female Sprague-Dawley rats aged 11 to 12 weeks, which we randomly divided into 6 groups. After the transplant procedures, we performed histopathological analysis, biochemical examinations, and statistical analysis.**

Results: **Significant damage was shown in the liver and kidney tissues of the ovarian transplant only and transplant plus corn oil groups compared with the control group, as evidenced by histopathological evaluation, increased BAX/BCL-2 intensity indicating apoptotic activity, and elevated interleukin 6 levels. However, treatment with curcumin, gallic acid, or their combination attenuated these adverse effects, suggesting potential protective effects against transplant-induced injury.**

Conclusions: **Our findings highlight the therapeutic potential of these compounds in mitigating tissue damage and inflammation associated with ovarian transplant.**

Key words: *Animal model, Distant organ damage, Ischemia-reperfusion injury, Transplant-induced injury*

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Introduction

Patients may have infertility and impairment of reproductive function as a result of cancer therapies such as surgery, radiation, and chemotherapy.[1] One of the most severe side effects of cytotoxic therapy in women who may recover from their malignancy is infertility.[2] As a common practice for preserving fertility, ovarian tissue cryopreservation and transplant have been included in many networks and initiatives.[2-4] Cryopreservation and ovarian tissue transplant in several experimental animals have also been investigated.[2,4-6]

Ovarian transplantation is a promising solution for women who have ovarian failure or infertility. However, ischemia-reperfusion injury (IRI) poses a major challenge to this procedure. Ischemia-reperfusion injury is caused by the temporary cessation of blood flow followed by the restoration of blood flow. This results in the overproduction of reactive oxygen species (ROS), leading to oxidative damage, inflammation, and tissue swelling.[1,2,7] Ischemia-reperfusion injury may spread from the ischemic region and adversely affect distant organs. Although local effects of IRI are observed in the affected organ, systemic effects can be observed predominantly in organs such as the lung, kidney, heart, and brain.[7-9]

The administration of antioxidants has consistently demonstrated beneficial effects on ovarian tissue subjected to IRI. Histopathological evaluations have revealed that antioxidants effectively attenuate tissue damage, preserve tissue architecture, and reduce the extent of necrosis.[7,10,11] Moreover, antioxidants have

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been shown to mitigate inflammation by reducing inflammatory cell infiltration and by suppressing proinflammatory cytokines. These interventions also modulate oxidative stress by inhibiting ROS production, enhancing endogenous antioxidant defense mechanisms, and preventing oxidative damage to macromolecules within ischemic ovarian tissue.[1,11]

Experimental studies with animal models have demonstrated the efficacy of antioxidants such as vitamin E, N-acetylcysteine, resveratrol, and melatonin in reducing oxidative stress and improving ovarian tissue viability following IRI.[2,5,10,11] Clinical studies, although limited, have reported encouraging results with the use of antioxidants as adjunct therapies in ovarian transplantation. These studies have shown improved graft survival rates, reduced ischemic injury, and enhanced ovarian function in patients receiving antioxidant treatments.[12-14]

Gallic acid and curcumin have recently gained attention for their potential as antioxidants and their ability to mitigate oxidative stress-induced damage in various disease models.[15-17] Ischemia-reperfusion injury triggers the release of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6), leading to tissue damage.[18] Gallic acid and curcumin have been shown to suppress the production and release of these cytokines and can downregulate TNF-α, IL-1β, and IL-6 gene expression, thus attenuating the inflammatory response.[1,15] By reducing inflammation, gallic acid and curcumin contribute to the overall protection of the transplanted ovarian tissue. Moreover, gallic acid and curcumin exhibit cytoprotective effects by preserving cellular integrity and function. Gallic acid and curcumin can prevent oxidative damage to macromolecules, including nucleic acids, lipids, and proteins, which are particularly vulnerable during ischemic conditions in liver and kidney function.[17] By maintaining cellular homeostasis, gallic acid and curcumin contribute to the viability and functionality of the transplanted ovarian tissue.[19]

Histopathological examinations have demonstrated the beneficial effects of gallic acid and curcumin in reducing tissue damage associated with IRI. These antioxidants have been shown to attenuate necrosis, preserve tissue architecture, and reduce inflammatory cell infiltration. Moreover, treatment with gallic acid and curcumin has been associated with decreased oxidative damage and improved antioxidant enzyme activity in experimental models.[17]

Gallic acid and curcumin are natural plant compounds with favorable safety profiles. They are widely available, cost-effective, and easily administered orally or through other routes. This makes them potentially feasible options for antioxidant therapy in clinical settings.[15] Their antioxidant, anti-inflammatory, and cytoprotective properties make them attractive candidates for reducing oxidative stress, preserving tissue viability, and improving the outcomes of ovarian transplantation.[20] However, further preclinical and clinical studies are needed to optimize the dosages, investigate treatment protocols, and evaluate long-term safety and efficacy in the context of ovarian transplantation.[17,21]

In this study, we hypothesized that antioxidant supplements used both before and after transplant may prevent potential injury to distant organs. We investigated the effects of curcumin and gallic acid on IRI-induced liver and kidney damage in rats receiving autologous intraperitoneal ovarian transplant.

Materials and Methods

Study and experimental design

The University of Health Sciences Hamidiye Local Ethics Committee for Animal Experiments (Istanbul, Turkey) (no. 2022-01/03) approved and found all experimental procedures and protocols conducted in this study to be ethically appropriate.

We used 42 adult female Sprague-Dawley rats aged 11 to 12 weeks in this study. We housed animals in stainless steel cages in rooms with a 12:12-hour light/dark cycle, maintained at a constant temperature of 22 to 23 °C and relative humidity of 45% to 55%. We provided rats with standard rodent chow and tap water ad libitum.

Ovarian transplantation

We randomly divided the 42 adult female Sprague Dawley rats into 6 groups. We placed rats under anesthesia (75 mg/kg intraperitoneally ketamine and 10 mg/kg intraperitoneally xylazine) on the opera-ting table in a supine position. After a midline incision, we opened the abdomen to perform bilateral oophorectomy. The ovaries, cleaned of adipose tissue and divided into 2 equal parts, were sutured to the vascular plexus of the peritoneal wall. We then closed the abdominal layers and skin.

The groups were as follows: group 1 (G1; n = 7) was the control (Sham) group that underwent abdominal incision and closure; group 2 (G2; n = 7) was the transplant only (TR) group; group 3 (G3; n = 7) was the transplant group that received corn oil (TR+OIL) as the solvent (0.5 mL/day); group 4 (G4; n = 7) was the transplant group that received curcumin (TR+CUR) (C1386, Sigma) (100 mg/kg/day); group 5 (G5; n = 7) was the transplant group that received gallic acid (TR+GA) (G7384, Sigma) (20 mg/kg/day); and group 6 (G6; n = 7) was the transplant group that received a combination of gallic acid and curcumin (TR+GA+CUR) (100 mg/kg/day and 20 mg/kg/day, respectively).

We selected doses of curcumin (dissolved in 0.5 mL corn oil) and gallic acid (dissolved in 0.5 mL distilled water) based on previous results. We administered doses orally via gavage to the rats 1 day before ovarian transplant and continued administration for 14 days after surgery. On postoperative day 14, all rats were euthanized under ketamine-xylazine anesthesia, and laparotomy was performed to collect liver and kidney tissues, which were then fixed in 10% buffered neutral formalin for histopathological examination.

Histopathological analyses

Liver and kidney tissues were fixed in 10% neutral buffered formalin for 48 hours. After fixation, we dehydrated tissues by using graded alcohols and then embedded tissues in paraffin blocks. We cut thin sections (5 μm) from the paraffin blocks containing liver and kidney tissues using a microtome (Thermo Scientific) and mounted sections on slides. We subjected the sections to physical deparaffinization in an oven and to chemical deparaffinization in xylene. Subsequently, we stained sections with hematoxylin and eosin (H&E) dye and periodic acid Schiff (PAS) stain and put coverslips on the slides.

We graded liver damage according to Suzuki and colleagues.[4] Kidney damage was graded according to the Solez score. We examined apoptosis markers BAX and BCL-2 immunohistochemically. We dewaxed sections mounted on positively charged slides in xylene, rehydrated samples in graded alcohol, and treated samples with a heat-induced epitope retrieval solution. Microwave irradiation was performed for 20 minutes, followed by cooling and washing in phosphate-buffered saline (PBS)-Tween 20. To block endogenous peroxidase, we incubated sections with 3% H_2O_2 for 30 minutes at room temperature. After washing sections in PBS-Tween 20, we performed blocking with normal goat serum for 1 hour at room temperature. We added primary antibodies, anti-BAX (1:100; mouse monoclonal antibody, sc-23959, Santa Cruz Biotechnology) and anti-BCL-2 (1:500; mouse monoclonal antibody, sc-7382, Santa Cruz Biotechnology), and incubated samples overnight at 4 °C. We prepared control slides by omitting the primary antibodies. After 4 washes in PBS-Tween 20, we incubated the sections with FITC-conjugated secondary antibodies (1:500; goat anti-mouse IgG-FITC, sc-2010, Santa Cruz Biotechnology) for 1 hour at 37 °C. After additional washes, we stained samples with 4′,6-diamidino-2-phenylindole for 15 minutes at room temperature and mounted them using a PBS-glycerol (1:1) solution.

We evaluated the stained liver and kidney tissues histopathologically and by using immunofluorescence microscopy. Photomicrographs of the stained tissues were captured using a digital camera (Zeiss, Axiocam 105 Color) mounted on a light microscope (Zeiss, Scope Al). We quantified fluorescence intensity with the image analysis software ImageJ.

Biochemical examination

Before homogenization, we weighed rat liver and kidney tissues. We minced tissues into small pieces and homogenized samples in PBS at a ratio of 1:9 (wt/vol) using a homogenizer (Qiagen TissueLyser LT II). We then centrifuged homogenates at 10 000 g for 5 minutes and collected resulting supernatants. We measured levels of IL-6 in the supernatant samples with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Bostonchem, Rat IL-6 ELISA Kit, BLS-1185Ra) following the manufacturer's protocol. We generated a standard curve graph and equation based on the absorbance values of the standard samples, allowing for the calculation of tissue IL-6 concentrations.

Statistical analyses

We used the SPSS program for statistical analysis. We reported quantitative variables as mean and standard deviation and categorical variables as frequencies and percentages. We examined variable distribution with the Shapiro-Wilk test. We assessed differences with 1-way analysis of variance and independent sample t test for customarily distributed variables. For nonnormally distributed variables, we examined differences with the Kruskal-Wallis H and

Mann-Whitney U tests. We analyzed dependence between categorical variables with the chi-square test. We assessed relationships between 2 variables with the Pearson correlation test (for variables showing normal distribution) or the Spearman correlation test (for variables not showing normal distribution). Effects between dependent and independent variables were investigated using regression analyses. $P < .05$ was considered statistically significant.

Results

Histopathological examination and evaluation were performed to assess distant organ damage in liver and kidney tissues of all ovarian transplant groups and control groups. Fourteen areas of liver and kidney tissues were examined from each group, and damage scoring was conducted. According to the Suzuki scoring system in the liver by histological observation, the Suzuki score was significantly higher in the ovarian transplant only group and the ovarian transplant group that received corn oil compared with the control and treatment groups ($P < .001$) (Table 1). The Suzuki score was shown to be lower in all 3 treatment groups than in the ovarian transplant group and the ovarian transplant group that received corn oil.

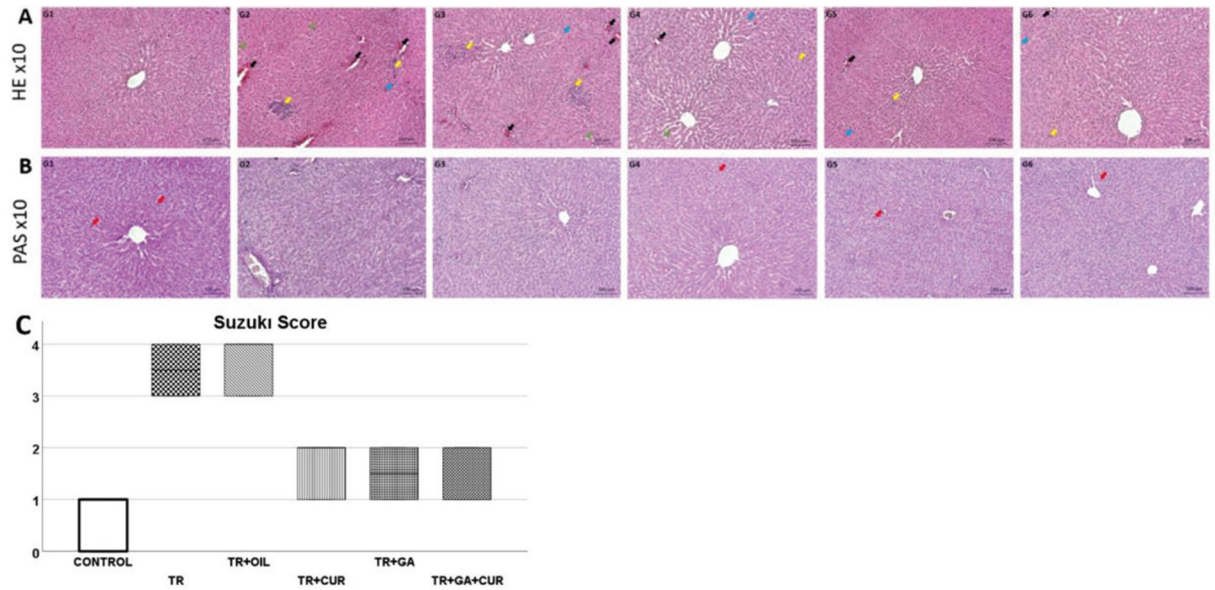
Several important observations were made from microscopic images of liver tissues stained with H&E (Figure 1A). Notably, areas of inflammation showed infiltration of immune cells into the liver parenchyma. Congestion in areas of inflammation suggested impaired blood flow because of IRI. Bleeding indicated extravasation of blood within the tissue, likely because of injury that incurred during the transplant process. In addition, we also observed vacuolization, which may indicate cellular damage and altered lipid metabolism. These findings collectively emphasize the extensive effect of IRI on the liver tissue.

Table 1. Suzuki Scoring in Rat Liver Tissue

Group	No.	Median Score (minimum-maximum)	*P*	Difference
G1 (control)	14	1 (0-1)	<.001	1<2; 1<3
G2 (TR)	14	3.5 (3-4)		4<2; 4<3
G3 (TR+OIL)	14	4 (3-4)		5<2; 5<3
G4 (TR+CUR)	14	2 (1-2)		6<2; 6<3
G5 (TR+GAL)	14	1.5 (1-2)		
G6 (TR+CUR+GAL)	14	1 (1-2)		

Abbreviations: G, group; TR, transplant only; TR+OIL, transplant plus corn oil; TR+CUR, transplant plus curcumin; TR+GA, transplant plus gallic acid; TR+GA+CUR, transplant plus curcumin plus gallic acid

Figure 1. Microscopic Images of the Liver



Abbreviations: G, group; H&E, hematoxylin and eosin; PAS, periodic acid Schiff; TR, transplant only; TR+OIL, transplant plus corn oil; TR+CUR, transplant plus curcumin; TR+GA, transplant plus gallic acid; TR+GA+CUR, transplant plus curcumin plus gallic acid

A, H&E staining of the liver, showing inflammation (yellow arrows), congestion (black arrows), hemorrhage (blue arrows), and vacuolization (green arrows). **B,** PAS staining of the liver. Severe PAS+ cells are observed in the G1 control group. TR and TR+OIL groups also show PAS+ cells. Treatment groups have moderate PAS+ cells (red arrows). **C,** Suzuki scores were based on vacuolation, congestion, and necrosis. Ten random fields per section were examined in each sample. Each bar represents mean ± SD.

In liver tissue sections with PAS staining, we noted variations in PAS staining intensity observed among the different groups. Group 1 (control group) displayed a notably high PAS+ cell presence. In contrast, groups 2 and 3 exhibited fewer PAS+ cells. Moderate PAS+ cells were observed in groups 4, 5, and 6. These variations in PAS staining highlighted potential differences in glycogen metabolism and storage capacities among the treatment groups.

The histological scoring system developed by Suzuki and colleagues was used to provide quantitative assessment of the observed histopathological changes (Figure 1C), which considers parameters such as vacuolation, congestion, and necrosis. Each section was analyzed in 10 random fields, and the resulting scores were averaged and presented as mean ± SD. This quantitative analysis further validated the trends observed in the microscopic images, offering comprehensive insight into the extent of liver damage and the potential protective effects of curcumin and gallic acid. Results showed that degree of injury and inflammation in kidney tissue, per the Solez score value, were significantly higher in the ovarian transplant only group and the ovarian transplant group that received corn oil compared with the control group ($P < .001$) (Table 2). Results showed that the score was lower in the treatment groups than in the ovarian transplant group.

Table 2. Solez Scoring in Rat Kidney Tissue

Group	No.	Median Score (minimum-maximum)	H	*P*	Difference
G1 (control)	14	0 (0-1)	21.743	<.001	1<2; 1<3
G2 (TR)	14	2 (1-3)			
G3 (TR+OIL)	14	2 (2-3)			
G4 (TR+CUR)	14	1 (1-3)			
G5 (TR+GAL)	14	2 (1-3)			
G6 (TR+CUR+GAL)	14	1 (0-2)			

Abbreviations: G, group; TR, transplant only; TR+OIL, transplant plus corn oil; TR+CUR, transplant plus curcumin; TR+GA, transplant plus gallic acid; TR+GA+CUR, transplant plus curcumin plus gallic acid

Microscopic images of H&E staining of the kidney revealed the presence of tubular dilatation, inflammation, and dilation of Bowman space (Figure 2A). Microscopic images of PAS staining of the kidney displayed tubular dilatation, Bowman space dilation, and interruptions in the basement membrane structure around the renal corpuscle (Figure 2B). Quantitative scoring was performed with the Solez scoring system, with scores assigned based on the extent of tubular dilatation, inflammation, dilatation of Bowman space, and interruptions in the basement membrane structure around the renal corpuscle. For each sample, 10 random fields per section were examined, and data were then represented as mean ± SD for each group. This comprehensive analysis provided valuable insight into the extent of kidney damage caused by IRI and the potential effects of curcumin and gallic acid.

Liver tissue showed significantly higher ($P < .001$) immunofluorescent BAX/BCL-2 intensity in the ovarian transplant group compared with the control group and group 6 (ovarian transplant group that received both gallic acid and curcumin) (Table 3; Figure 3). In addition, the ovarian transplant group that received corn oil also showed signi-ficantly higher intensity compared with the control group. Notably, the BAX/BCL-2 intensity value was lower in the treatment groups than in the ovarian and ovarian transplant groups receiving corn oil.

Kidney tissue showed significantly higher ($P = .02$) immunofluorescent BAX/BCL-2 intensity in the ovarian transplant group compared with the control group and group 6 (Table 4; Figure 4). In all treatment groups, immunofluorescent BAX/BCL-2 intensity value was lower than results shown in the ovarian transplant group.

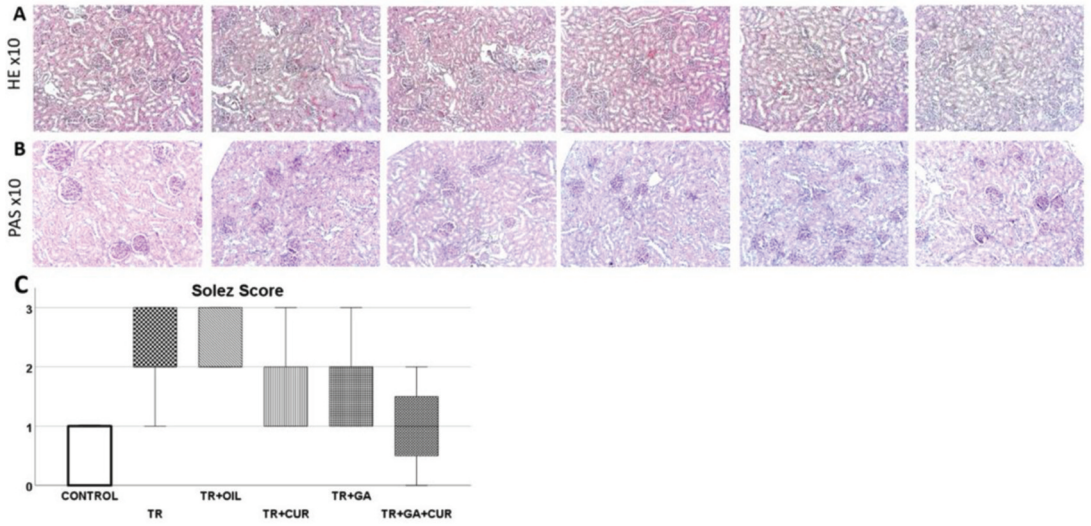
The IL-6 levels in kidney and liver tissues were measured using an ELISA procedure. Interleukin 6 levels were significantly increased in all ovarian

Table 3. Bax/Bcl-2 Intensity of Immunfloresence in Rat Liver Tissue

Group	No.	Median Score (minimum-maximum)	H	*P*	Difference
G1 (control)	6	0.36 (0.23-1.11)	22.763	<.001	1<3; 1<2; 6<2
G2 (TR)	6	2.4 (2.05-2.96)			
G3 (TR+OIL)	6	1.71 (0.89-4.7)			
G4 (TR+CUR)	6	0.65 (0.32-1.76)			
G5 (TR+GAL)	6	0.82 (0.19-1.87)			
G6 (TR+CUR+GAL)	6	0.44 (0.3-0.87)			

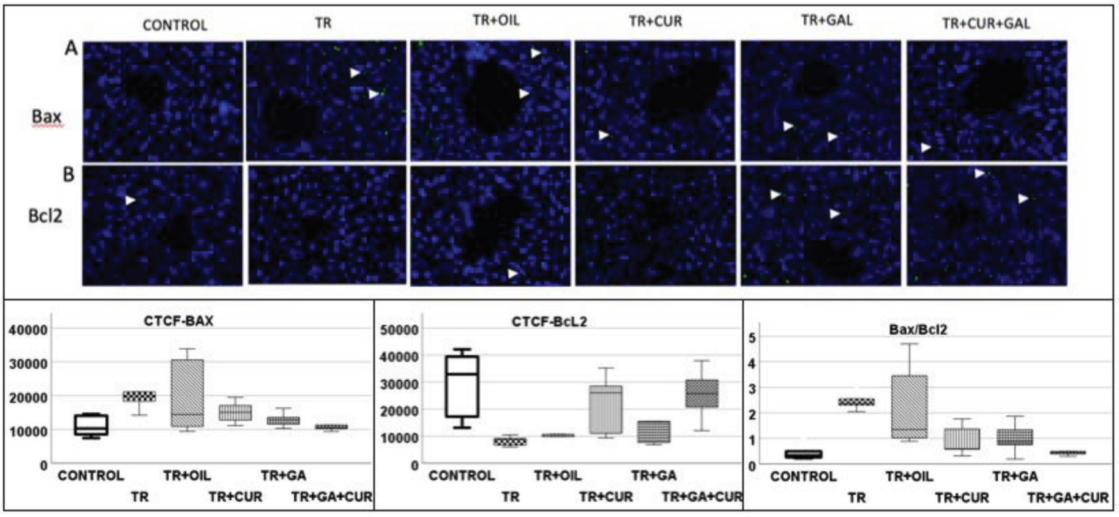
Abbreviations: G, group; TR, transplant only; TR+OIL, transplant plus corn oil; TR+CUR, transplant plus curcumin; TR+GA, transplant plus gallic acid; TR+GA+CUR, transplant plus curcumin plus gallic acid

Figure 2. Microscopic Images of the Kidney



Abbreviations: G, group; H&E, hematoxylin and eosin; PAS, periodic acid Schiff; TR, transplant only; TR+OIL, transplant plus corn oil; TR+CUR, transplant plus curcumin; TR+GA, transplant plus gallic acid; TR+GA+CUR, transplant plus curcumin plus gallic acid
A, H&E staining of the kidney, showing tubular dilatation, inflammation, and Bowman space. **B,** PAS staining of the kidney, showing tubular dilatation, Bowman space dilation, and interruption in the basement membrane structure surrounding the renal corpuscle. **C,** Solez scores were based on tubular dilatation, inflammation, dilatation of Bowman space, and interruptions in the basement membrane structure surrounding the renal corpuscle. Ten random fields per section were examined in each sample. Each bar represents mean ± SD.

Figure 3. Microscopic Images of Immunflourescence Staining of BAX and BCL2 in Liver and Normalized Total Cell Fluorescence Results



Abbreviations: G, group; TR, transplant only; TR+OIL, transplant plus corn oil; TR+CUR, transplant plus curcumin; TR+GA, transplant plus gallic acid; TR+GA+CUR, transplant plus curcumin plus gallic acid
A, Immunflourescence staining of Bax in liver. **B,** Immunflourescence staining of Bcl2 in liver. White arrows show Bax+ and Bcl2+ cells. Green indicates Bax and Bcl-2. Blue indicates 4′,6-diamidino-2-phenylindole. Light microscopy magnification = ×20. **C,** Corrected total cell fluorescence (CTCF) was calculated by normalising the fluorescence intensity with the mean fluorescence of background.

transplant groups compared with the control group ($P < .001$) (Table 5).

Discussion

This study evaluated the effects of curcumin and gallic acid on liver and kidney damage caused by IRI in rats undergoing autologous intraperitoneal ovarian transplant. This study also evaluated the histopathologic results and distant organ damage in liver and kidney tissues of different treatment groups.

Histological results of liver tissues, assessed with the Suzuki scoring system, showed that the ovarian transplant group and the transplant group that

Table 4. Bax/Bcl-2 Intensity of Immunfloresence in Rat Kidney Tissue

Group	No.	Median Score (minimum-maximum)	H	*P*	Difference
G1 (control)	5	0.3 (0.14-1.1)	18.662	.02	1<2; 6<2
G2 (TR)	5	1.71 (1.09-9.97)			
G3 (TR+OIL)	5	1.58 (0.85-24.26)			
G4 (TR+CUR)	5	0.64 (0.36-1.36)			
G5 (TR+GAL)	5	0.62 (0.38-0.92)			
G6 (TR+CUR+GAL)	5	0.46 (0.3-0.67)			

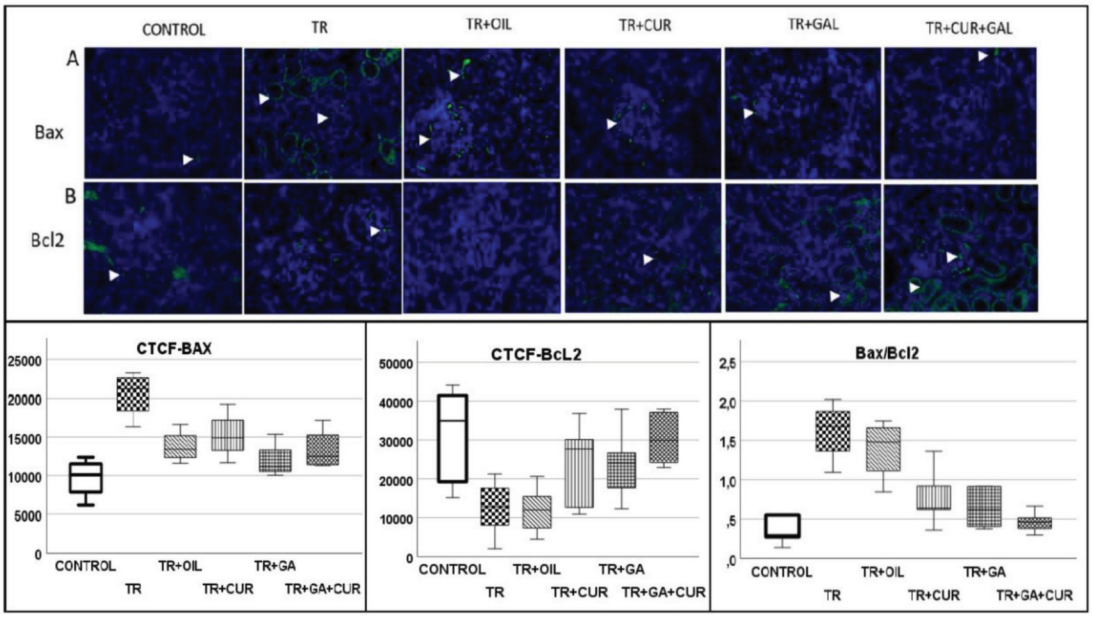
Abbreviations: G, group; TR, transplant only; TR+OIL, transplant plus corn oil; TR+CUR, transplant plus curcumin; TR+GA, transplant plus gallic acid; TR+GA+CUR, transplant plus curcumin plus gallic acid

Table 5. Interleukin 6 Levels in Rat Kidney and Liver Tissues

Group	N.	Level, mean ± SD, pg/mL	*P*	Difference
Kidney				
G1 (control)	5	400.38 ± 41.56	<0.001	1<2; 3; 4; 5; 6
G2 (TR)	6	682.19 ± 179.55		
G3 (TR+OIL)	6	811.32 ± 89.1		
G4 (TR+CUR)	7	807.26 ± 86.87		
G5 (TR+GAL)	6	810.98 ± 53.69		
G6 (TR+CUR+GAL)	6	750.03 ± 81.26		
Liver				
G1 (control)	5	401.05 ± 30.57	<0.001	1<2; 3; 4; 5; 6
G2 (TR)	6	664.69 ± 96.28		
G3 (TR+OIL)	6	750.69 ± 77.67		
G4 (TR+CUR)	7	696.76 ± 121.6		
G5 (TR+GAL)	6	699.48 ± 121.47		
G6 (TR+CUR+GAL)	6	712.94 ± 101.97		

Abbreviations: G, group; TR, transplant only; TR+OIL, transplant plus corn oil; TR+CUR, transplant plus curcumin; TR+GA, transplant plus gallic acid; TR+GA+CUR, transplant plus curcumin plus gallic acid

Figure 4. Microscopic Images of Immunflourescence Staining of BAX and BCL2 in Kidney and Normalized Total Cell Fluorescence Results



Abbreviations: G, group; TR, transplant only; TR+OIL, transplant plus corn oil; TR+CUR, transplant plus curcumin; TR+GA, transplant plus gallic acid; TR+GA+CUR, transplant plus curcumin plus gallic acid

A, Immunflourescence staining of Bax in kidney. **B,** Immunflourescence staining of Bcl2 in kidney. White arrows show Bax+ and Bcl2+ cells. Green indicates Bax and Bcl-2. Blue indicates 4′,6-diamidino-2-phenylindole. Light microscopy magnification = ×20. **C,** Corrected total cell fluorescence (CTCF) was calculated by normalizing the fluorescence intensity with the mean fluorescence of background.

received corn oil had significantly higher Suzuki scores than the control and curcumin and gallic acid treatment groups. All 3 treatment groups had lower Suzuki scores than the ovarian transplant group and the transplant group that received corn oil. This finding suggests that treatment with curcumin and gallic acid helped reduce liver damage caused by IRI.

Immunohistochemistry results (Figure 1) provided a comprehensive assessment of the effects of curcumin and gallic acid on liver tissue subjected to IRI during autologous intraperitoneal ovarian transplant. Hematoxylin and eosin staining results revealed significant histopathological changes, with observations of inflammation, congestion, bleeding, and vacuolization. These alterations underscored the severity of IRI-induced damage to liver tissue. The presence of inflammation suggested an immune response triggered by the injury, possibly involving immune cell infiltration and cytokine release. The

congestion and hemorrhage results signified compromised blood flow and vascular damage associated with IRI. Vacuolization pointed to cellular alterations likely caused by oxidative stress and lipid metabolism disruption.

Results from PAS staining offered insight into glycogen metabolism alterations. The varying intensity of PAS staining among different groups indicated changes in glycogen accumulation. Group 1 (control group) exhibited high PAS+ cell presence, suggesting elevated glycogen accumulation. In contrast, reduced PAS+ cells in group 2 and group 3 may indicate impaired glycogen storage. The moderate PAS+ cells in groups 4, 5, and 6 imply balanced glycogen metabolism or potential protective effects of curcumin and gallic acid against excessive glycogen accumulation.

The Suzuki scoring system provided a rigorous assessment of tissue damage. The scores aligned with observations from microscopic images and PAS staining. The lowest score in control group 1 substantiated the lower tissue damage evident in H&E staining. Conversely, elevated scores in group 2 and group 3, possibly caused by IRI-induced damage, and distinctively lower scores in groups 4, 5, and 6 suggested potential mitigation of tissue damage, perhaps attributed to the treatments.

The integrated findings suggested that treatment with curcumin and gallic acid may confer protective effects against liver tissue damage induced by IRI. The collective evidence from H&E staining and PAS staining and the quantitative scoring system provided a comprehensive understanding of the histopathological changes and potential treatment effects. These results emphasize the need for further investigation into the underlying mechanisms by which these compounds exert their protective effects and their clinical implications in managing IRI-associated liver damage in the context of autologous intraperitoneal ovarian transplantation.

The Solez score, a histological scoring system for assessing injury and inflammation that we used to investigate rat kidney tissue, was significantly higher in the ovarian transplant group and the transplant group that received corn oil compared with the control group. However, the curcumin and gallic acid treatment groups had lower Solez scores compared with the ovarian transplant group, indicating that curcumin and gallic acid reduced kidney damage caused by IRI.

Pretreatment with either curcumin or gallic acid, as well as their combination, have been reported to help counteract the adverse effects of tramadol, an analgesic, and can induce oxidative stress in the liver and kidney due to its metabolism by the cytochrome P450 system. The activities and protein expression of cytochrome P450 and antioxidant enzymes were restored to normal levels in the liver and kidney after pretreatment with these antioxidants.[16] As evidenced by a previous study, proanthocyanidins, gallic acid, curcumin, and carotene decreased ROS levels in ovarian tissue compared with the control group. The study concluded that proanthocyanidins and gallic acid are potential antioxidants that can effectively reduce oxidative stress, specifically in ovarian tissue.[22] Another study revealed that, whereas gallic acid alone might induce oxidative stress and affect liver and renal function, the simultaneous administration of curcumin and gallic acid can counteract these effects. The antioxidative properties of curcumin can provide a protective role against gallic acid-induced changes in antioxidant enzymes and oxidative stress markers in the rat liver.[17]

The microscopic images from H&E staining demonstrated prominent pathological changes within the kidney tissues following IRI. Notably, the presence of tubular dilatation, indicative of impaired renal tubular function, and inflammation signified an inflammatory response as a result of injury. Bowman space dilation further indicated normal glomerular function and fluid filtration disruption.
The corresponding PAS staining images corroborated the findings from H&E staining. The notable tubular dilatation and Bowman space dilation accentuated the adverse effect of IRI on the renal microarchi-tecture. Moreover, interruptions in the basement membrane structure surrounding the renal corpuscle suggested compromised structural integrity of the glomerulus, likely contributing to impaired filtration function.

The observed tubular dilatation, inflammation, and disruptions in glomerular structures collectively signified functional and structural impairment, which can potentially lead to compromised renal function. The lowest score in control group 1 substantiated the lower tissue damage, whereas elevated scores in groups 2 and 3 evidently supported IRI-induced kidney injury. The use of curcumin and gallic acid in the treatment groups (groups 4, 5, and 6) could have mitigated some harmful effects. However, further investigation is

required to determine the extent of their protective effects on kidney tissue against IRI.

Overall, the microscopic and quantitative assessments in our study provide valuable insight into the pathophysiological changes induced by IRI and the potential efficacy of the tested compounds in ameliorating kidney damage. The findings underscore the importance of interventions that target the intricate interplay between inflammation, tissue integrity, and functional outcomes in the context of IRI-induced kidney injury.

The BAX and BCL-2 markers, analyzed with immunohistochemistry in liver and kidney tissues from rats that underwent ovarian transplantation, are essential proteins in regulating apoptosis, a process of programmed cell death. BAX is a proapoptotic protein that promotes cell death by initiating mitochondrial membrane permeabilization, which releases cytochrome c and subsequent activation of caspases. The increased BAX intensity that we observed with immunohistochemistry staining suggested an upregulation of proapoptotic signaling or an imbalance in the BAX-to-BCL-2 ratio, indicating a tendency toward apoptosis induction. Elevated intensity of BCL-2, an anti-apoptotic protein that inhibits cell death by preventing mitochondrial membrane permeabilization and maintaining the integrity of the mitochondria, detected by immunohistochemistry staining, may suggest a cellular attempt to counteract apoptosis or promote cell survival.[23,24]

Our analyses of the expression and intensity of BAX and BCL-2 in liver and kidney tissues of rats undergoing ovarian transplant aimed to investigate the apoptotic response and the potential modulation of apoptosis-related pathways after treatment with curcumin, gallic acid, and their combination. Evaluation of the BAX and BCL-2 markers provided insight into the balance between proapoptotic and antiapoptotic processes within the liver and kidney tissues, offering a deeper understanding of the cellular responses and potential therapeutic effects of the administered treatments in the context of ovarian transplant.

Immunofluorescent staining was performed to evaluate the BAX/BCL-2 intensity, which indicates apoptosis regulation. In the liver tissue, the BAX/BCL-2 intensity was significantly higher in the ovarian transplant group compared with the control group and the group that received both gallic acid and curcumin. Similarly, the ovarian transplant group that received corn oil also had significantly higher BAX/BCL-2 intensity than the control group. However, the treatment groups had lower BAX/BCL-2 intensity values than the ovarian transplant only group and the transplant group that received corn oil, indicating that curcumin and gallic acid treatment reduced apoptosis in the liver tissue.

In the kidney tissue, the BAX/BCL-2 intensity was significantly higher in the ovarian transplant group compared with the control group and the group that received both gallic acid and curcumin. However, all treatment groups had lower BAX/BCL-2 intensity values than the ovarian transplant group, suggesting that the treatment helped reduce apoptosis in the kidney tissue.

The results suggested that the treatment groups (groups 4, 5, and 6), which received curcumin, gallic acid, and their combination, had lower levels of BAX and BCL-2 compared with the transplant group that received corn oil (group 3) and the transplant only group (group 2).

The lower levels of BAX and BCL-2 in the treatment groups indicated a potential downregulation of apoptotic signaling, suggesting a reduction in the tendency toward apoptosis induction. This could imply that the administered treatments (curcumin, gallic acid, and their combination) may have exerted antiapoptotic effects within the liver and kidney tissues of rats undergoing ovarian transplant.

Group 1, which was the control group that underwent abdominal incision and closure without transplant or treatment, had the lowest levels of BAX and BCL-2 in both liver and kidney tissues. This finding suggested that the surgical procedure alone, without transplant or therapy, had minimal effects on apoptotic signaling.

Notably, group 6, which received the combination of gallic acid and curcumin, exhibited the second lowest levels of BAX and BCL-2 among all groups in liver and kidney tissues. This finding suggested a potential synergistic effect of gallic acid and curcumin in modulating apoptotic pathways, leading to a further reduction in apoptotic signaling compared with that shown in the individual treatment groups (group 4 and group 5).

Curcumin, gallic acid, and their combination may have had antiapoptotic effects in the liver and kidney tissues of rats undergoing ovarian transplant. Downregulation of BAX and BCL-2 levels suggested

a protective mechanism against apoptosis induction, which could contribute to preservation of cellular integrity and function in these tissues. Further investigation is necessary to elucidate the specific molecular mechanisms underlying these effects and to assess the functional consequences on tissue health and transplant outcomes.

With regard to levels of IL-6, a proinflammatory cytokine,[25,26] in the kidney and liver tissues, all groups that underwent ovarian transplant showed a significant increase in IL-6 levels compared with the control group. The significance of IL-6 levels in rat liver and kidney tissues with ovarian transplant indicated an inflammatory response or immune activation in these organs.[27] Interleukin 6 is a proinflammatory cytokine crucial in various physiological processes, including immune responses and tissue inflammation. The observed increase in IL-6 levels suggested that the ovarian transplant procedure may have triggered an inflammatory reaction in the liver and kidney tissues. This finding highlighted the potential effect of ovarian transplant on the local inflammatory milieu of these rat organs, providing valuable insight into the immune-related changes associated with this experimental intervention.[27] Further investigation and characterization of the specific mechanisms and implications of elevated IL-6 levels in these tissues are warranted to better understand the physiological and pathological consequences of ovarian transplant.

Levels of IL-6 are not solely indicative of an inflammatory response. Although IL-6 is critical in promoting inflammation, it has a broader range of functions and implications in various physiological and pathological processes. In addition to inflammation, IL-6 is involved in immune regulation, hematopoiesis, tissue regeneration, and the acute phase response. It also plays a role in modulating metabolic processes, such as glucose and lipid metabolism. In addition, IL-6 has been associated with the development and progression of certain diseases, including autoimmune disorders, cardiovascular diseases, cancer, and neurodegenerative conditions. Therefore, elevated IL-6 levels can signify not only inflammation but also immune dysregulation, tissue damage, altered metabolic homeostasis, and the presence of certain pathological conditions.[25,27] The interpretation of IL-6 levels should be considered in the context of the specific research or clinical scenario being investigated.

Therefore, elevated levels of IL-6 in the liver and kidney tissues of rats with ovarian transplant indicate an essential role of IL-6 in the response of these organs to the transplant procedure. Interleukin 6 is known to have pleiotropic effects on various cell types, including hepatocytes and renal cells. In the context of ovarian transplant, elevated levels of IL-6 in the liver and kidney may suggest the involvement of IL-6 in orchestrating inflammatory and immune responses within these tissues.

The increased IL-6 levels could reflect an immune system activation in response to the transplant procedure, potentially contributing to the recruitment and activation of immune cells in the liver and kidney. Introducing the transplanted ovaries or surgical manipulation may trigger this immune response. Furthermore, elevated levels of IL-6 in the liver and kidney tissues may also indicate tissue damage or injury from the transplant procedure.

The importance of elevated IL-6 levels in liver and kidney tissues of rats with ovarian transplant requires further investigation. Additional studies are needed to elucidate the specific mechanisms by which IL-6 affects these organs, the downstream signaling pathways involved, and the overall effect on tissue function and homeostasis. Such research can provide valuable insights into the complex interplay between IL-6, inflammation, immune responses, and tissue responses in the context of ovarian transplant.

In addition to further investigation of IL-6 levels in the liver and kidney tissues of rats with ovarian transplant, further investigations are needed to explore other serological markers to gain a comprehensive understanding of the underlying processes and potential implications. Alongside IL-6, measuring other proinflammatory cytokines such as TNF-α, IL-1β, and IL-8 can provide insight into the extent and nature of the inflammatory response. Examining the balance between proinflammatory and anti-inflammatory cytokines would further enhance the understanding of the immune modula-tion in liver and kidney tissues.

In summary, the results of this study indicate that treatment with curcumin and gallic acid can mitigate liver and kidney damage caused by IRI in a rat model of autologous intraperitoneal ovarian transplant. The treatments were associated with lower histological scores, reduced apoptosis, and potentially attenuated inflammation, as indicated by lower IL-6 levels. These

findings are consistent with the current literature on the potential protective effects of curcumin and gallic acid in various experimental models of organ damage and highlight their potential therapeutic value in ischemia-related conditions. However, it is essential to note that further studies and clinical trials are necessary to validate these findings and evaluate their relevance to human health.

Conclusions

Ovarian transplantation holds great promise for women who have infertility or ovarian failure. However, IRI remains a significant obstacle to the success of this procedure. The overproduction of ROS during ischemic conditions leads to oxidative damage, inflammation, and tissue swelling. Although several approaches have been studied to minimize the effects of IRI, further research is still required to determine the most effective method for reducing oxidative damage and improving the chances of successful ovarian transplantation.

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