Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2024;13(1):202-8

Protective effects of carvedilol against ovarian ischemia-reperfusion injury in rats

Savas Karakus[1], Mustafa Ozkaraca[2]

[1]Cumhuriyet University, Faculty of Medicine, Department of Obstetrics and Gynecology, Sivas, Türkiye
[2]Cumhuriyet University, Faculty of Veterinary Medicine, Department of Pathology, Sivas, Türkiye

Received 22 October 2023; Accepted 12 February 2024
Available online 28.02.2024 with doi: 10.5455/medscience.2023.10.212

Abstract

Ovarian torsion is a gynecological emergency that can lead to ovarian ischemia-reperfusion injury (IRI). This study aimed to investigate the potential protective effects of carvedilol against ovarian IRI in rats. A total of 24 female rats were divided into four groups: control, ischemia (I), ischemia-reperfusion (IR), and ischemia-reperfusion with carvedilol treatment (IR-C). Ovarian ischemia was induced by clamping the ovarian pedicle for 4 hours, followed by reperfusion for 4 hours. The IR-C group received carvedilol treatment 30 minutes before reperfusion. After the experiment, the ovaries were removed and evaluated for hemorrhage, edema, and immunopositivity of Caspase 3, apoptosis-inducing factor (AIF), Microtubule-associated protein 1 light chain 3b (LC3B), and Tumor necrosis factor-alpha (TNFa). In the staining performed with Caspase 3 and AIF, moderate immunopositivity was observed in the I and IR groups ($p<0.001$), while the IR-C group showed mild immunopositivity. In the stainings performed with LC3B and TNFa, the I and IR groups exhibited severe immunopositivity ($p<0.001$), whereas in the IR-C group, TNFa showed moderate immunopositivity ($p<0.001$), and LC3B showed mild immunopositivity ($p<0.001$). The results suggest that carvedilol may have protective effects against ovarian IRI in rats.

Keywords: Carvedilol, ovarian ischemia-reperfusion, rats, immunopositivity

Introduction

Ischemia-reperfusion injury (IRI) is a multifaceted physiological phenomenon that arises when tissue regains blood flow subsequent to ischemia or oxygen deprivation. This process involves local and systemic effects, including cell energy depletion during ischemia and oxidative and microcirculatory stress, inflammation, and apoptosis during reperfusion [1,2].

Ovarian torsion is a gynecological emergency that can lead to ovarian IRI. Reestablishing blood flow to the ischemic ovary is crucial for averting irreversible cellular damage; nonetheless, the process of reperfusion itself can exacerbate tissue injury beyond the extent caused by ischemia in isolation. The restoration of blood flow to the ischemic tissue results in severe metabolic dysregulation and destructive alterations in tissue structure, ultimately causing dysfunction or complete failure of the ovary [3,4].

In various organs, including the heart, liver, and kidney, carvedilol, a non-selective beta-blocker endowed with antioxidant and anti-inflammatory properties, has exhibited a protective effect against IRI [5,6]. Although the potential protective impact of carvedilol for ovarian IRI has not been extensively studied, its mechanism of action in other organs may provide insights into how it could prevent ovarian IRI.

Carvedilol's antioxidant properties are attributed to its ability to scavenge reactive oxygen species (ROS) and suppress ROS generation [7]. ROS has a significant role in the pathogenesis of IRI, leading to oxidative stress, inflammation, and apoptosis [8]. By reducing ROS levels, carvedilol may help alleviate the oxidative stress and inflammation associated with IRI. In addition to its antioxidant effects, carvedilol has been shown to modulate the balance between pro- and anti-inflammatory cytokines [9]. This modulation may help reduce inflammation and tissue damage during the reperfusion phase of IRI. Furthermore, carvedilol has been found to improve calcium homeostasis and reduce the detrimental effects of ischemia and reperfusion injury in cardiomyocytes [10].

Caspase 3 [11,12], apoptosis-inducing factor (AIF) [13],

CITATION
Karakus S, Ozkaraca M Protective effects of carvedilol against ovarian ischemia-reperfusion injury in rats. Med Science. 2024;13(1):202-8.

Corresponding Author: Savas Karakus, Cumhuriyet University, Faculty of Medicine, Department of Obstetrics and Gynecology, Sivas, Türkiye
Email: karakussavas@yahoo.com

DOI: 10.5455/medscience.2023.10.212

Microtubule-associated protein 1 light chain 3b (LC3B) [12], and Tumor necrosis factor-alpha (TNFa) [12] are markers that play a significant role in cell death. Therefore, in this study, the immunopositivity of these markers was evaluated in rats to investigate the potential protective effects of carvedilol against ovarian IRI. While the specific mechanism of carvedilol in preventing ovarian IRI remains to be elucidated, its antioxidant, anti-inflammatory, and calcium homeostasis-improving properties may contribute to its protective effects. Therefore, the aim of this study is to evaluate the efficacy of carvedilol in preventing ovarian IRI in rats. The findings of this study may offer a foundation for future investigations exploring the potential therapeutic application of carvedilol in treating ovarian IRI.

Material and Methods

Experimental animals

This study involved the inclusion of 24 female adult Wistar Albino rats, with a weight range of 150 to 250 grams and an age range of 10 to 15 weeks. The experimental protocols were conducted at the Animal Research Laboratory affiliated with Cumhuriyet University. Food was made available to the rats without any restrictions (ad libitum), and they were kept in a controlled environment with a temperature of 22±2°C. The humidity was regulated, and the rats were exposed to a 12-hour light and dark cycle.

The study protocol received approval from the Ethical Committee on Animal Research at Cumhuriyet University (Approval No. 610/14.07.2023).

Surgical procedures and groups

Random assignment was employed to distribute the female rats into four groups:

Control group, referred to as the control group, comprised 6 rats. For this group, the abdominal wall was opened and closed without subjecting the rats to ovarian torsion and detorsion.

I group, referred to as the torsion ischemia (I) group, comprised 6 rats, and a laparotomy was conducted to induce ovarian ischemia. This was achieved by clamping the bilateral ovarian arteries for a period of 4 hours, followed by bilateral oophorectomy.

IR group, referred to as the ischemia/reperfusion (IR) group, consisted of 6 rats, and ischemia was induced by clamping the bilateral ovarian arteries for 4 hours. Subsequently, reperfusion was initiated by releasing the clamps for an additional 4 hours. The rats did not receive any medication. Bilateral oophorectomy was carried out as well.

IR-C group, referred to as the torsion/detorsion carvedilol prophylaxis group (IR-C), comprised 6 rats. Bilateral ovarian torsion was induced in this group. Thirty minutes prior to the start of the ovarian torsion procedure, the rats received a single oral (p.o.) dose of 10 mg/kg carvedilol. Detorsion was performed after 4 hours of ischemia. Bilateral oophorectomy was conducted after 4 hours of detorsion. The carvedilol utilized in the study was obtained from (Carvedilol; Lot No: P500265, Sigma Aldrich, China) and was administered via orogastric tubes after dissolving it in 1 mL of saline, following the same method employed in previous studies.

In the experimental group, the rats were administered a single 10 mg/kg dosage. For a continuous duration of three days preceding the onset of ischemia, the rats were orally given carvedilol at a dose of 10 mg/kg each day. Furthermore, the administration was timed to occur 30 minutes prior to the reperfusion stage.

A combination of ketamine hydrochloride (50 mg/kg Ketax; Wem, İstanbul, Türkiye) and injection 50 mg/kg sodium thiopental (Pental®, İE Ulugay İlaç Sanayi AŞ) was utilized to induce anesthesia in the rats. Subsequently, the abdominal region underwent depilation and was then disinfected using a povidone-iodine scrub. To visualize the uterine horns and adnexa, a laparotomy procedure was performed by making a 20 mm longitudinal incision in the central area of the lower abdomen.

To induce ischemia, a torsion model was employed, wherein atraumatic vascular clips were applied and rotated 360 degrees clockwise to the vascular pedicle 1 cm above and below each ovary bilaterally for a duration of 4 hours, similar to the methodology employed in previous research. The abdominal wall, encompassing the muscle-aponeurotic plane and the skin, was surgically closed using 3-0 vicryl (polyglactin 910, Ethicon, Somerville, New Jersey). A bilateral oophorectomy was performed on all groups one week following the initial surgery to facilitate histological scoring and biochemical evaluation. Biochemical markers were measured by collecting a 5-mL blood sample from the vena cava of each rat. To conclude the experiment, euthanasia of the rats was performed by administering an overdose of anesthetic agents.

Histopathological method

The ovarian tissues of the rats were identified by performing necropsies and then fixed in a 10% neutral formalin solution. The tissues were processed through routine alcohol-xylene steps and embedded in paraffin blocks. 5 µm-thick sections were taken onto poly-lysine-coated slides and stained with hematoxylin-eosin. Randomly selected 6 fields were assessed for hemorrhage and edematous changes according to Table 1.

Table 1. Histopathological scoring

Hemorrhage	Edema
Absent (-)	Absent (-)
<10% of the total area (+)	<5% of the total area (+)
10-30% of the total area (++)	5-10% of the total area (++)
>30% of the total area (+++)	>10% of the total area (+++)

Immunohistochemical method

5 µm-thick sections taken onto poly-lysine-coated slides will be passed through xylene and alcohol series, washed with PBS, and then subjected to 10 minutes of treatment in a 3% H2O2 solution to ensure endogenous peroxidase inactivation. An antigen

DOI: 10.5455/medscience.2023.10.212

retrieval solution will be applied for 2x5 minutes at 500 watts to expose the antigen in the tissues. Subsequently, the washed tissues with PBS were incubated overnight at +4ºC with primary antibodies at a dilution ratio of 1/200 for Caspase 3 (ElabScience, Catalog No. E-AB-30004), LC3B (Santa Cruz, Catalog No. sc-271625), IL1β (Santa Cruz, Catalog No. sc-52012), and TNFa (Santa Cruz, Catalog No. sc-133192). Following the manufacturer's instructions, the Large Volume Detection System: anti-Polyvalent, HRP (Thermofisher, Catalog No. TP-125-HL) was utilized as a secondary step. DAB (3,3'-Diaminobenzidine) was then employed as the chromogen for visualization. After counterstaining with Mayer's Hematoxylin, the slides were coated with Entellan and examined under a light microscope. Immunopositivity was assessed semiquantitatively, with the following categories: absent (-), mild (+), moderate (++), and severe (+++).

Statistical analysis

The obtained histopathological and immunohistochemical data were analyzed using SPSS 22.00 software. The non-parametric Kruskal-Wallis test was employed to assess the differences between the groups, and The Dunn test, a post hoc test, was utilized to identify the group that crea-ted the difference defined as having a p-value less than 0.05 ($p<0.05$).

Results

Histopathological findings

As shown in Table 2, statistically significant differences were found among the groups in the histopathological evaluation.

Statistically significant differences were found among the groups in the histopathological evaluation (Table 2). The ovaries in the control group exhibited a normal histological appearance. In the I and IR groups, severe levels of hemorrhage and edema were observed. In the IR-C group, hemorrhage was mild, while edema was moderate. The p values for hemorrhage and edema were found to be 0.001, indicating statistical significance.

Table 2. Values for hemorrhage and edema in different groups

Groups	Hemorrhage Median (min-max)	Edema Median (min-max)
Control	0 (0-0)[a]	0 (0-0)[a]
I	3 (2-3)[b]	3 (2-3)[b]
IR	3 (2-3)[b]	3 (2-3)[c]
IR-C	1 (1-1)[c]	1 (1-1)[c]
p	0.001***	0.001***

(*) The letters a, b, c indicates significant differences between groups in the same column ($p<0.05$*, $p<0.01$**, $p<0.001$***). I: ischemia, IR: ischemia-reperfusion, IR-C: ischemia-reperfusion/carvedilol. Kruskal wallis test, dunn test

The ovaries in the control group exhibited a normal histological appearance. In the I and IR groups, severe levels of hemorrhage and edema were observed. In the IR-C group, hemorrhage was mild, while edema was moderate (Figure 1).

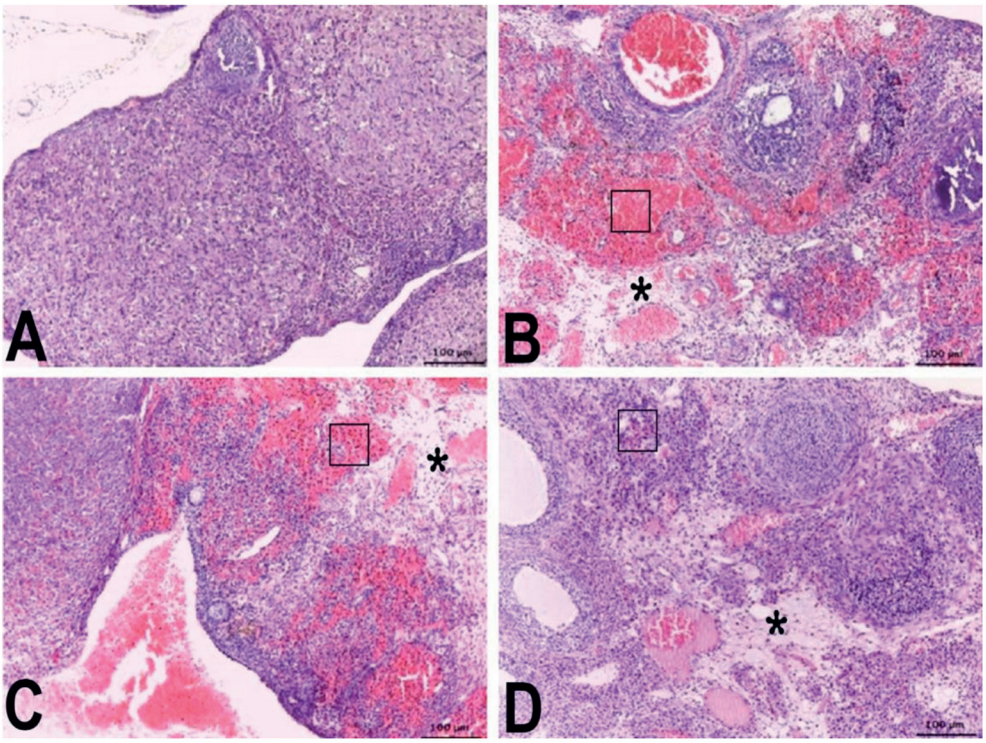

Figure 1. A. Control group: It shows a normal histological appearance, **1B.** (I) group: Severe hemorrhage (□) and edema (*) are observed, **1C.** (IR) group: Severe hemorrhage (□) and edema (*) are observed, **1D.** (IR-C) group: Mild hemorrhage (□) and moderate edema (*) are observed

DOI: 10.5455/medscience.2023.10.212

Immunohistochemical findings

Statistically significant differences were found in the immunohistochemical staining. The results showed moderate immunopositivity in the I and IR groups for Caspase 3 and AIF, while the IR-C group showed mild immunopositivity. In the case of LC3B and TNFa, the I and IR groups exhibited severe immunopositivity, whereas the IR-C group showed moderate immunopositivity for TNFa and mild immunopositivity for LC3B. (Figures 2-5). The p values for Caspase 3, AIF, LC3B, and TNFa were all found to be 0.001, indicating statistical significance. (Table 3).

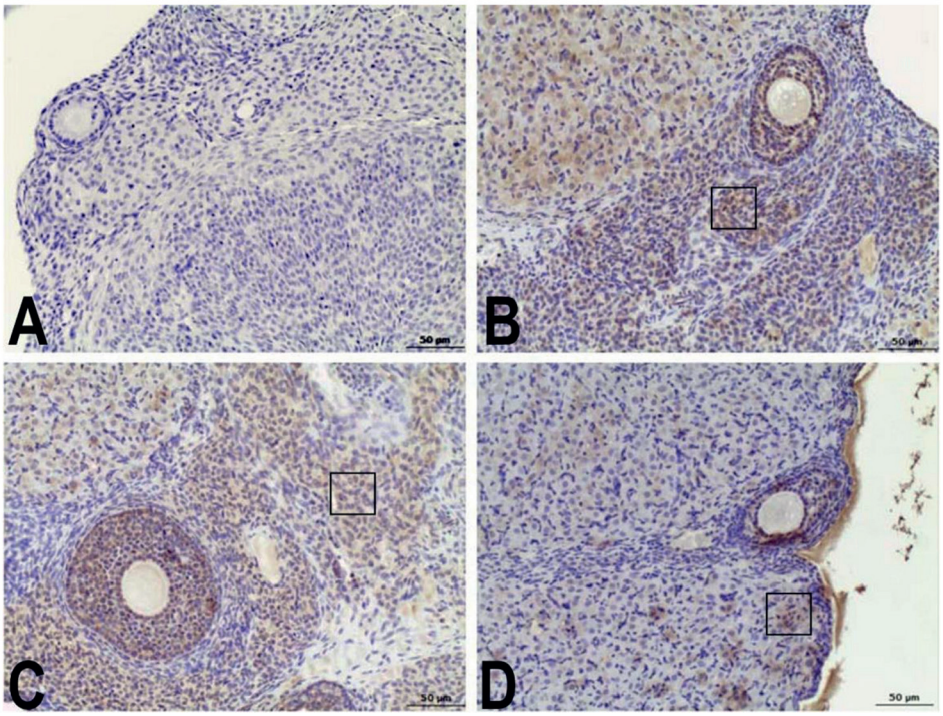

Figure 2. A. Control group: Immunonegativity is observed, **2B.** (I) group: Moderate level of Caspase 3 immunopositivity (□) is observed, **2C.** (IR) group: Moderate level of Caspase 3 immunopositivity (□) is observed, **2D.** (IR-C) group: Mild level of Caspase 3 immunopositivity (□) is observed

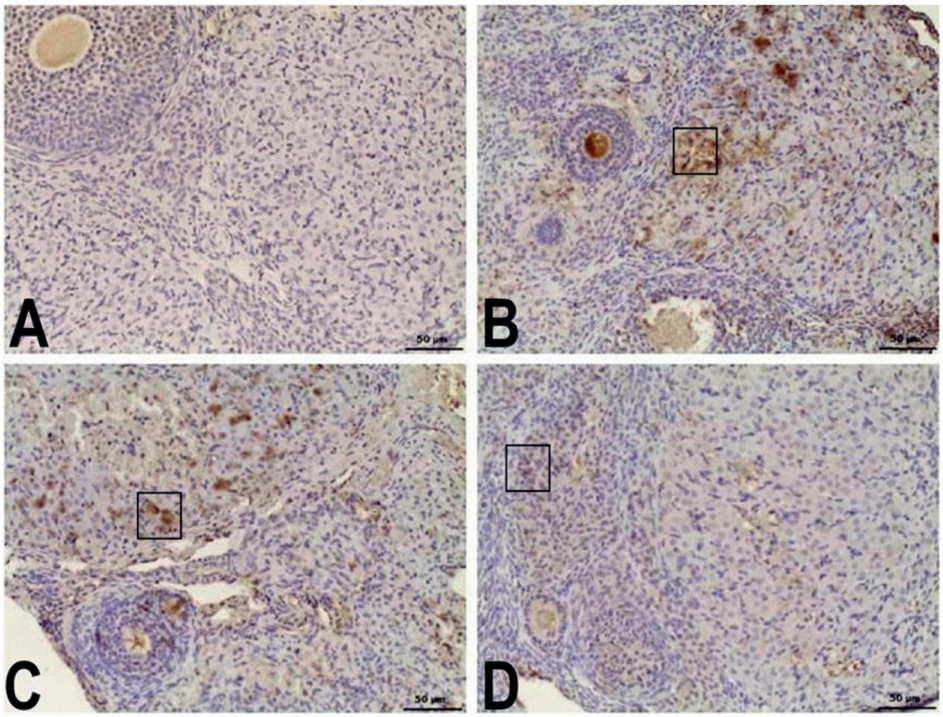

Figure 3. A. Control group: Immunonegativity is observed, **3B.** (I) group: Moderate level of AIF immunopositivity (□) is observed, **3C.** (IR) group: Moderate level of AIF immunopositivity (□) is observed, **3D.** (IR-C) group: Mild level of AIF immunopositivity (□) is observed

DOI: 10.5455/medscience.2023.10.212

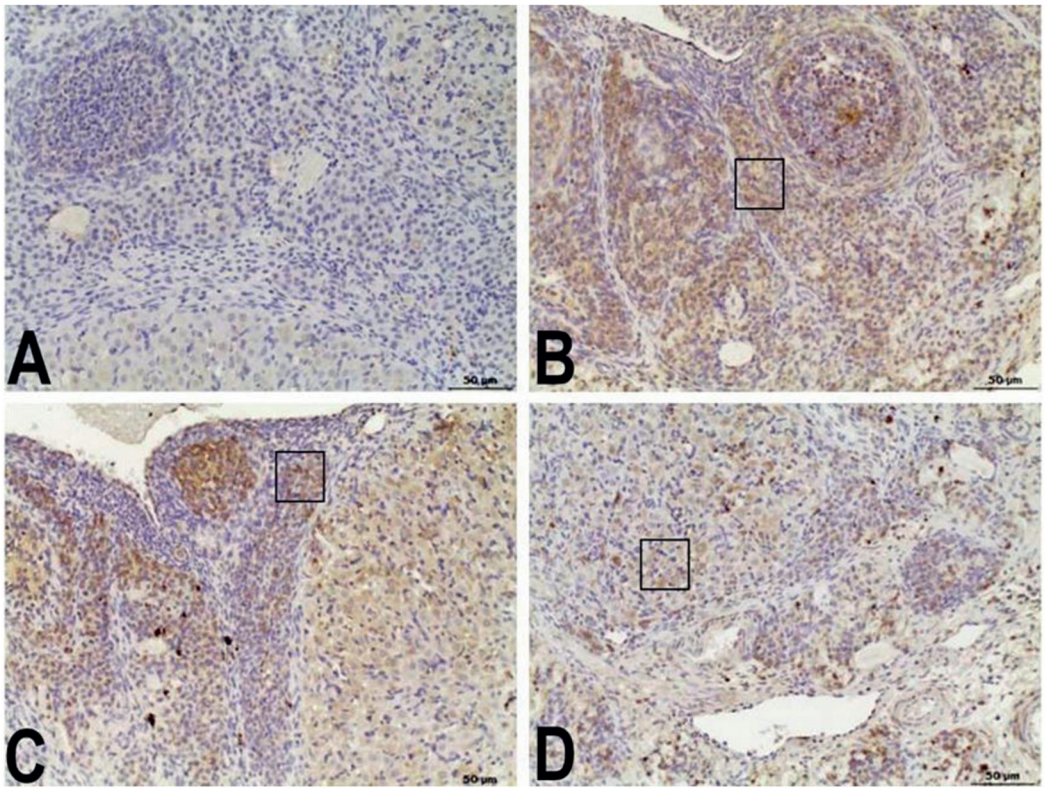

Figure 4. A. Control group: Immunonegativity is observed, **4B.** (I) group: Severe level of LC3B immunopositivity (□) is observed, **4C.** (IR) group: Severe level of LC3B immunopositivity (□) is observed, **4D.** (IR-C) group: Mild level of LC3B immunopositivity (□) is observed

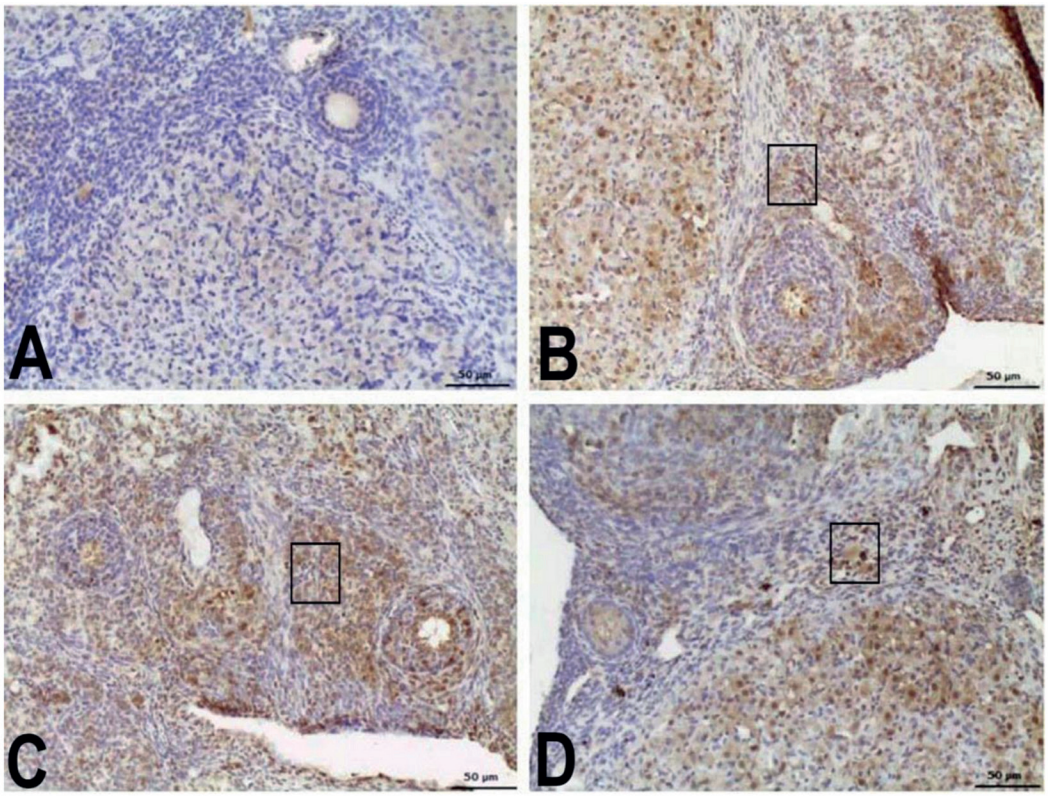

Figure 5. A. Control group: Immunonegativity is observed, **5B.** (I) group: Severe level of TNFa immunopositivity (□) is observed, **5C.** (IR) group: Severe level of TNFa immunopositivity (□) is observed, **5D.** (IR-C) group: Moderate level of TNFa immunopositivity (□) is observed

DOI: 10.5455/medscience.2023.10.212

Table 3. Values for Caspase 3, AIF, LC3B, and TNFa in different groups

Groups	Caspase 3 Median (min-max)	AIF Median (min-max)	LC3B Median (min-max)	TNFa Median (min-max)
Control	0 (0-0)[a]	0 (0-0)[a]	0 (0-0)[a]	0 (0-1)[a]
I	2 (1-2)[b]	2 (2-2)[b]	3 (2-3)[b]	3 (3-3)[b]
IR	2 (1-2)[b]	2 (2-2)[c]	3 (2-3)[b]	3 (3-3)[b]
IR-C	1 (0-1)[c]	1 (0-1)[c]	1 (1-2)[c]	2 (2-3)[c]
p	0.001***	0.001***	0.001***	0.001***

The letters a, b, and c indicate significant differences between groups in the same column ($p<0.05$*, $p<0.01$**, $p<0.001$***) kruskall Wallis, dunn test

Discussion

The objective of the study was to assess the effectiveness of carvedilol in preventing ovarian IRI in rats. Therefore, the results suggest that carvedilol could be potentially effective in mitigating ovarian ischemia-reperfusion damage in rats. Carvedilol improved calcium homeostasis under hypoxia conditions and improved the function of the post-ischemia left ventricle in mouse hearts. This study also found that carvedilol reduced the size of the infarcted myocardium and improved cardiac functions [14]. This is similar to the findings in our study, where carvedilol reduced the severity of damage in the ovarian tissue. Another study found that carvedilol administration was associated with a decrease in dilatation and congestion in the vena centralis, along with regenerative changes observed in hepatocyte cells. This study also found that carvedilol demonstrated a preventive effect against the initiation of oxidative stress, induction of inflammation, and progression of apoptosis [15]. These findings are consistent with the results in our study, where carvedilol reduced the severity of hemorrhage and edema and decreased the immunopositivity of markers of cell death and inflammation.

Another study found mild histopathological changes related to ovarian tissue injury in the acute phase [16]. This is similar to the findings in our study, where carvedilol reduced the severity of damage in the ovarian tissue. Another study found that carvedilol ameliorated IRI and improved renal function [17]. This is consistent with the results in our study, where carvedilol reduced the severity of ovarian IRI. Therefore, our findings are consistent with the existing literature on the protective effects of carvedilol in IRI.

Regarding the immunohistochemical findings, one study found that carvedilol was shown to have a cardio protective effect by increasing miR-133 expression and inhibiting caspase-9 and the subsequent apoptotic pathways in cardiomyocytes. This study demonstrated that carvedilol reduced the expression of caspase-3 in the presence of H2O2, which is similar to the findings in our study, where carvedilol reduced the immunopositivity of Caspase 3 in the ovarian tissue of rats [18].

Another study performed immunohistochemical staining for Caspase 3, AIF, LC3B, and TNFa and mentioned that carvedilol has antioxidant properties and may offer protection against cardiac mitochondrial toxicity induced by doxorubicin. The results suggest that carvedilol may have a beneficial effect in protecting cells from damage, which is consistent with the findings in our study, where carvedilol reduced the severity of ovarian IRI [19]. These studies suggest that carvedilol has potential protective effects against cell damage, which is consistent with our findings. Our study found statistically significant differences in the immunohistochemical staining performed with Caspase 3, AIF, LC3B, and TNFa. The I and IR groups showed moderate to severe immunopositivity for these markers, indicating a high cellular stress and damage level. However, the IR-C group showed only mild to moderate immunopositivity, suggesting that carvedilol may have reduced the cellular stress and damage associated with ischemia and reperfusion.

The mechanism by which carvedilol exerts these protective effects is likely multifactorial. Carvedilol, characterized as a non-selective beta-blocker with antioxidant and anti-inflammatory properties, has been reported to offer protection against IRI in multiple organs, including the heart, liver, and kidney [20]. Carvedilol's protective effects against IRI are attributed to its ability to ROS and suppress ROS generation. By doing so, it reduces oxidative stress, inflammation, and apoptotic processes associated with IRI [15].In the context of our study, carvedilol's antioxidant properties may have helped alleviate the oxidative stress and inflammation associated with IRI in the ovarian tissue. By reducing ROS levels, carvedilol may have mitigated the damaging effects of oxidative stress, thereby reducing inflammation and apoptosis. In addition to its antioxidant effects, carvedilol has been shown to modulate the balance between pro- and anti-inflammatory cytokines [21]. This modulation may have helped reduce inflammation and tissue damage during the reperfusion phase of IRI in our study. The reduction in inflammation could be reflected in the mild immunopositivity observed for TNFa, a cell signaling protein involved in systemic inflammation, in the IR-C group treated with carvedilol.

Carvedilol has also been found to improve calcium homeostasis and reduce the detrimental effects of ischemia and reperfusion injury in cardiomyocytes [22]. While the specific mechanism of carvedilol in preventing ovarian IRI remains to be elucidated, its antioxidant, anti-inflammatory, and calcium homeostasis-improving properties may contribute to its protective effects.

DOI: 10.5455/medscience.2023.10.212

Furthermore, carvedilol has been shown to suppress autophagy and promote apoptosis in hepatic stellate cells, which could potentially explain the observed moderate immunopositivity for Caspase 3 and AIF, markers associated with apoptosis, in the IR-C group [23]. LC3B, a marker associated with autophagy, also showed mild immunopositivity in the IR-C group, suggesting that carvedilol may have suppressed autophagy in the ovarian tissue [24].

Our study has some limitations. Firstly, the study was conducted on rats, and the results may not necessarily apply to humans. Secondly, the sample size was relatively small, with only 24 rats included in the study. Finally, the study only evaluated the efficacy of carvedilol in preventing ovarian IRI and did not compare it to other treatments or interventions. It is important to note that while the results of this study are consistent with previous research on the protective effects of carvedilol in IRI, further research is needed to determine the efficacy of carvedilol in humans and to compare it to other treatments or interventions. Additionally, the small sample size and the fact that the study was conducted on rats may limit the generalizability of the findings to other populations.

Conclusion

The study suggests that carvedilol may effectively prevent ovarian IRI in rats. The results showed that the severity of hemorrhage and edema was reduced, and the immunopositivity of Caspase 3, AIF, LC3B, and TNFa was decreased in the group that received carvedilol. However, further research is needed to determine the efficacy of carvedilol in humans and to compare it to other treatments or interventions.

Conflict of Interests
The authors declare that there is no conflict of interest in the study.

Financial Disclosure
The authors declare that they have received no financial support for the study.

Ethical Approval
The study protocol received approval from the Ethical Committee on Animal Research at Cumhuriyet University (Approval No. 610/14.07.2023).

References

1. Soares ROS, Losada DM, Jordani MC, et al. Ischemia/reperfusion injury revisited: an overview of the latest pharmacological strategies. Int J Mol Sci. 2019;20:5034.

2. Kirmizi DA, Baser E, Okan A, et al. The effect of a natural molecule in ovary ischemia reperfusion damage: does lycopene protect ovary? Exp Anim. 2021;70:37-44.

3. Meng X, Xie W, Xu Q ,et al. Neuroprotective effects of radix scrophulariae on cerebral ischemia and reperfusion injury via MAPK pathways. Molecules. 2018;23:2401.

4. Kalyoncu Ş, Yilmaz B, Demir M, et al. Melatonin attenuates ovarian ischemia reperfusion injury in rats by decreasing oxidative stress index and peroxynitrite. Turk J Med Sci. 2020;50:1513-22.

5. Wang D, Chen Y, Jiang J, et al. Carvedilol has stronger anti-inflammation and anti-virus effects than metoprolol in murine model with coxsackievirus B3-induced viral myocarditis. Gene. 2014;547:195-201.

6. Ayashi S, Assareh AR, Jalali MT, et al. Role of antioxidant property of carvedilol in mild to moderate hypertensive patients: a prospective open-label study. Indian J Pharmacol. 2016;48:372-6.

7. Cleveland KH, Yeung S, Huang KM, et al. Phosphoproteome profiling provides insight into the mechanism of action for carvedilol-mediated cancer prevention. Mol Carcinog. 2018;57:997-1007.

8. Orellana-Urzúa S, Briones-Valdivieso C, Chichiarelli S, et al. Potential role of natural antioxidants in countering reperfusion injury in acute myocardial infarction and ischemic stroke. Antioxidants (Basel). 2023;12:1760.

9. Dandona P, Ghanim H, Brooks DP. Antioxidant activity of carvedilol in cardiovascular disease.J Hypertens. 2007;25:731-41.

10. Christopher TA, Lopez BL, Yue TL, et al. Carvedilol, a new beta-adrenoreceptor blocker, vasodilator and free-radical scavenger, exerts an anti-shock and endothelial protective effect in rat splanchnic ischemia and reperfusion. J Pharmacol Exp Ther. 1995;273:64-71.

11. Yurtcu N, Karademir D, Ergul M, et al. Anticancer activity of Heat Shock Protein 70 (HSP70) Inhibitor, JG-98, against human cervical cancer HeLa and ovarian cancer SKOV-3 cells. Med Science. 2023;12:20-5.

12. Park W, Wei S, Kim BS, et al. Diversity and complexity of cell death: a historical review. Exp Mol Med. 2023;55:1573-94. Erratum in: Exp Mol Med. 2023;55:2083.

13. Sevrioukova IF. Apoptosis-inducing factor: structure, function, and redox regulation. Antioxid Redox Signal. 2011;14:2545-79.

14. Hu H, Li X, Ren D, et al. The cardioprotective effects of carvedilol on ischemia and reperfusion injury by AMPK signaling pathway. Biomed Pharmacother. 2019;117:109106.

15. Kafadar MT, Gök MA. Effects of Carvedilol on liver ischemia-reperfusion injury in rats. Ulus Travma Acil Cerrahi Derg. 2022;28:885-93.

16. Parlaktas BS, Atilgan D, Gencten Y, et al. The effects of carvedilol on ischemia-reperfusion injury in the rat testis. Int Braz J Urol. 2014;40:109-17.

17. Hayashi T, De Velasco MA, Saitou Y, et al. Carvedilol protects tubular epithelial cells from ischemia-reperfusion injury by inhibiting oxidative stress. Int J Urol. 2010;17:989-95.

18. Xu C, Hu Y, Hou L, et al. β-Blocker carvedilol protects cardiomyocytes against oxidative stress-induced apoptosis by up-regulating miR-133 expression. J Mol Cell Cardiol. 2014;75:111-21.

19. Oliveira PJ, Bjork JA, Santos MS, et al. Carvedilol-mediated antioxidant protection against doxorubicin-induced cardiac mitochondrial toxicity. Toxicol Appl Pharmacol. 2004;200:159-68.

20. Özsoy AZ, Nursal AF, Arıcı A, et al. Effects of carvedilol on an ischemia/reperfusion model: biochemical, histopathological and immunohistochemical evaluation. J Obstet Gynaecol Res. 2016;42:1132-40.

21. Elfakhrany A, Abo-Elsoud RQEA, Kareem HMA, et al. Autophagy and oxidative balance mediate the effect of Carvedilol and Glibenclamide in a rat model of renal ischemia-reperfusion injury. Open Access Macedonian Journal of Medical Sciences. 2022;10:1402-10.

22. Ragone MI, Bayley M, Colareda GA, et al. Cardioprotective mechanisms of hypothyroidism on ischemia/reperfusion in rats and effects of Carvedilol: energetic study. J Cardiovasc Pharmacol Ther. 2020;25:72-85.

23. Küçük M, Çakıcı ÖU, Işık Gönül İ, et al. Comparison of the preventive effects of Carvedilol and Nebivolol on kidney ischemia reperfusion injury. Turk J Nephrol. 2021;30:9-16.

24. Yue TL, Ma XL, Wang X, et al. Possible involvement of stress-activated protein kinase signaling pathway and Fas receptor expression in prevention of ischemia/reperfusion-induced cardiomyocyte apoptosis by carvedilol. Circ Res. 1998;82:166-74.