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Research Article
Role of Oxidant and Proinflammatory Cytokines and the Effect of Leflunomide in the Pathogenesis of Ovarian Ischemia-Reperfusion Injury

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Abstract

Background and Objective: Ischemia-reperfusion (IR) causes tissue damage by inducing oxidative stress and inflammation. In this study, the protective ability of leflunomide, which is reputed to have antioxidant and anti-inflammatory effects, against IR-induced ovarian damage in rats was researched. **Materials and Methods:** Twenty-four albino Wistar female rats were divided into 4 groups of six each: Healthy (HG), sham-operated (SOG), ovarian IR (OIR) and leflunomide+ovarian IR (LOIR). One hour before the IR procedure, the LOIR group received 10 mg/kg leflunomide orally. Ovaries of rats in OIR and LOIR groups were subjected to 3 hrs ischaemia and 6 hrs reperfusion. For six days, leflunomide were implemented once a day. On day seven, the animals were euthanized and their ovaries were collected. The tissues were examined biochemically and histopathologically. **Results:** Leflunomide treatment significantly suppressed IR-induced rise in malondialdehyde (MDA), Tumor Necrosis Factor-Alpha (TNF-α) and Interleukin 6 (IL-6) and the decline in Total Glutathione (tGSH), superoxide dismutase (SOD) and catalase (CAT) ($p<0.001$). Severe stromal hemorrhage, edema and follicular hemorrhage were noted in the ovaries of the IR group. Leflunomide treatment significantly prevented IR-induced ovarian tissue damage ($p<0.05$). **Conclusion:** Leflunomide could protect the ovaries with antioxidant and anti-inflammatory activity in ovarian IR injury.

Key words: Leflunomide, ischemia-reperfusion, oxidative stress, inflammation, ovarian damage, rat

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ischemia results from a restriction or total blockage of the blood supply to tissues or organs. It results in the deficiency or complete absence of oxygen and other nutrients in organs and tissues[1]. The delay in reperfusion of an ischemic area results in irreversible damage to organs and tissues. Thus, the first step in ischemic tissue treatment is to restore reperfusion[2]. The reperfusion of ischemic tissue, however, exacerbates ischemic tissue damage by inducing oxidative stress and inflammatory responses[3]. It is well-recognized that xanthine oxidase (XO) cannot convert hypoxanthine to xanthine in ischemic tissue (in the absence of oxygen). Therefore, XO and hypoxanthine accumulate in ischemic tissues excessively. When ischemic tissue is reoxygenated by reperfusion, XO accumulated in the tissue converts hypoxanthine to xanthine, resulting in the overproduction of reactive oxygen radicals (ROS)[4]. By oxidizing lipids in the cell membrane (LPO), ROS, also known as reperfusion mediator, produces toxic products such as malondialdehyde[5]. Moreover, reperfusion after ischemia (IR) results in oxidative and inflammatory damage to organs and tissues, as it decreases antioxidants and increases proinflammatory cytokines[6,7]. The IR contributes to morbidity and mortality in several disease states, including myocardial infarction, intestinal injury, trauma, circulatory arrest and sleep apnea. It is also a fundamental problem in organ transplantation and many surgical procedures[8]. Clinically, ovarian IR injury is a condition that occurs after the detorsion of ovaries that have been torn for various reasons[9]. The literature indicates that late detection and treatment of ovarian torsion can lead to severe ovarian injury and infertility[10]. Based on the literature, it appears that antioxidants and anti-inflammatory agents may be useful in treating the pathogenesis of IR injuries.

The compound leflunomide (N-[4-trifluoromethylphenyl]-methylisoxazole-4-carboxamide; HWA 486) tested against ovarian IR injury belongs to the malononitrile amide family[11]. As a Disease-Modifying Antirheumatic Drug (DMARD), leflunomide is approved by the FDA for the treatment of individuals with rheumatoid arthritis, exhibiting anti-inflammatory and immunomodulatory properties[12]. Leflunomide has a broad spectrum of antiviral action and is used to treat rheumatoid arthritis, lupus nephritis, primary Sjögren's syndrome, psoriatic arthritis, granulomatosis, giant cell arteritis and Takayasu arteritis[11]. In addition, leflunomide is preferred for the treatment of rheumatic diseases prevalent in women of childbearing age[13]. The antirheumatic activity of leflunomide and its metabolites has been reported in association with the inhibition of proinflammatory cytokines, including Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and Tumor Necrosis Factor-Alpha (TNF-α)[14]. As a result of its antioxidant properties, leflunomide has been reported to conserve intestinal tissue from IR injury[15]. The literature on the impact of leflunomide on IR injury is limited and leflunomide has not previously been tested in ovarian IR injury. The purpose of this trial was to evaluate the preventive effect of leflunomide on IR-related ovarian injury in rats biochemically and histopathologically.

MATERIALS AND METHODS

Study area: The experimental procedures of this study were carried out in the laboratories of Experimental Animals Application and Research Centre, Erzincan Binali Yildirim University, Turkey in May, 2024.

Animals: In total, 24 albino Wistar rats (270-282 g) were acquired from Medical Experimental Application and Research Center, Erzincan Binali Yildirim University, Turkey. Rats were kept in groups in the laboratory with 22-23°C, 12 hrs light/dark cycle and fed without restriction. The protocols and applications were approved by the local Animal Experimentation Ethics Committee (Date: 28.03.2024, meeting no: 15/11).

Drugs: Leflunomide (20 mg tablets) was purchased from Abdi İbrahim (Istanbul, Turkey) and ketamine (500 mg/10 mL solution for injection) from Pfizer (Istanbul, Turkey), sevoflurane (250 mL inhalation solution) from Abbvie (Istanbul, Turkey).

Animal groups: There were four groups of albino Wistar female rats (n = 6/each group): Healthy (HG), sham-operated (SOG), ovarian IR (OIR) and leflunomide+ovarian IR (LOIR).

Experimental procedure: The LOIR rats received leflunomide (10 mg/kg) orally 1 hr before anesthesia. In both the HG, SOG and OIR groups, pure water was administered in equal volumes via the same method. Ketamine (60 mg/kg) was administered intraperitoneally (i.p.) and sevoflurane was sniffed at appropriate intervals to anesthetize the animals. Surgical operations were conducted under anaesthesia while the rats remained supine and immobile[16]. A 2-2.5 cm long vertical opening was formed in the lower abdomen of SOG,

OIR and LOIR groups to allow access to the ovaries to perform surgical procedures. In the SOG group, the ovaries were sutured with surgical thread without any procedure being administered. The right ovaries, fallopian tubes and vasculature of OIR and LOIR rats were torsion clockwise 360 degrees and the ovaries were ischemic for 3 hrs using atraumatic clamps. A six-day detorsion and reperfusion procedure followed the removal of the clamps at the end of this period. For six days, leflunomide and pure water were administered once a day. On the seventh day, all animals were sacrificed with ketamine (120 mg/kg, i.p.) and their ovaries were collected. Malondialdehyde (MDA), Total Glutathione (tGSH), superoxide dismutase (SOD), catalase (CAT), TNF-α, IL-1β and IL-6 levels were determined in the excised ovarian tissues. Histopathological assessments were also conducted on the tissues. The biochemical and histopathological results obtained from all groups were compared and evaluated.

Biochemical analysis

MDA, GSH, SOD, CAT and protein analysis: To measure MDA (product no: 706002), GSH (product no: 703002) and SOD (product no: 10009055), Enzyme-Linked Immunosorbent Assay (ELISA) kits were used and kit instructions were respected for each assay (Cayman Chemical Company, Ann Arbor, Michigan, USA). The CAT was assayed using the technique proposed by Goth[17]. A spectrophotometric determination of protein at 595 nm was performed in accordance with the method of Bradford[18].

TNF-α, IL-1β and IL-6 analysis: The TNF-α, IL-1β and IL-6 levels were detected using Eastbiopharm Co. Ltd., ELISA rat kits (Hangzhou, Zhejiang, China) and kit instructions were respected for each assay.

Histopathologic method: The ovarian tissues were preserved in 10% formaldehyde following necropsy. The tissues were followed by alcohol-xylol and embedded in paraffin. Sections (5 µ) were colored with Hematoxylin and Eosin (H&E) and evaluated semi-quantitatively as absent (0), mild (1), moderate (2) and severe (3) in terms of stromal hemorrhage, follicular hemorrhage, follicular degeneration and congestion in 6 different random areas.

Statistical analysis: All statistical procedures were done with the statistical program "SPSS for Windows, 22.0". The biochemical results were analyzed using one-way ANOVA followed by Tukey's test. The data were expressed as "Mean±Standard Deviation" ($\bar{X}\pm SD$). The histopathologic results were analyzed with Kruskal-Wallis test followed by Mann-Whitney U test. The data were shown as median (quartile 1-quartile 3). The $p<0.05$ was regarded as statistical significance.

RESULTS

MDA, tGSH, SOD and CAT analysis results in ovarian tissues: The MDA amount in ovarian rats that underwent IR procedures (3.54±0.27) was higher than those of healthy (1.53±0.20) and sham-operated (1.61±0.25) rats ($p<0.001$). Leflunomide pretreatment inhibited IR-induced MDA increase ($p<0.001$). There was a resemblance between HG, SOG and LOIR (1.74±0.21) in terms of MDA data ($p>0.05$) (Fig. 1a).

As can be seen in Fig. 1(b-d), the increase in oxidants was accompanied by a decrease in antioxidants in the OIR group. The tGSH, SOD and CAT levels in the OIR ovaries (2.36±0.13, 3.18±0.12, 2.29±0.24, respectively) were noted to be decreased compared to the HG (4.56±0.17, 5.44±0.18, 4.68±0.12, respectively) and SOG (4.54±0.11, 5.33±0.17, 4.46±0.20, respectively) groups ($p<0.001$). Leflunomide significantly reduced IR-induced antioxidant decrease ($p<0.001$). The HG, SOG and LOIR data (4.34±0.12, 5.22±0.16, 4.41±0.17, respectively) were found to be similar in terms of SOD and CAT activities ($p>0.05$).

TNF-α, IL-1β and IL-6 analysis results in ovarian tissues: The TNF-α, IL-1β and IL-6 levels were found to be increased only in the group that underwent IR procedure (4.84±0.08, 4.38±0.17, 5.88±0.10, respectively) compared to the healthy (2.65±0.17, 2.27±0.17, 3.36±0.24, respectively) and sham-operated (2.79±0.18, 2.43±0.17, 3.49±0.33, respectively) groups ($p<0.001$). Leflunomide pretreatment was noted to prevent this IR-induced increase ($p<0.001$). Furthermore, TNF-α, IL-1β and IL-6 levels in the HG, SOG and LOIR (2.78±0.25, 2.33±0.59, 3.51±0.33, respectively) groups were observed to be similar ($p>0.05$) (Fig. 2a-c).

Histopathologic evaluation: As illustrated in Table 1 and Fig. 3a, the ovarian sections of the HG group had a normal histological appearance. In the sham operation group, no abnormality was found except mild stromal hemorrhage (Fig. 3b). Severe stromal hemorrhage, edema and follicular hemorrhage were noted in the ovaries of the IR group (Fig. 3c). Leflunomide treatment significantly prevented IR-induced ovarian tissue damage ($p<0.05$). Only mild stromal hemorrhage was observed in the LOIR group (Fig. 3d).

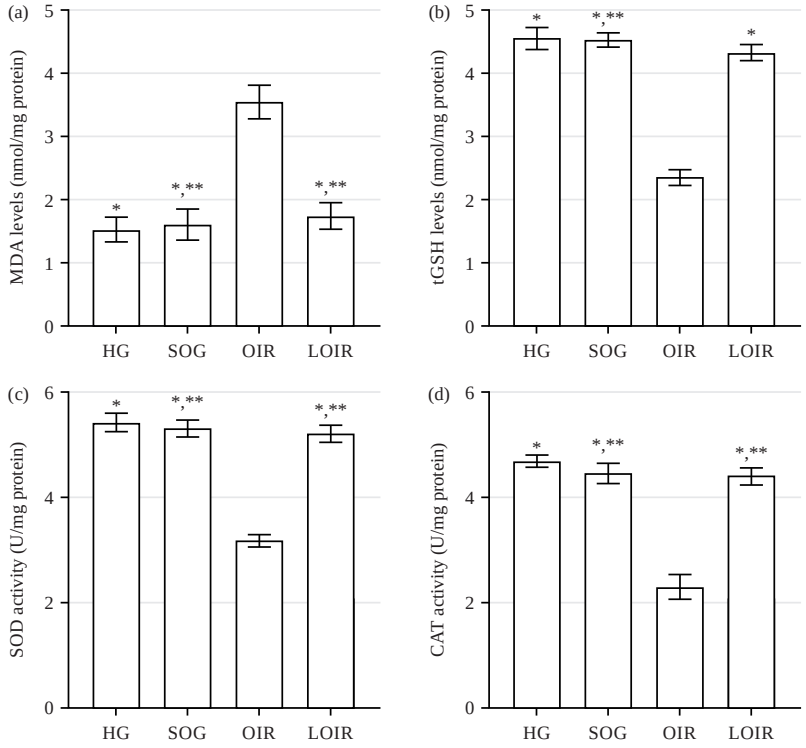

Fig. 1(a-d): Oxidant and antioxidant levels in ovarian tissues

Bars show Mean±Standard Deviation, n = 6, *p<0.001 vs OIR, SOG, **p>0.05 vs HG, MDA: Malondialdehyde, tGSH: Total Glutathione, SOD: Superoxide dismutase, CAT: Catalase, HG: Healthy group, SOG: Sham-operated group, OIR: Ovarian IR and LOIR: Leflunomide+ovarian IR

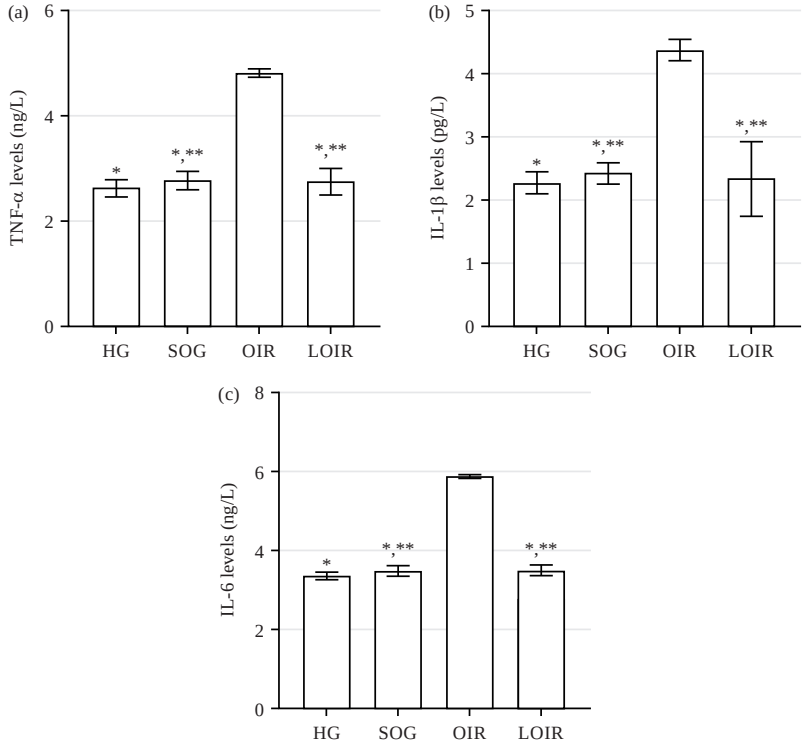

Fig. 2(a-c): Proinflammatory cytokine levels in ovarian tissues

Bars show Mean±Standard Deviation, n = 6, *p<0.001 vs OIR, SOG, **p>0.05 vs HG, TNF-α: Tumor Necrosis Factor-Alpha, IL-1β: Interleukin-1β, IL-6: Interleukin-6, HG: Healthy group, SOG: Sham-operated group, OIR: Ovarian IR and LOIR: Leflunomide+ovarian IR

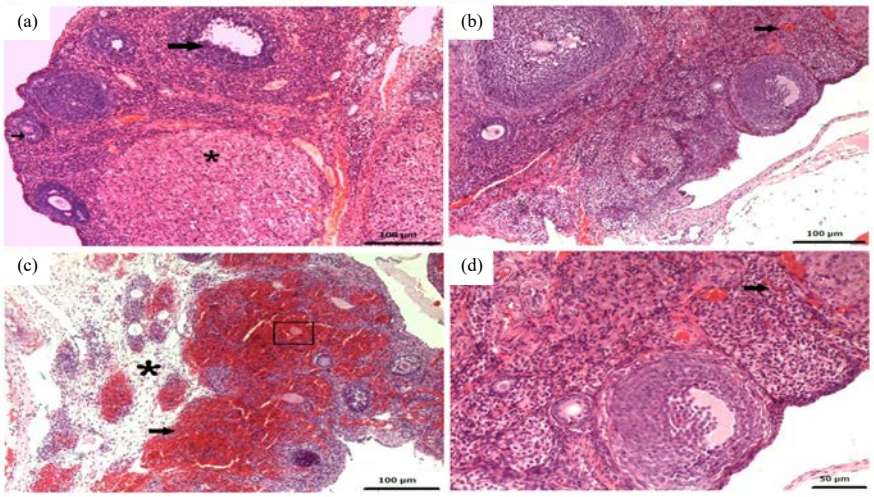

Fig. 3(a-d): Histopathological appearances of ovarian tissues obtained from the study groups, (a) HG, (b) SOG, (c) OIR and (d) LOIR group

(a) n = 6, Normal histological appearance. Corpus luteum (star), Primary follicle (thin arrow), Secondary follicle (thick arrow) view (H&E ×10), (b) n = 6, Appearance of mild stromal hemorrhage (arrow) (H&E ×10), (c) n = 6, Severe stromal hemorrhage (arrow), edema (asterisk) and follicular hemorrhage (square) appearance (H&E ×10), (d) n = 6, Appearance of mild stromal hemorrhage (arrow) (H&E ×20), HG: Healthy group, SOG: Sham-operated group, OIR: Ovarian IR and LOIR: Leflunomide+ovarian IR

Table 1: Histopathological grading analysis results of ovarian tissues

	Median (quartile 1-quartile 3)			
Groups (n = 6/each group)	Stromal haemorrhage	Follicular haemorrhage	Follicular degeneration	Congestion
HG	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
SOG	0.5 (0-1)	0 (0-0)	0 (0-0)	3 (3-3)
OIR	3 (2-3)*	3 (2-3)*	0 (0-0)	3 (2-3)*
LOIR	1 (0-1)	0 (0-0)**	0 (0-0)	0 (0-0)**

Histopathological grading: 0: Absent, 1: Mild damage, 2: Moderate damage, 3: Severe damage, *p<0.001 vs HG, SOG and LOIR, **p>0.05 vs HG and SOG, HG: Healthy group, SOG: Sham-operated group, OIR: Ovarian IR, LOIR: Leflunomide+ovarian IR and statistical analysis was performed by Kruskal Wallis and Mann-Whitney U test

DISCUSSION

Clinically, ovarian torsion is most often seen in women of reproductive age. In the past, ovarian resection was the treatment of choice, however, the current approach recommends detorsion of torsion ovaries[19]. Reperfusion of ovarian tissues exposed to ischemia by torsion may, however, result in increased tissue damage. According to the literature, tissue damage resulting from ovarian IR has been attributed to oxidative and inflammatory processes[20]. Consequently, this study investigated the protective effects of leflunomide, which is an antioxidant and anti-inflammatory agent, against IR-induced ovarian injury in rats[14,15].

With reperfusion, molecular oxygen is abundantly available to ischemic tissue, increasing ROS levels. Increased radicals attack cell membranes and damage cell integrity with LPO. As an important indicator of oxidative stress, MDA is a toxic metabolite produced during LPO[6,21,22]. According to the findings of this study, MDA amount in ovaries increased after exposure to IR. According to Ulug *et al.*[6], tissue MDA levels increased after the detorsion of torsion ovaries. By contrast, this increase in MDA levels was significantly suppressed in the ovaries of rats treated with leflunomide prior to IR. No information on the effect of leflunomide on oxidant and antioxidants in ovarian tissue was found in the literature. A previous study by Karaman *et al.*[23] investigated the preventive effects of leflunomide on liver IR injury and it was found that it prevented the increase in tissue MDA levels and that it possessed radical scavenging properties.

It is well known that the cellular defense system against oxidative damage is activated by increasing levels of nonenzymatic compounds such as GSH and the activity of enzymatic antioxidants such as SOD and CAT[21]. Based on experimental findings, the increase in oxidants in the ovaries of the IR group was accompanied by a decrease in tGSH levels. In living tissues, GSH is one of the most important and well-known antioxidants. In addition to detoxifying hydrogen peroxide and organic peroxides, GSH protect cells from the

damaging effects of ROS[9]. The amount of GSH in tissues exposed to IR has been shown to be decreased in previous study of Dincer *et al.*[21]. Following IR, a decrease in SOD and CAT activities was detected in ovarian tissues in the present study. There is a cooperative relationship between these antioxidants. The SOD converts the superoxide radical into the less reactive hydrogen peroxide. The CAT catalyzes the detoxification reaction in which hydrogen peroxide is converted into water and oxygen[24]. Additionally, Çolak *et al.*[24] observed a decline in antioxidants (GSH, SOD and CAT) in ovarian tissues exposed to IR and this decrease was attributed to excessive antioxidant consumption due to increased ROS levels. The literature search did not identify any studies investigating the impact of leflunomide on antioxidant capacity in ovarian oxidative damage. However, leflunomide was used in oxidative liver injury and reversed the IR-related decline in SOD and CAT. The authors concluded that leflunomide exerted its antioxidant properties by inhibiting ROS production and/or by preventing neutrophil activation[23].

There is evidence that an elevated oxidant level triggers inflammation in IR injuries[6,24]. Inflammatory events are initiated by migrating leukocytes, inflammatory chemokines and cytokines (such as TNF) to the site of tissue damage[24]. The results of the current study were consistent with past reports that found an increase in TNF-α levels in the ovaries of the IR group[6,20,24]. An increase in ROS production leads to increased transcription of various proinflammatory cytokines such as IL-1β and IL-6 along with TNF-α via stimulation of nuclear factor kappa-B[21]. It has been reported in the literature that prolonged overproduction of IL-1β during an inflammatory response may lead to tissue damage through the overactivation of immune cells[25]. In this study, IL-1β and IL-6 levels were elevated in the IR group versus the healthy group, supporting previous studies indicating a role for inflammation in ovarian IR damage[6,20,21]. On the other hand, leflunomide administered before the IR procedure significantly inhibited these increases in inflammatory markers. Recent evidence supported that leflunomide exerted its anti-inflammatory and immunomodulatory effects through suppression of proinflammatory cytokines[23]. Leflunomide has been known to protect tissues from inflammatory damage by suppressing TNF-α related signaling pathways[26]. The literature on the influence of leflunomide on inflammatory processes in IR-induced injury is quite limited. However, leflunomide has been shown to attenuate lipopolysaccharide-related increases in TNF-α and IL-6 in peritoneal macrophages[27]. Similarly, in the study by Magari *et al.*[28], leflunomide inhibited the increase in TNF-α, IL-1β and IL-6 induced by T cell activation.

The results of the histopathological evaluation of the ovaries were consistent with those of the biochemical analysis. There was severe stromal hemorrhage, follicular hemorrhage and congestion in the OIR rat's ovaries, as well as a rise in oxidative and inflammatory markers and a reduction in antioxidants. In rat ovaries, Yuksel *et al.*[29] previously observed hemorrhage, necrosis, edema and vascular dilatation following the administration of IR. In the leflunomide group, follicular hemorrhage and congestion were completely prevented, while stromal hemorrhage was significantly reduced. Previously, leflunomide treatment was shown to significantly reduce necrosis and congestion detected in tissues with increased amounts of MDA and decreased levels of SOD and CAT and leflunomide was found to possess radical scavenging, antioxidant and anti-inflammatory properties[23].

The fact that ovarian tissues were not analyzed immunohistochemically is among the limitations of the study. It is also important to perform follicle counting in rat ovaries and to evaluate the rats in terms of fertility.

It is the recommended detorsion procedure for ovarian torsion. However, the ovaries are exposed to oxidative and inflammatory damage, although more so after detorsion. Therefore, additional approaches are needed to protect the ovaries from free radical attack and inflammatory damage during ischemia and reperfusion. The current study demonstrated the protective effect of leflunomide treatment in rats undergoing ovarian IR procedures. It is hoped that this study will pave the way for clinical studies.

CONCLUSION

The increase in oxidant (MDA) and reduction in antioxidants (tGSH, SOD and CAT) in the ovaries of the IR group indicates that oxidative stress is developing in the tissues and the elevation of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) indicates that inflammation also plays a role in the damage process. Histopathological examination of the ovarian tissues revealed damage caused by IR treatment. Leflunomide suppressed the increase in MDA, TNF-α, IL-1β and IL-6 and the decrease in tGSH, SOD and CAT and significantly prevented histopathologic damage. Our biochemical and histopathological findings suggest that leflunomide acts as an antioxidant and anti-inflammatory to protect ovarian tissues against IR-induced ovarian injury.

SIGNIFICANCE STATEMENT

Ovarian IR injury is a pathological condition which may result in severe ovarian damage and infertility and for which oxidative stress and accompanying inflammation are responsible for its pathogenesis. When the pathogenesis of IR injury is taken into consideration, agents such as

leflunomide with antioxidant and anti-inflammatory activity come to mind first. Therefore, in the present study, the protective effect of leflunomide against ovarian IR injury in rats was investigated. Leflunomide showed antioxidant activity by suppressing oxidant increase and antioxidant decrease in ovarian tissues exposed to IR and also significantly inhibited the increase in proinflammatory cytokines. In addition, histopathological analysis of ovarian tissues of rats receiving leflunomide treatment revealed the protective effect of leflunomide.

REFERENCES

1. Ding, Y., Y. Zhang, W. Zhang, J. Shang, Z. Xie and C. Chen, 2021. Effects of lipoic acid on ischemia-reperfusion injury. Oxid. Med. Cell. Longevity, Vol. 2021. 10.1155/2021/5093216.
2. Zhang, M., Q. Liu, H. Meng, H. Duan and X. Liu *et al*., 2024. Ischemia-reperfusion injury: Molecular mechanisms and therapeutic targets. Signal Transduction Targeted Ther., Vol. 9. 10.1038/s41392-023-01688-x.
3. Zang, X., J. Zhou, X. Zhang, Y. Han and X. Chen, 2020. Ischemia reperfusion injury: Opportunities for nanoparticles. ACS Biomater. Sci. Eng., 6: 6528-6539.
4. Wu, M.Y., G.T. Yiang, W.T. Liao, A.P.Y. Tsai and Y.L. Cheng *et al*., 2018. Current mechanistic concepts in ischemia and reperfusion injury. Cell. Physiol. Biochem., 46: 1650-1667.
5. Yapca, O.E., B. Borekci and H. Suleyman, 2013. Ischemia-reperfusion damage. Eurasian J. Med., 45: 126-127.
6. Ulug, P., U. Nayki, R. Mammadov, S. Bulut, T.B. Tastan, T.A. Coban and H. Suleyman, 2024. Protective effect of ramipril against oxidant and proinflammatory cytokine damage induced by ischemia-reperfusion in ovarian tissue in rats. Transplant. Proc., 56: 215-222.
7. Liu, C. and X. Zhang, 2024. Bosentan, an endothelin receptor-1 antagonist, exerts cardioprotective effects during myocardial ischemia-reperfusion injury in experimental rats. Int. J. Pharmacol., 20: 611-621.
8. Eltzschig, H.K. and T. Eckle, 2011. Ischemia and reperfusaion-from mechanism to translation. Nat. Med., 17: 1391-1401.
9. Kadıoğlu, B.G., B. Gündoğdu, N. Kurt, H. Süleyman and Z. Süleyman, 2020. Effect of desloratadine on oxidative and inflammatory ovarian ischemia-reperfusion injury in female rats: Biochemical and histopathological evaluation. Anal. Quant. Cytol. Histol., 42: 61-68.
10. Bayer, A.I. and A.K. Wiskind, 1994. Adnexal torsion.: Can the adnexa be saved? Am. J. Obstet. Gynecol., 171: 1506-1511.
11. Zheng, K., Y. Chen, S. Liu, C. He and Y. Yang *et al*., 2023. Leflunomide: Traditional immunosuppressant with concurrent antiviral effects. Int. J. Rheumatic Dis., 26: 195-209.
12. Singh, H., G. Sukhija, V. Tanwar, S. Arora and J. Bhutani, 2016. Rare occurrence of drug induced subacute cutaneous lupus erythematosus with leflunomide therapy. J. Clin. Diagn. Res., 10: OD06-OD07.
13. Pfaller, B., A. Pupco, T. Leibson, D. Aletaha and S. Ito, 2020. A critical review of the reproductive safety of leflunomide. Clin. Rheumatol., 39: 607-612.
14. Ju, D.W., Q.Y. Zheng, H.B. Wang and J. Fang, 1994. Leflunomide inhibits cytokine-induced DNA synthesis of rabbit synovial cells in culture. Acta Pharmacol. Sin., 15: 223-226.
15. Yildiz, Y., H. Kose, S. Cecen, K. Ergin, E.M. Demir and M. Serter, 2010. Protective effects of leflunomide on intestinal ischemia-reperfusion injury: Leflunomide against intestinal ischemia-reperfusion. Digestive Dis. Sci., 55: 245-252.
16. Demiryilmaz, I., M.I. Turan, A. Kisaoglu, M. Gulapoglu, I. Yilmaz and H. Suleyman, 2014. Protective effect of nimesulide against hepatic ischemia/reperfusion injury in rats: Effects on oxidant/antioxidants, DNA mutation and COX-1/COX-2 levels. Pharmacol. Rep., 66: 647-652.
17. Góth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. Clin. Chim. Acta, 196: 143-151.
18. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
19. Cass, D.L., 2005. Ovarian torsion. Semin. Pediatr. Surg., 14: 86-92.
20. Bingul, E., E. Hizal, B. Suleyman, R. Mammadov and S. Bulut *et al*., 2023. Effect of cinnamon extract on ovarian torsion detorsion damage in rats. Int. J. Pharmacol., 19: 842-851.
21. Dincer, B., I. Cinar, M. Yayla and E. Toktay, 2022. Evaluation of the protective effects of gossypin for ischemia/reperfusion injury in ovary tissue. J. Obstet. Gynaecol., 48: 748-756.
22. Zheng, H., X. Ping, R. Wu, C. Lei and H. Mao *et al*., 2024. Interventional effects of *Lactobacillus plantarum* Hfy04 isolated from naturally fermented yak yogurt on oxazolone-induced colitis in BALB/c mice. Int. J. Pharmacol., 20: 817-824.
23. Karaman, A., E. Fadillioglu, E. Turkmen, E. Tas and Z. Yilmaz, 2006. Protective effects of leflunomide against ischemia-reperfusion injury of the rat liver. Pediatr. Surg. Int., 22: 428-434.
24. Çolak, S., K. Koc, S. Yıldırım and F. Geyikoğlu, 2022. Effects of boric acid on ovarian tissue damage caused by experimental ischemia/reperfusion. Biotech. Histochem., 97: 415-422.
25. Beyazit, F., B. Büyük, H. Turkon, S. Elmas and M. Uzun, 2019. Adalimumab mitigates ovarian ischemia-reperfusion injury in rats by regulating oxidative stress, apoptosis and resolution of inflammation. J. Obstet. Gynaecol. Res., 45: 358-367.

26. Manna, S.K., A. Mukhopadhyay and B.B. Aggarwal, 2000. Leflunomide suppresses TNF-induced cellular responses: Effects on NF-κB, activator protein-1, c-Jun N-terminal protein kinase, and apoptosis. J. Immunol., 165: 5962-5969.
27. Li, W.D., G.X. Ran, H.L. Teng and Z.B. Lin, 2002. Dynamic effects of leflunomide on IL-1, IL-6, and TNF-α activity produced from peritoneal macrophages in adjuvant arthritis rats. Acta Pharmacol. Sin., 23: 752-756.
28. Magari, K., S. Miyata, F. Nishigaki, Y. Ohkubo and S. Mutoh, 2004. Comparison of anti-arthritic properties of leflunomide with methotrexate and FK506: Effect on T cell activation-induced inflammatory cytokine production *in vitro* and rat adjuvant-induced arthritis. Inflammation Res., 53: 544-550.
29. Yuksel, T.N., Z. Halici, E. Cadirci, E. Toktay, B. Ozdemir and A. Bozkurt, 2023. Effect of trimetazidine against ovarian ischemia/reperfusion injury in rat model: A new pathway: JAK2/STAT3. Iran. J. Basic Med. Sci., 26: 1370-1379.