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# Therapeutic Potential of Cepharanthine in Ovarian Ischemia-Reperfusion Injury: Insights from a Rat Ovarian Torsion-Detorsion Model

## Over İskemi-Reperfüzyon Hasarında Sefarantin'in Terapötik Potansiyeli: Rat Over Torsiyon-Detorsiyon Modelinden Elde Edilen Bulgular

Burak BİRCAN[1]
Ayhan TANYELİ[2]
Ersen ERASLAN[3]
Fazile Nur EKİNCİ AKDEMİR[4]
Şahin YAZICI[5]
Derya GÜZEL ERDOĞAN[6]
Elif POLAT[7]
Mustafa Can GÜLER[2]

[1]Department of Medical Sciences and Techniques, Healthcare Vocational School, Osmaniye Korkut Ata University, Osmaniye, Türkiye
[2]Department of Physiology, Faculty of Medicine, Atatürk University, Erzurum, Türkiye
[3]Department of Physiology, Faculty of Medicine, Bandırma Onyedi Eylül University, Bandırma, Türkiye
[4]Department of Physiology, Erzurum Medicine Faculty, University of Health Sciences, Erzurum, Türkiye
[5]Department of Obstetrics and Gynecology, Erzurum City Hospital, Erzurum, Türkiye
[6]Department of Physiology, Faculty of Medicine, Sakarya University, Sakarya, Türkiye
[7]Department of Nutrition and Dietetics, Faculty of Health Sciences, Erzurum Technical University, Erzurum, Türkiye

## ABSTRACT

This study evaluated the protective effect of Cepharanthine (CEP) against ovarian ischemia-reperfusion (I/R) injury induced by torsion-detorsion in rats, and its effects on histopathological damage and markers of oxidative stress and inflammation. Twenty-four female Sprague-Dawley rats were randomized into three experimental groups: sham, T/D, and CEP 10 mg/kg. The study examined ovarian tissue samples to measure oxidative stress biomarkers, such as malondialdehyde (MDA), myeloperoxidase (MPO), and superoxide dismutase (SOD), as well as proinflammatory mediators, specifically tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). The T/D group exhibited significant oxidative stress, characterized by elevated MDA and MPO levels and low SOD activity, along with heightened inflammatory responses, as indicated by elevated TNF-α and IL-1β levels ($p < .05$ vs. Sham). CEP administration mitigated these deleterious effects, significantly reducing oxidative stress and inflammatory cytokine levels while restoring SOD activity ($p < .05$ vs. T/D). CEP effectively attenuated oxidative stress and inflammation in ovarian I/R injury. The preclinical findings support the potential therapeutic effect of CEP, but further studies are needed for clinical applications.

**Keywords:** Cepharanthine, Inflammation, Ischemia reperfusion, Oxidative stress, Ovarian torsion detorsion.

## ÖZ

Bu çalışma, Sefarantin'in (CEP)'in, sıçanlarda torsiyon–detorsiyon ile oluşturulan over iskemi–reperfüzyon (İ/R) hasarına karşı koruyucu etkisini ve histopatolojik hasar ile oksidatif stres ve inflamasyon göstergeleri üzerindeki etkisini değerlendirmiştir. Yirmi dört dişi Sprague-Dawley sıçanı rastgele üç deney grubuna ayrılmıştır: sham, T/D ve CEP 10 mg/kg. Çalışma, over doku örneklerinde malondialdehit (MDA), miyeloperoksidaz (MPO) ve süperoksit dismutaz (SOD) gibi oksidatif stres biyobelirteçleri ile tümör nekroz faktör-alfa (TNF-α) ve interlökin-1 beta (IL-1β) gibi proinflamatuar mediyatörleri ölçmüştür. T/D grubu, yüksek MDA ve MPO seviyeleri ile düşük SOD aktivitesi ile karakterize önemli oksidatif stres ve yüksek TNF-α ve IL-1β seviyelerinin gösterdiği artmış inflamatuar yanıtlar sergiledi. CEP uygulaması bu zararlı etkileri azalttı, oksidatif stresi ve inflamatuar sitokin seviyelerini önemli ölçüde azaltırken SOD aktivitesini restore etti. CEP, over İ/R hasarında oksidatif stres ve inflamasyonu etkili bir şekilde azaltmıştır. Elde edilen preklinik bulgular, CEP'nin potansiyel terapötik etkisini desteklemektedir, ancak klinik uygulamalar için ileri çalışmalara ihtiyaç vardır.

**Anahtar kelimeler:** İnflamasyon, İskemi reperfüzyon, Oksidatif stres, Over torsiyon detorsiyon, Sefarantin.

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**Sorumlu Yazar/Corresponding author:** Mustafa Can Güler
**E-mail:** mustafacan.guler@atauni.edu.tr

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## Introduction

Adnexal torsion (AT) is a critical gynecological emergency that poses significant risks to reproductive health and fertility (Stickland & Phillips, 2021). It occurs when the ovary and sometimes the fallopian tube twists around a central axis formed by the infundibulopelvic and tubo-ovarian ligaments. This torsional event results in at least one complete rotation, leading to compromised blood flow to the affected structures (Thiyagalingam et al., 2024). Given that acute pelvic pain is one of the most common reasons for emergency gynecological consultations, AT remains a condition of considerable clinical importance (Franco et al., 2023). Timely diagnosis of AT is crucial for preventing severe complications, such as loss of ovarian function or adnexal necrosis, which can significantly impact fertility, even in young women (Kumari et al., 2024). In this context, the surgical detorsion itself can precipitate a distinct pathological process—ischemia–reperfusion (I/R) injury—which may further amplify ovarian damage.

The pathophysiology of AT is primarily driven by an ischemic process that can rapidly progress to severe vascular complications if not promptly managed (Baron & Mathai, 2025). Torsion disrupts normal ovarian perfusion, causing venous and lymphatic congestion, leading to ovarian swelling and pelvic fluid accumulation (Dixon et al., 2025). If arterial circulation is also compromised, hemorrhagic necrosis ensues, further complicating the clinical scenario (Amirbekian & Hooley, 2014). Given the rapid progression of these pathological changes, delays in diagnosis and intervention can substantially worsen patient outcomes.

Surgical management remains the mainstay of treatment for AT, aiming to untwist the affected adnexa and restore perfusion. However, even when timely intervention is performed, I/R injury continues to pose a significant clinical challenge (Armin Akış et al., 2024). I/R injury arises following the restoration of blood flow after a temporary interruption in oxygen supply (ischemia), triggering a cascade of inflammatory responses and oxidative stress, which can exacerbate tissue damage and impair ovarian function (Tanyeli et al., 2022).

Current investigations have concentrated on exploring promising treatment options to mitigate I/R-induced damage (Erbaş et al., 2024; Yigit et al., 2024). Notably CEP has garnered attention as a potential therapeutic agent owing to its diverse range of reported pharmacological effects. These include antibacterial, antioxidant, anticancer, and anti-inflammatory activities (Liang et al., 2022; Liu et al., 2023).

Oxidative stress is characterized by the accumulation of ROS beyond cellular tolerance. It leads to molecular and tissue damage, contributing to the I/R injury pathophysiology (Ekinci Akdemir et al., 2024). Endogenous free radicals generated during oxidative metabolism can induce significant cellular and DNA damage if left unchecked (Güler et al., 2023). CEP is known for its potent oxidative stress preventive capacity by decreasing ROS levels, which are excessively produced at sites of inflammation (Chen et al., 2023). The protective effects of CEP are attributed to its membrane-stabilizing and antiperoxidative properties, which counteract oxidative stress and inflammation (Liu et al., 2023). Such findings highlight CEP's potential as a therapeutic candidate to alleviate I/R-associated ovarian tissue injury.

Here, we evaluated the protective potential of CEP in a rat model of ovarian T/D-related I/R injury. We hypothesized that CEP could attenuate I/R-induced damage, thereby preserving ovarian structure and function.

## Methods

### Chemicals

The disinfection process involved the application of a 10% povidone-iodine solution (Batticon; Adeka). Anesthetic induction was achieved using xylazine hydrochloride (Rompun®, Bayer, Istanbul) and ketamine (Ketalar®, Pfizer, Istanbul). The compound CEP (Figure 1, purity ≥ 98.0%, CAS Number: 481-49-2) was procured from Sigma Aldrich (Germany).

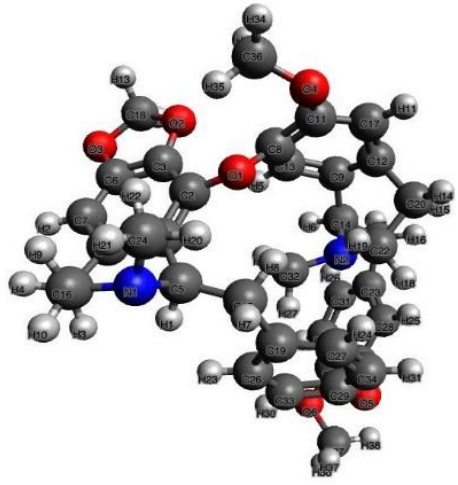

**Figure 1.** 3D chemical structure of cepharanthine (Created with Avogadro version 1.2.0., http://avogadro.cc/).

**Şekil 1.** Sefarantin'in (CEP) 3D kimyasal yapısı (Avogadro sürüm 1.2.0 ile oluşturulmuştur, http://avogadro.cc/).

**Ethics Committee Approval**

In accordance with the ethical principles of the Declaration of Helsinki, the study protocol received approval from the Ethics Committee (Approval No: 5, Date: 30.06.2017) at Atatürk University. All experimental animals were sourced from the Laboratory Animal Research and Application Center at Atatürk University.

**Animals**

The animals were housed in standard rat cages under controlled environmental conditions, with room temperature, 55± 5% humidity level, and a 12-hour light-dark cycle. They had unrestricted access to standard pellet food and water *ad libitum*. All rats were fasted overnight and free to reach water before the experimental model application.

**Preoperative Preparation**

The rats were positioned in a supine anatomical posture and secured for the procedure. The abdominal area was carefully shaved to ensure a clean surgical field. Disinfection was performed using a 10% povidone-iodine solution. Anesthesia was induced with a combination of ketamine and xylazine to provide adequate sedation and analgesia. The administered ketamine/xylazine dosage (100/15 mg/kg body weight (BW), intraperitoneally (i.p.)) was selected based on a previously established experimental rat model (Güler et al., 2024).

**Animals and Experimental Design**

24 female *Wistar albino* rats (12–16 weeks old, 200–250 g) were divided into three groups (n=8). Figure 2 summarizes the experimental process.

**Group I (Sham):** A median laparotomy incision (1–2 cm) was made, and the incision was closed using 3/0 silk sutures without inducing torsion-detorsion (T/D) or administering any pharmacological intervention (Figure 2a).

**Group II (T/D):** After performing the abdominal incision, the ovaries, ovarian vessels, and fallopian tubes were rotated 360° clockwise to induce bilateral ovarian torsion. The torsion was maintained for 3 hours using atraumatic microvascular clamps. Following the torsion phase, the clamps were removed to restore blood flow for an additional 3 hours (detorsion phase), and the incision was closed (Figure 2b) (Armin Akış et al., 2024).

**Group III (CEP 10 mg/kg):** All procedures were performed as in the T/D group. In addition, a 10 mg/kg CEP was administered i.p. 30 minutes before detorsion (Figure 2c). The CEP dosage was determined according to prior experimental studies (Zhao et al., 2020).

After completing the 3-hour detorsion period, anesthesia was re-administered, and the rats were euthanized using cervical dislocation. The blood samples were obtained via cardiac puncture, and ovarian tissue samples were harvested for biochemical analysis.

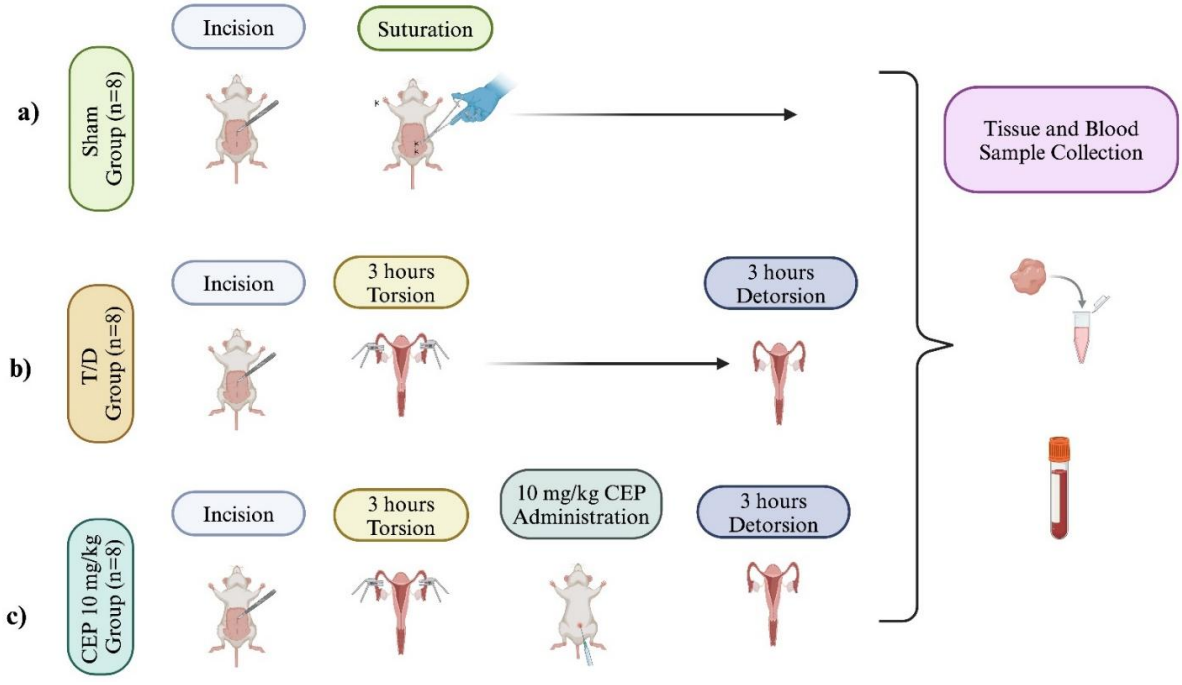

**Figure 2.** The summarization of the experimental process, a) the sham group; only incision and suturation was performed, b) the T/D group; the T/D model was performed, c) the CEP 10 mg/kg group; 10 mg/kg CEP was administered i.p. 30 minutes before detorsion (Created in https://BioRender.com, CEP= Cepharanthine, T/D= Torsion/detorsion).

**Şekil 2.** Deneysel sürecin özeti, a) sham grubu; yalnızca insizyon ve sütürasyon yapılmıştır, b) T/D grubu; T/D modeli uygulanmıştır, c) CEP 10 mg/kg grubu; detorsiyondan 30 dakika önce 10 mg/kg CEP intraperitoneal olarak uygulanmıştır (https://BioRender.com ile oluşturulmuştur, CEP= Sefarantin, T/D= Torsiyon/detorsiyon).

**Biochemical Analysis**

A high-speed homogenizer (IKA, Staufen, Germany) was used to homogenize 100 mg of ovarian tissue samples on ice at 10.000 rpm for 5 minutes. The homogenization was carried out in 2 mL of 10% phosphate buffer solution. After homogenization, the samples were centrifugated at 4°C, 3.000×g for 30 minutes. The resulting supernatant was collected for biochemical assessments, including malondialdehyde (MDA), myeloperoxidase (MPO), total oxidant status (TOS), total antioxidant status (TAS), and superoxide dismutase (SOD). Additionally, proinflammatory cytokines, interleukin-1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) were quantified with a rat-specific ELISA kit (Elabscience, Wuhan, China).

**MDA Determination**

MDA levels were quantified following the method outlined by Ohkawa et al. (1979). Tetramethoxypropane served as standard, and lipid peroxidation levels were reported in nanomoles of MDA. The interaction between MDA and thiobarbituric acid

(TBA) was assessed spectrophotometrically using a PowerWave™ XS Biotek spectrophotometer.

For the assay, the supernatant was mixed with butylated hydroxytoluene (BHT) in methanol, followed by the addition of TBA solutions and phosphoric acid. The mixture was vortexed and incubated at 95°C for 60 minutes to allow the reaction. Following incubation, the samples were centrifuged for 3 minutes at 10.000 × *g*. The supernatant was carefully collected, transferred to a cuvette, and analyzed for absorbance at 532 nm using a spectrophotometer.

**MPO Activity Assay**

MPO activity was found via the technique proposed by Bradley et al. (1982). The tissue suspensions were subjected to centrifugation for 15 minutes at 40.000×g, and the obtained supernatant was utilized for analysis. This assay is based on the oxidative reaction between MPO and *o*-dianisidine in the presence of hydrogen peroxide ($H_2O_2$), forming a yellow-orange complex. The absorbance of this complex was assessed spectrophotometrically at 460 nm using a PowerWave™ XS Biotek spectrophotometer.

**SOD Activity Assay**

A method described by Sun et al. was preferred for determining the SOD activity (Sun et al., 1988) This assay is based on the formation of a blue formazan dye, which is produced when xanthine and superoxide radicals reduce nitro blue tetrazolium (NBT) in the presence of xanthine oxidase. The reaction product's optical density (OD) was measured at 560 nm using a PowerWave™ XS Biotek spectrophotometer. A single unit of SOD activity was characterized as the enzyme amount necessary to reduce NBT reduction by 50%.

**The Analysis of Oxidative Stress Index (OSI), Total Antioxidant Status (TAS), and Total Oxidant Status (TOS)**

The TAS assay operates on the principle that antioxidants in the sample diminish the dark blue-green 2,2'-azinobis radical to its reduced form. A reduction in absorbance at 660 nm is indicative of the total antioxidant capacity. TAS levels were quantified by a commercial assay kit (RL0024; Rel Assay Diagnostics, Gaziantep, Türkiye).

In the TOS assay, the ferrous ion-chelator complex is oxidized by oxidizing agents in the sample, producing ferric ions that form a colored complex in an acidic medium. The intensity of the resulting color was measured spectrophotometrically, reflecting the total oxidant content. TOS levels were determined through a commercial kit (RL0005; Rel Assay Diagnostics, Gaziantep, Türkiye).

The OSI was calculated by dividing TOS by TAS using the formula: OSI = TOS/TAS (Akdemir et al., 2024).

**Statistical Analysis**

Data are summarized as mean ± standard error of the mean (SEM) for variables meeting parametric assumptions and as median [interquartile range, IQR] otherwise.

Normality was assessed with the Shapiro–Wilk test and homogeneity of variances with Levene's test. When assumptions were satisfied, one-way ANOVA followed by Tukey's HSD (which adjusts for multiple pairwise comparisons) was used. When assumptions were violated, the Kruskal–Wallis test was applied; if significant, pairwise Mann–Whitney U tests with Holm–Bonferroni multiplicity adjustment were conducted to identify group differences. Two-sided, multiplicity-adjusted $p < .05$ were considered statistically significant. Analyses were performed in SPSS v16.0 (SPSS Inc., Chicago, IL).

## Results

### Oxidative Stress and Antioxidant Activity Parameters

### MDA, SOD Levels, and MPO Activity

Figure 3 demonstrates the MDA, SOD levels, and MPO activity. The findings validate the successful development of the T/D model. Compared to the sham group, the T/D group exhibited a significant increase in MDA and MPO levels (Figure 3A and Figure 3C, $p < .05$). 10 mg/kg CEP treatment resulted in a marked reduction in MDA and MPO levels compared to the T/D group (Figure 3A and Figure 3C, $p < .05$).

Additionally, The T/D group exhibited significantly declined SOD levels compared to the sham group (Figure 3B, $p < .05$), indicating oxidative stress induction. Treatment with 10 mg/kg CEP significantly elevated in SOD levels relative to the T/D group (Figure 3B, $p < .05$), suggesting an enhancement in antioxidant defense mechanisms. These findings indicate that 10 mg/kg CEP mitigated oxidative stress by reducing lipid peroxidation and MPO activity while enhancing antioxidant enzyme (SOD) levels.

### TAS, TOS and OSI Levels

The variations in total antioxidant and oxidant levels closely paralleled the trends observed in MDA, SOD, and MPO levels. The T/D group demonstrated significantly low TAS levels relative to the sham group (Figure 4A, $p < .05$). However, administration of 10 mg/kg CEP significantly elevated TAS levels compared to the T/D group (Figure 4A, $p < .05$).

Furthermore, TOS and OSI levels increased in the T/D group relative to the sham group (Figure 4B and Figure 4C, $p < .05$). However, treatment with 10 mg/kg CEP significantly reduced TOS and OSI levels compared to the T/D group (Figure *4B and Figure 4C,* $p < .05$).

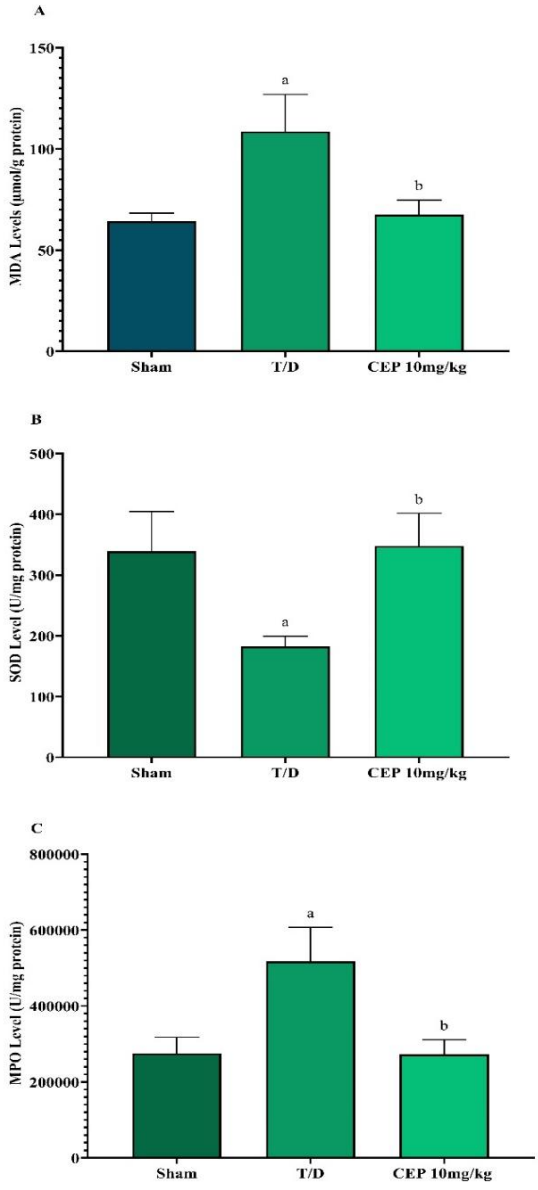


**Figure 3.** The levels of MDA and the activities of MPO and SOD in the different experimental groups. Data are expressed as SEM. Different superscript letters indicate statistically significant differences between groups ($p < .05$). Groups sharing the same letter are not significantly different ($^a$ = indicates a significant difference compared to the sham group, $^b$ = represents a significant difference between the 10 mg/kg CEP group and the T/D group. MDA= Malondialdehyde, MPO= Myeloperoxidase, SOD= Superoxide dismutase, CEP= Cepharanthine, SEM= Standard error of the mean, T/D= Torsion/detorsion).

**Şekil 3.** Farklı deney gruplarındaki MDA düzeyleri ve MPO ile SOD enzim aktiviteleri. Veriler SEM olarak ifade edilmiştir. Farklı üst simge harfleri gruplar arasında istatistiksel olarak anlamlı farklılıkları göstermektedir ($p <$ ,05). Aynı harfi paylaşan gruplar anlamlı ölçüde farklı değildir ($^a$ = T/D grubunda sham grubuna kıyasla anlamlı

artışı belirtir, [b]= 10 mg/kg CEP grubunda T/D grubuna kıyasla anlamlı azalmayı belirtir. MDA= Malondialdehit, MPO= Miyeloperoksidaz, SOD= Süperoksit dismutaz, CEP= Sefarantin, SEM= Ortalama standart hatası, T/D= Torsiyon/detorsiyon).

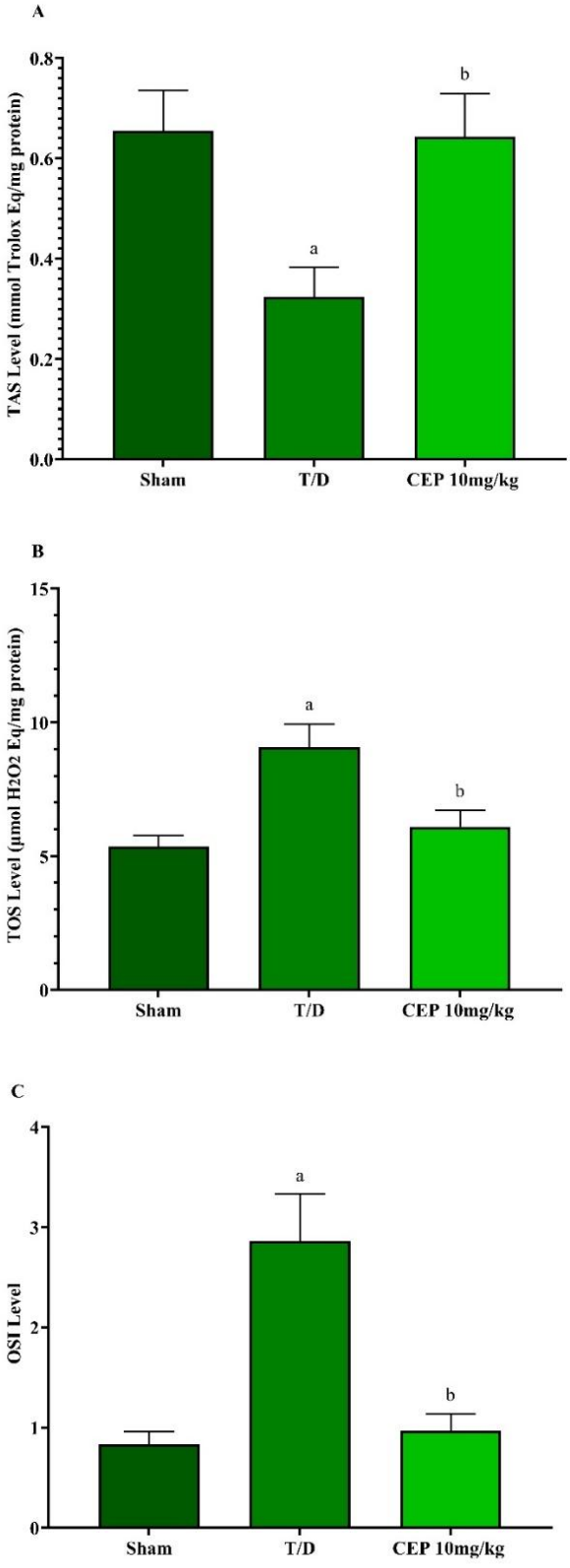


**Figure 4.** The levels of TAS, TOS, and OSI in the different experimental groups. Data are expressed as SEM. Different superscript letters indicate statistically significant differences between groups ($p$< .05). Groups sharing the same letter are not significantly different ([a] = indicates a significant difference compared to the sham group, [b] = represents a significant difference between the 10 mg/kg CEP group and the T/D group. TAS= Total antioxidant status, TOS= Total oxidant status, OSI= Oxidative stress index, CEP= Cepharanthine, SEM= Standard error of the mean, T/D= Torsion/detorsion).

**Şekil 4.** Farklı deney gruplarındaki TAS, TOS ve OSI düzeyleri. Veriler SEM olarak ifade edilmiştir. Farklı üst simge harfleri gruplar arasında istatistiksel olarak anlamlı farklılıkları göstermektedir ($p$< ,05). Aynı harfi paylaşan gruplar anlamlı ölçüde farklı değildir ([a] = T/D grubunda sham grubuna kıyasla anlamlı artışı belirtir, [b]= 10 mg/kg CEP grubunda T/D grubuna kıyasla anlamlı azalmayı belirtir. TAS= Toplam antioksidan statüsü (kapasitesi), TOS= Toplam oksidan statüsü (kapasitesi), OSI= Oksidatif stres indeksi, CEP= Sefarantin, SEM= Ortalama standart hatası, T/D= Torsiyon/detorsiyon).

**Proinflammatory Cytokines (TNF-α and IL-1β)**

In our study, TNF-α and IL-1β levels were evaluated by ELISA to assess the changes in proinflammatory cytokines. TNF-α and IL-1β showed elevated levels in the T/D group compared to the sham group and CEP treatment alleviated TNF-α and IL-1 levels (Table 1, Figure 5A and Figure 5B, $p$< .05).

**Table 1.** Comparison of IL-1β and TNF-α levels among the sham, T/D, and CEP 10 mg/kg groups.

**Tablo 1.** Sham, T/D ve CEP 10 mg/kg grupları arasında IL-1β ve TNF-α düzeylerinin karşılaştırılması.

| Group | TNF-α (pg/mg protein) | IL-1β (pg/mg protein) |
|---|---|---|
| Sham | 32.5 ± 2.1 | 28.7 ± 1.9 |
| T/D | 65.8 ± 3.4[a] | 59.3 ± 2.8[a] |
| CEP 10 mg/kg | 41.2 ± 2.6[b] | 37.6 ± 2.3[b] |

TNF-α and IL-1β levels in different groups. Data are expressed as SEM. Different superscript letters indicate statistically significant differences between groups ($p$< .05). [a] = indicates a significant difference compared to the sham group, [b] = represents a significant difference between the 10 mg/kg CEP group and the T/D group.

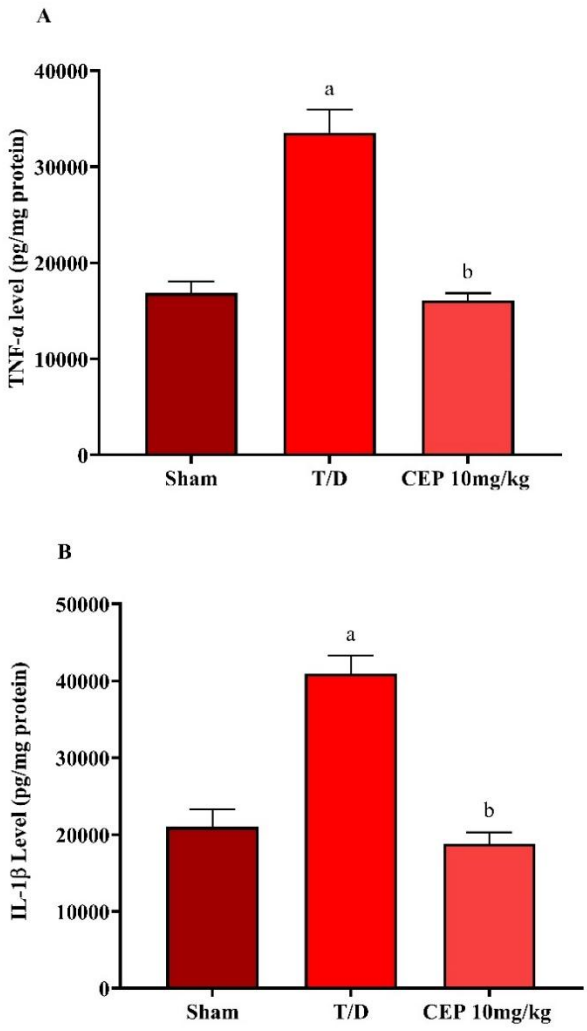


**Figure 5.** Comparison of IL-1β and TNF-α levels among the sham, T/D, and CEP 10 mg/kg groups. Data are expressed as SEM. Different superscript letters indicate statistically significant differences between groups ($p$< .05). Groups sharing the same letter are not significantly different ([a] = indicates a significant difference compared to the sham group, [b] = represents a significant difference between the 10 mg/kg CEP group and the T/D group. IL-1β= Interleukin-1 beta, TNF-α= Tumor necrosis factor-alpha, CEP= Cepharanthine, SEM= Standard error of the mean, T/D= Torsion/detorsion).

**Şekil 5.** Sham, T/D ve CEP 10 mg/kg grupları arasında IL-1β ve TNF-α düzeylerinin karşılaştırılması. Veriler SEM olarak ifade edilmiştir. Farklı üst simge harfleri gruplar arasında istatistiksel olarak anlamlı farklılıkları göstermektedir ($p$< ,05). Aynı harfi paylaşan gruplar anlamlı ölçüde farklı değildir ([a] = T/D grubunda sham grubuna kıyasla anlamlı artışı belirtir, [b]= 10 mg/kg CEP grubunda T/D grubuna kıyasla anlamlı azalmayı belirtir. IL-1β= İnterlökin-1 beta, TNF-α= Tümör nekroz faktörü-alfa, CEP= Sefarantin, SEM= Ortalama standart hatası, T/D= Torsiyon/detorsiyon).

## Discussion

This research focused on evaluating the protective properties of CEP on ovarian T/D-related I/R injury in a rat model. With the continuous advancements in organ-preserving surgical techniques across various medical fields, addressing I/R injuries remains a critical concern. The prevention and mitigation of these injuries have been extensively documented in the literature (Aktepe et al., 2024; Osmanlıoğlu et al., 2023; Ulug et al., 2024). However, effective therapeutic strategies to counteract the oxidative and inflammatory damage induced by I/R injury in ovarian tissue remain limited.

Ovarian torsion is a gynecological emergency that, when treated via detorsion, frequently results in I/R injury, emphasizing the necessity for protective interventions (Güler et al., 2020). This condition is characterized by a cascade of oxidative stress and inflammation, leading to substantial tissue damage. Previous studies have primarily focused on key biochemical markers associated with I/R injury, SOD, MDA, MPO, TAS, TOS, IL-1β, and TNF-α. For instance, Tanyeli et al. (2022) demonstrated that ovarian I/R injury increases oxidative stress markers such as OSI, MPO, and pro-inflammatory cytokines like IL-1β and TNF-α while reducing antioxidant enzyme levels in distant organs such as the lung. Similarly, Arslan et al. (2023) reported that I/R injury in ovarian tissue is associated with elevated levels of MDA, MPO, TOS, and inflammatory cytokines, coupled with a reduction in TAS levels, which was ameliorated by the administration of *Passiflora incarnata*.

CEP has been recognized for its pharmacological activities, like free lipid peroxidation inhibition and radical scavenging (Liang et al., 2022). The ability of CEP to counteract oxidative damage is primarily attributed to its role in neutralizing ROS, thereby preventing cellular injury. Additionally, its anti-inflammatory effects stem from the modulation of cytokine release and inhibition of inflammatory signaling pathways, which may be crucial in mitigating I/R-induced ovarian damage (Kao et al., 2015; Liu et al., 2023).

In this study, CEP administration led to a notable decrease in oxidative stress and inflammatory markers. Specifically, CEP treatment lowered MPO and MDA levels, enhanced SOD activity, and diminished pro-inflammatory cytokines IL-1β and TNF-α. These findings strongly indicate that CEP

prevented by attenuating oxidative damage and suppressing the inflammatory response. Given its dual mechanism of action, CEP could represent a promising pharmacological intervention in managing ovarian T/D.

The potential clinical implications of these findings are substantial. Ovarian T/D is a critical condition that requires prompt surgical intervention, yet the associated I/R injury can lead to significant morbidity, including loss of ovarian function (Güler & Tanyeli, 2020). The incorporation of CEP as an adjunctive treatment could help preserve ovarian viability and improve post-detorsion outcomes in affected individuals.

Nevertheless, this study has certain limitations. While CEP consistently attenuated histopathological injury and modulated oxidative–inflammatory markers in this rat T/D model, translation to clinical ovarian torsion requires caution. First, CEP was administered before reperfusion; clinically, a feasible window is the perioperative period (on admission or immediately before detorsion). Second, the dose, route, and pharmacokinetics for this indication in humans remain undefined and warrant dose-finding and safety studies. Future work should extend endpoints beyond acute injury to ovarian reserve and fertility outcomes. Finally, the interaction of CEP with standard surgical care should be evaluated in randomized preclinical designs to inform early-phase clinical trials.

## Conclusion

This research represents the first attempt to investigate the potential of CEP in mitigating ovarian T/D-induced I/R injury. The observed anti-inflammatory and antioxidant effects highlight its therapeutic potential for gynecological emergencies involving T/D-related tissue injury. Additional studies are needed to clarify its exact mechanisms of action and to establish its clinical applicability. These findings lay the groundwork for future investigations into using CEP and other natural compounds to protect ovarian function in the setting of I/R injury.

**Ethics Committee Approval:** In accordance with the ethical principles of the Declaration of Helsinki, the study protocol received approval from the Ethics Committee (Approval No: 5, Date: 30.06.2017) at Atatürk University. All experimental animals were sourced from the Laboratory Animal Research and Application Center at Atatürk University.

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