

Protective Effect of Active Anionic Oxygen Administration on Testicular Tissue in Rats with Experimental Sepsis

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ABSTRACT

This study aimed to investigate the effects of active anionic oxygen (AAO) on oxidative stress, inflammation, and steroidogenesis in sepsis-induced testicular injury in rats. Thirty male Wistar Albino rats were randomly assigned to four groups: Control, Sepsis (lipopolysaccharide, LPS), AAO, and Sepsis+AAO. Testicular tissues were analyzed for oxidative stress markers (reduced glutathione [GSH], malondialdehyde [MDA], superoxide dismutase [SOD], and catalase [CAT]), inflammatory cytokines (interleukin-6 [IL-6], tumor necrosis factor-alpha [TNF- α], transforming growth factor-beta 1 [TGF- β 1], and interleukin-1 beta [IL-1 β]), and steroidogenic markers (3 β -hydroxysteroid dehydrogenase [3 β -HSD], 17 β -hydroxysteroid dehydrogenase [17 β -HSD], and steroidogenic acute regulatory protein [StAR]). Sepsis significantly decreased GSH ($p < .0001$), SOD ($p < .01$), and CAT ($p < .0001$) levels, while increasing MDA ($p < .0001$) levels compared with the control group. In addition, IL-6 ($p < .0001$), TNF- α ($p < .001$), TGF- β 1 ($p < .05$), and IL-1 β ($p < .0001$) levels were significantly elevated, whereas the expression of 3 β -HSD ($p < .01$), 17 β -HSD ($p < .05$), and StAR ($p < .01$) was significantly reduced. Compared with the sepsis group, AAO treatment significantly improved antioxidant status (all $p < .05$), suppressed inflammatory cytokine levels (all $p < .01$), and enhanced steroidogenic marker expression (all $p < .05$). These findings demonstrate that AAO exerts potent antioxidant and anti-inflammatory effects, attenuates sepsis-induced testicular injury, and preserves steroidogenesis, suggesting its potential as a protective agent for sepsis-associated testicular dysfunction.

Keywords: Active anionic oxygen, Inflammation, Oxidative stress, Sepsis, Steroidogenesis.

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Introduction

Sepsis is a public health problem that develops with a dysregulated host response to infection, manifests with complex physiological and biochemical responses, can result in death, and poses a significant global threat (Jarczack et al., 2021; Rudd et al., 2020; Singer et al., 2016). It is one of the most common causes of hospital mortality, and the incidence of sepsis is increasing day by day (Rudd et al., 2020; Singer et al., 2016). Sepsis can be caused by any type of infectious agent, and its clinical manifestations vary according to geographical regions. Eighty percent of cases are community-acquired, and the most common infection focus is the lungs (64%). In terms of etiology, Gram-positive and Gram-negative bacteria, particularly *Staphylococcus aureus* (*S. aureus*), *Pseudomonas spp.*, and *Escherichia coli* (*E. coli*), are observed at approximately equal rates. The pathophysiology of sepsis is shaped by the uncontrolled host response to infection. In this process, microbes are recognized via Toll-like receptors (TLRs) on innate immune cells, leading to activation of the Nuclear Factor kappa B (NF- κ B) pathway. Consequently, both pro- and anti-inflammatory cytokines are released. It is currently accepted that early excessive

inflammation and late immunosuppression may overlap. Organ damage is associated with factors such as vascular disorders, reduced oxygen utilization, and mitochondrial injury (Cecconi et al., 2018).

Active Anionic Oxygen (AAO) is defined as a water-based, natural biocide that eliminates infectious agents by stimulating the body's own antibody production without the need for chemicals such as vaccines or antibiotics. This substance, which has no irritating properties and is harmless to human health, is a broad-spectrum octaoxidant that destroys all pathogens through anionic active oxygen atoms. Its efficacy has been proven by laboratory and clinical studies. It is an organic molecule that exhibits rapid action against all mutated bacteria, viruses, and fungal agents, including coronavirus. By virtue of its high oxidation-reduction potential (ORP), it oxidizes microorganisms by stripping electrons from their atoms and eliminates them through oxidative burst. It also oxidizes and kills the biofilm layer and the pathogens concealed beneath it. It is reported to oxidize and destroy cancer cells as well. Containing no chemicals such as cortisone, alcohol, or antibiotics, it is completely organic and has a neutral pH as safe as water. It is non-toxic, non-allergenic, and non-irritant (Gerek et al., 2021).

Oxygen taken via the respiratory tract is reduced in the mitochondria, generating reactive oxygen species (ROS). The resulting ROS cause significant damage in many tissues throughout the organism and are therefore referred to as oxidants or free radicals. Antioxidants are utilized as a defense system to prevent ROS formation and the damage they cause in the organism. Oxidative stress, on the other hand, occurs due to an imbalance between oxidants and antioxidants (Kükürt et al., 2021). Although numerous molecular mechanisms are involved in the progression of sepsis, a significant portion of these arise from the production of ROS and the lack of sufficient cellular antioxidants. In septic conditions, excessive production of oxygen-derived free radicals contributes to the development of multi-organ failure and death. Numerous studies have demonstrated that oxidative balance is disrupted and oxidative stress levels are increased in sepsis patients and experimental models. This condition results from the combination of multiple factors, including hypotension, microvascular thrombosis, oxidative damage-

induced mitochondrial injury, and systemic inflammation (Sahoo et al., 2024).

Methods

Ethical Approval

Ethical approval for this study was obtained from the Animal Experiments Local Ethics Committee of Kafkas University (Date: May 8, 2026, No: KAÜ-HADYEK/2026-102).

Animals and Experimental Design

All experimental animals underwent a one-week adaptation period at the Experimental Animal Center of Kafkas University, during which they had free access to standard chow and water. They were housed under controlled environmental conditions (12 h light/12 h dark cycle, 22 ± 2 °C). Male Wistar Albino rats (*Rattus norvegicus*), weighing 250–300 g (approximately 10–12 weeks old), were used in this study. Animals were randomly divided into experimental groups using a simple randomization procedure. A total of 30 animals were randomly divided into four groups. Group 1 (control) included six animals ($n = 6$). Groups 2, 3, and 4 each included eight animals ($n = 8$).

All subjects were subjected to identical environmental and physiological stress conditions throughout the experiment to ensure the comparability of the obtained data. Animals in the control group received drinking water via oral gavage daily for 14 days. On day 14, they were injected intraperitoneally with physiological saline, concurrently with the LPS administration to the other groups (Aksak Karamese et al., 2025). Groups 2, 3, and 4 received AAO at a dose of 8 mL/kg/day via oral gavage for 14 days (Gerek et al., 2021). In the fourth group, following the 14-day AAO administration, a single dose of 7.5 mg/kg LPS was administered intraperitoneally to induce endotoxemia, as previously described by Aksak Karamese et al. (2025). This dose and route of administration were selected based on established protocols in the literature that reliably induce a systemic inflammatory response comparable to the early phase of sepsis, without causing rapid mortality. Tissue samples were collected at [18 hours] after LPS injection, when pro-inflammatory cytokine levels are known to peak.

Tissue Preparation

Tissue Homogenization

At the end of the experimental period, animals were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally. Following anesthesia, testicular tissue were excised, rinsed with ice-cold saline, and stored at -20°C until biochemical analysis. For biochemical assays, tissues were homogenized in ice-cold phosphate-buffered saline (PBS, pH 7.4) at a 1:10 (w/v) ratio using a bead mill homogenizer (Benchmark Scientific, USA) at 4,000 rpm for three cycles of 40 seconds each. The homogenates were then centrifuged at $14,000 \times g$ for 15 min at 4°C . The resulting supernatants were collected for further analyses. Protease inhibitors were not used in this study. The resulting supernatants were collected for further analyses. Protein concentrations were determined using the Bradford method with bovine serum albumin (BSA) as the standard (Bradford, 1976). GSH and MDA levels were partially improved to tissue weight and expressed as nmol/g tissue. SOD and CAT activities were partially improved to total protein content and expressed as U/g protein and U/mg protein, respectively.

Testicular Oxidative Stress Parameters

Testicular tissue was homogenized for the determination of GSH (Beutler et al., 1963), MDA (Yoshioka et al., 1979), CAT (Aebi, 1974), SOD (Sun et al., 1988) activities, which were analyzed according to their respective methods. MDA levels were determined using the Yoshioka method. In this assay, MDA, an aldehyde product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to form a pink-colored complex. The absorbance of this complex was measured at 532 nm using a spectrophotometer (Bio-Tek Gen5 Epoch, USA). According to the Beutler method, DTNB [5,5'-Dithiobis(2-nitrobenzoic) acid] is reduced by compounds containing sulfhydryl groups, and the resulting yellow color was measured at 412 nm using a spectrophotometer (Bio-Tek Gen5 Epoch, USA). CAT activity was determined according to the method of Aebi (1974). This method is based on the decomposition rate of hydrogen peroxide (H_2O_2), and the change in absorbance was measured at 240 nm spectrophotometrically. SOD(SOD) activity was

determined according to the method of Sun et al. (1988). This method is based on the inhibition of superoxide radical-induced reactions by SOD, and the degree of inhibition was measured spectrophotometrically at 560 nm.

Determination of Testicular Cytokine Levels

Testicular tissue samples were homogenized and centrifuged, and the supernatants were used for cytokine analysis. The levels of IL-1 β , TNF- α , IL-6, and TGF- β 1 were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Wayon-Bio Co., Ltd., China) according to the manufacturer's instructions. The catalog numbers for the kits were as follows: IL-1 β (WOB-30206), TNF- α (WOB-31063), IL-6 (WOB-30219), and TGF- β 1 (WOB-31073). Optical densities were measured at 450 nm using a microplate spectrophotometer (Bio-Tek Gen5 Epoch, USA).

Assessment of Testicular Steroidogenesis Markers

The levels of StAR, 3 β -HSD, and 17 β -HSD in testicular tissue were determined using ELISA kits (Wayon-Bio Co., Ltd., China) according to the manufacturer's instructions. The catalog numbers for the kits were as follows: 3 β -HSD (WOB-34721), 17 β -HSD (WOB-39021), and StAR (WOB-34809). Optical densities were measured at 450 nm using a microplate spectrophotometer (Bio-Tek Gen5 Epoch, USA).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). Data were presented as mean $\pm$ Standard Error of the Mean (SEM). Statistical significance was determined using a one-way analysis of variance (One-Way ANOVA). Before performing the one-way ANOVA, the assumptions of normality and homogeneity of variances were tested using appropriate statistical tests. Normality was assessed using the Shapiro–Wilk test, and homogeneity of variances was assessed using Levene's test. One-way ANOVA was applied to assess differences among groups, followed by Tukey's post hoc test. Results with p -values less than .05 were considered statistically significant.

Results

Effects on Testicular Oxidative Stress Parameters

GSH Levels

As shown in Figure 1A, testicular GSH levels were significantly decreased in the sepsis group compared to the control group ($p < .0001$). AAO administration alone also led to a significant reduction in GSH levels relative to the control group ($p < .05$). In the Sepsis+AAO group, GSH levels were also significantly lower than those in the control group ($p < .0001$). Compared with the sepsis group, both the AAO and Sepsis+AAO groups exhibited significantly increased GSH levels ($p < .0001$ for both). Moreover, the difference between the AAO and Sepsis+AAO groups was also statistically significant ($p < .001$). These findings indicate that sepsis-induced oxidative stress suppresses testicular GSH levels, whereas AAO protective partially restores these levels.

MDA Levels

As shown in Figure 1B, testicular MDA levels were significantly increased in the sepsis group compared to the control group ($p < .0001$). AAO administration alone also led to a significant change in MDA levels relative to the control group ($p < .05$). In the Sepsis+AAO group, MDA levels were significantly different from those in the control group ($p < .05$). Compared with the sepsis group, both the AAO and Sepsis+AAO groups exhibited significantly decreased MDA levels ($p < .05$ for both). Moreover, the difference between the AAO and Sepsis+AAO groups was also statistically significant ($p < .05$). These findings indicate that sepsis-induced oxidative stress increases testicular MDA levels, whereas AAO protective partially restores these levels.

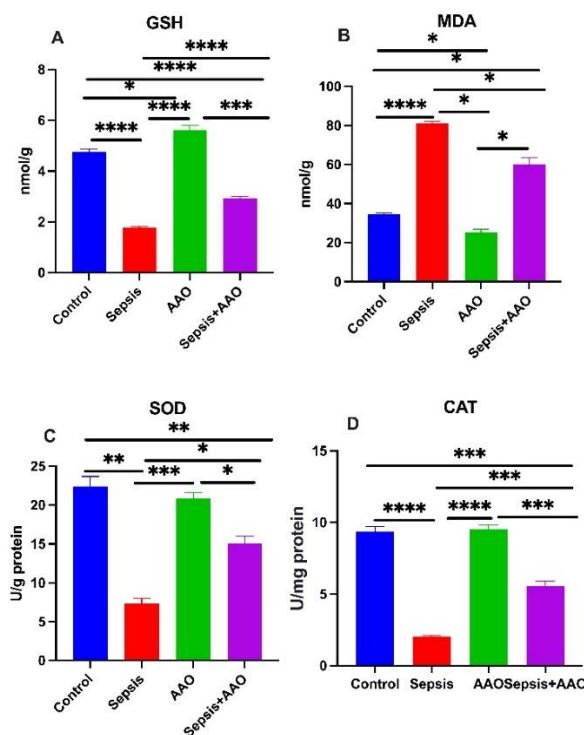
SOD Activity

As shown in Figure 1C, testicular SOD levels were significantly decreased in the sepsis group compared to the control group ($p < .01$). AAO administration alone did not cause a significant change in SOD levels relative to the control group ($p > .05$). In the Sepsis+AAO group, SOD levels were significantly lower than those in the control group ($p < .01$). Compared with the sepsis group, both the AAO and Sepsis+AAO groups exhibited significantly increased SOD

levels ($p < .001$ and $p < .05$, respectively). Furthermore, the difference between the AAO and Sepsis+AAO groups was also statistically significant ($p < .05$). These findings indicate that sepsis-induced oxidative stress suppresses testicular SOD levels, whereas AAO protective partially restores these levels.

Figure 1.

Oxidative stress parameters in testicular tissue. (A) GSH, (B) MDA, (C) SOD, (D) CAT. GSH: Glutathione; MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; AAO: Active anionic oxygen.



Note: (A) Testicular tissue GSH levels, (B) testicular tissue MDA levels, (C) testicular tissue SOD activity, and (D) testicular tissue CAT activity are shown. Data are presented as mean $\pm$ standard error of the mean (SEM). The study included the following sample sizes: control group ($n = 6$), sepsis group ($n = 8$), AAO group ($n = 8$), and sepsis + AAO group ($n = 8$). Statistical significance is indicated as * $p < .05$, ** $p < .01$, *** $p < .001$, and **** $p < .0001$.

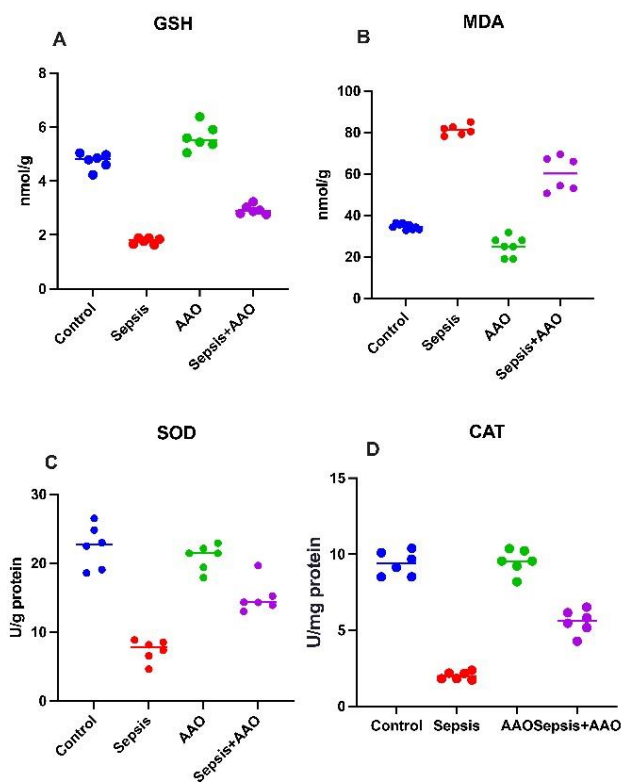
CAT Activity

As shown in Figure 1D, testicular CAT levels were significantly decreased in the sepsis group compared to the control group ($p < .0001$). AAO administration alone did not cause a significant change in CAT levels relative to the control group ($p > .05$). In the Sepsis+AAO group, CAT levels were significantly lower than those in the control group ($p < .001$). Compared with the sepsis group, both the AAO and

Sepsis+AAO groups exhibited significantly increased CAT levels ($p < .0001$ and $p < .001$, respectively). Furthermore, the difference between the AAO and Sepsis+AAO groups was also statistically significant ($p < .001$). These findings indicate that sepsis-induced oxidative stress suppresses testicular CAT levels, whereas AAO protective partially restores these levels. The distribution of oxidative stress-related biomarkers, including glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT), is presented in Figure 2.

Figure 2.

Effect of AAO treatment on oxidative stress markers in testicular tissue. (A) GSH, (B) MDA, (C) SOD, (D) CAT levels. Data are presented as mean $\pm$ SEM. Individual data points for each animal are shown in the figure. GSH: Glutathione; MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; AAO: Active anionic oxygen.



Changes in Testicular Cytokine Levels

IL-6 Levels

As shown in Figure 3A, testicular IL-6 levels were markedly increased in the sepsis group compared to the control group ($p < .0001$). AAO administration alone did not cause a significant change in IL-6 levels relative to the control group ($p > .05$). In the Sepsis+AAO group, IL-6 levels were

significantly higher than those of the control group ($p < .001$). Compared with the sepsis group, IL-6 levels were significantly lower in both the AAO group ($p < .0001$) and the Sepsis+AAO group ($p < .01$). Furthermore, the difference between the AAO and Sepsis+AAO groups was also statistically significant ($p < .001$). These findings indicate that sepsis-induced inflammatory response markedly increases testicular IL-6 levels, whereas AAO protective significantly suppresses IL-6 levels.

TNF- α Levels

As shown in Figure 3B, testicular TNF- α levels were significantly increased in the sepsis group compared to the control group ($p < .01$). AAO administration alone did not cause a significant change in TNF- α levels relative to the control group ($p > .05$). In the Sepsis+AAO group, TNF- α levels were significantly higher than those of the control group ($p > .05$). Compared with the sepsis group, TNF- α levels were significantly lower in both the AAO group ($p < .01$) and the Sepsis+AAO group ($p < .01$). Furthermore, the difference between the AAO and Sepsis+AAO groups was not statistically significant ($p > .05$). These findings indicate that sepsis-induced inflammatory response significantly increases testicular TNF- α levels, whereas AAO protective significantly suppresses TNF- α levels.

TGF- β 1 Levels

As shown in Figure 3C, testicular TGF- β 1 levels were significantly increased in the sepsis group compared to the control group ($p < .05$). AAO administration alone did not cause a significant change in TGF- β 1 levels relative to the control group ($p > .05$). In the Sepsis+AAO group, TGF- β 1 levels were significantly higher than those of the control group ($p > .05$). Compared with the sepsis group, TGF- β 1 levels were significantly lower in both the AAO group ($p < .01$) and the Sepsis+AAO group ($p < .001$). Furthermore, the difference between the AAO and Sepsis+AAO groups was not statistically significant ($p > .05$). These findings indicate that sepsis-induced inflammatory response significantly increases testicular TGF- β 1 levels, whereas AAO protective significantly suppresses TGF- β 1 levels.

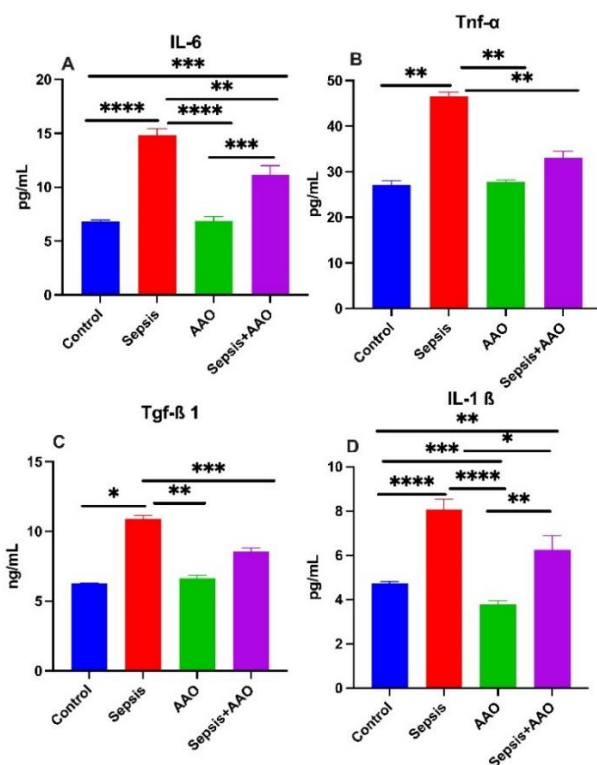
IL-1 β Levels

As shown in Figure 3D, testicular IL-1 β levels were significantly increased in the sepsis group compared to the control group ($p < .0001$). AAO administration alone also

led to a significant increase in IL-1 β levels relative to the control group ($p < .001$). In the Sepsis+AAO group, IL-1 β levels were significantly higher than those of the control group ($p < .01$). Compared with the sepsis group, IL-1 β levels were significantly lower in both the AAO group ($p < .0001$) and the Sepsis+AAO group ($p < .05$). Furthermore, the difference between the AAO and Sepsis+AAO groups was also statistically significant ($p < .001$). These findings indicate that sepsis-induced inflammatory response significantly increases testicular IL-1 β levels, whereas AAO protective significantly suppresses IL-1 β levels. The distribution of inflammatory cytokine levels in testicular tissue, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), transforming growth factor-beta 1 (TGF- β 1), and interleukin-1 beta (IL-1 β), is presented in Figure 4.

Figure 3.

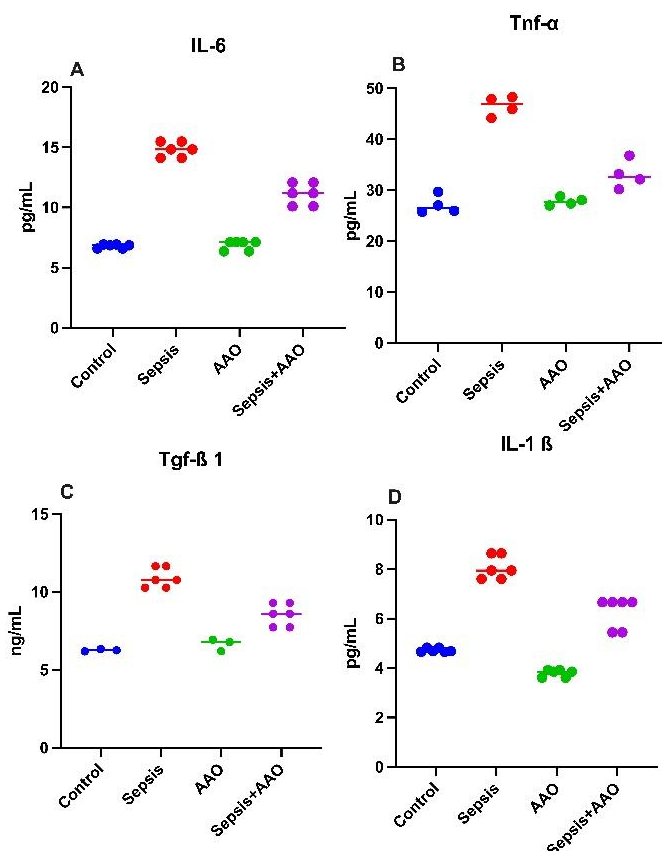
Inflammatory cytokine levels in testicular tissue. (A) IL-6, (B) TNF- α , (C) TGF- β 1, (D) IL-1 β . IL-6: Interleukin-6; TNF- α : Tumor necrosis factor-alpha; TGF- β 1: Transforming growth factor-beta 1; IL-1 β : Interleukin-1 beta; AAO: Active anionic oxygen.



Note: (A) Testicular tissue IL-6 levels, (B) testicular tissue TNF- α levels, (C) testicular tissue TGF- β 1 levels, and (D) testicular tissue IL-1 β levels are shown. Data are presented as mean $\pm$ standard error of the mean (SEM). The study included the following sample sizes: control group ($n = 6$), sepsis group ($n = 8$), AAO group ($n = 8$), and sepsis + AAO group ($n = 8$). Statistical significance is indicated as * $p < .05$, ** $p < .01$, *** $p < .001$, and **** $p < .0001$.

Figure 4.

Effect of AAO treatment on inflammatory cytokine levels in testicular tissue. (A) IL-6, (B) TNF- α , (C) TGF- β 1, (D) IL-1 β levels. Data are presented as mean $\pm$ SEM. Individual data points for each animal are shown in the figure. IL-6: Interleukin-6; TNF- α : Tumor necrosis factor-alpha; TGF- β 1: Transforming growth factor-beta 1; IL-1 β : Interleukin-1 beta; AAO: Active anionic oxygen.



Effects on Testicular Steroidogenesis Markers

3 β -HSD Levels

As shown in Figure 5A, testicular 3 β -HSD levels were significantly decreased in the sepsis group compared to the control group ($p < .01$). AAO administration alone did not cause a significant change in 3 β -HSD levels relative to the control group ($p > .05$). In the Sepsis+AAO group, 3 β -HSD levels were also significantly lower than the control group, albeit to a lesser extent ($p < .05$). Compared with the sepsis group, 3 β -HSD levels were significantly increased in the AAO group ($p < .01$) and markedly increased in the Sepsis+AAO group ($p < .0001$). Furthermore, the difference between the AAO and Sepsis+AAO groups was statistically significant ($p < .05$). These findings indicate that sepsis-

induced suppression of steroidogenesis reduces 3 β -HSD levels, whereas AAO protective significantly increases 3 β -HSD levels.

17 β -HSD Levels

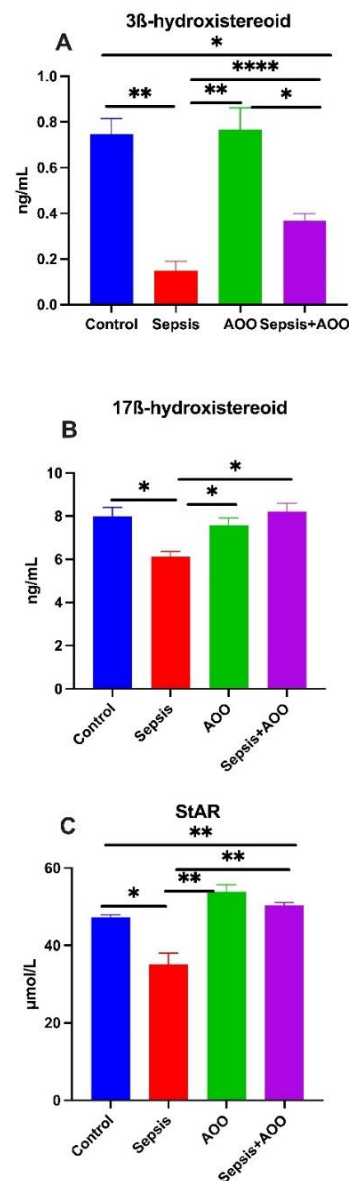
As shown in Figure 5B, testicular 17 β -HSD levels were significantly decreased in the sepsis group compared to the control group ($p < .05$). AAO administration alone did not cause a significant change in 17 β -HSD levels relative to the control group ($p > .05$). No significant difference was observed between the Sepsis+AAO and control groups ($p > .05$). Compared with the sepsis group, 17 β -HSD levels were significantly increased in both the AAO group ($p < .05$) and the Sepsis+AAO group ($p < .05$). Furthermore, no significant difference was found between the AAO and Sepsis+AAO groups ($p > .05$). These findings indicate that sepsis-induced suppression of steroidogenesis reduces 17 β -HSD levels, whereas AAO protective significantly increases 17 β -HSD levels.

StAR Levels

As shown in Figure 5C, testicular StAR levels were significantly decreased in the sepsis group compared to the control group ($p < .05$). AAO administration alone did not cause a significant change in StAR levels relative to the control group ($p > .05$). In the Sepsis+AAO group, StAR levels were significantly lower than those of the control group ($p < .01$). Compared with the sepsis group, StAR levels were significantly increased in both the AAO group ($p < .01$) and the Sepsis+AAO group ($p < .01$). Furthermore, no significant difference was found between the AAO and Sepsis+AAO groups ($p > .05$). These findings indicate that sepsis-induced suppression of steroidogenesis reduces StAR levels, whereas AAO protective significantly increases StAR levels. Figure 6 shows the distribution of steroidogenic marker expression in testicular tissue, including 3 β -hydroxysteroid dehydrogenase (3 β -HSD), 17 β -hydroxysteroid dehydrogenase (17 β -HSD), and steroidogenic acute regulatory protein (StAR), across the experimental groups.

Figure 5.

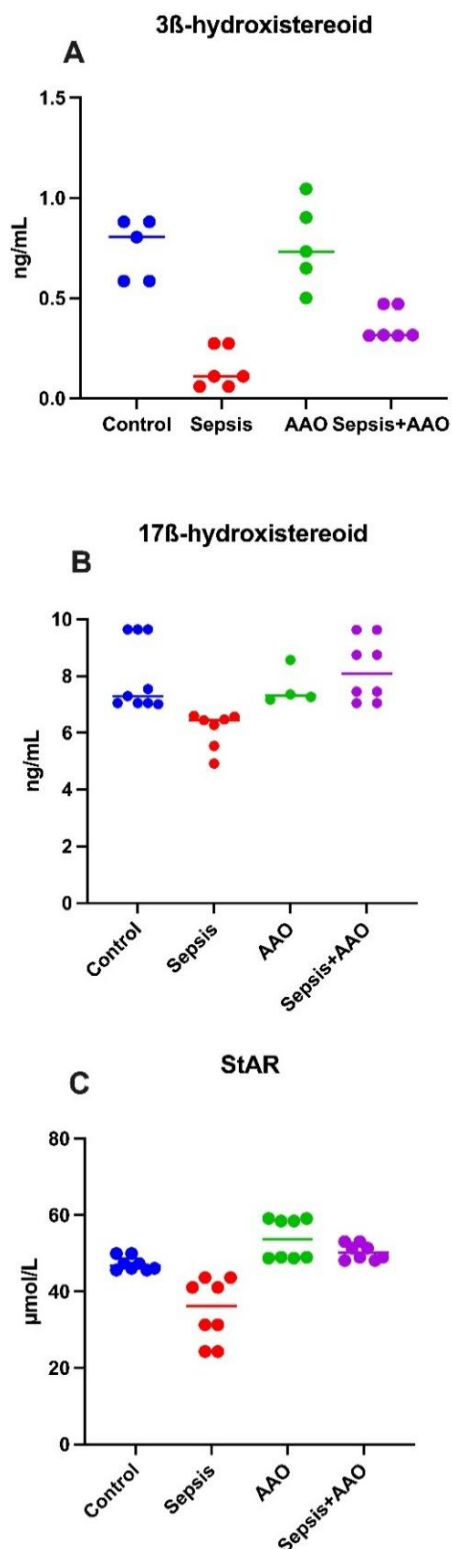
Steroidogenesis markers in testicular tissue. (A) 3 β -HSD, (B) 17 β -HSD, (C) StAR. 3 β -HSD: 3 β -Hydroxysteroid dehydrogenase; 17 β -HSD: 17 β -Hydroxysteroid dehydrogenase; StAR: Steroidogenic acute regulatory protein; AAO: Active anionic oxygen.



Note: (A) Testicular tissue 3 β -HSD levels, (B) testicular tissue 17 β -HSD levels, and (C) testicular tissue StAR levels are shown. Data are presented as mean $\pm$ standard error of the mean (SEM). The study included the following sample sizes: control group ($n = 6$), sepsis group ($n = 8$), AAO group ($n = 8$), and sepsis + AAO group ($n = 8$). Statistical significance is indicated as * $p < .05$, ** $p < .01$, *** $p < .001$, and **** $p < .0001$.

Figure 6.

Effect of AAO treatment on steroidogenic marker expression in testicular tissue. (A) 3 β -HSD, (B) 17 β -HSD, (C) StAR levels. Data are presented as mean $\pm$ SEM. Individual data points for each animal are shown in the figure. 3 β -HSD: 3 β -Hydroxysteroid dehydrogenase; 17 β -HSD: 17 β -Hydroxysteroid dehydrogenase; StAR: Steroidogenic acute regulatory protein; AAO: Active anionic oxygen.



Discussion

The ability of experimental endotoxemia models to reflect clinical sepsis has long been a subject of debate. Acute LPS-induced endotoxemia differs markedly from clinical sepsis, which is characterized by a polymicrobial and dynamically evolving pathophysiology while the LPS model triggers an early and excessive cytokine release, the inflammatory response in clinical sepsis is slower, more sustained, and more complex. Therefore, experimental findings based on LPS should be interpreted with caution, considering the failure of immunotherapies in clinical sepsis (Remick et al., 2000). In the present study, it was determined that LPS-induced sepsis led to increased oxidant status and decreased antioxidant defense mechanisms in rat testicular tissue, resulting in a marked elevation of oxidative stress. Furthermore, AAO administration partially improved this oxidative damage and significantly reduced oxidative stress in testicular tissue. Previous studies have demonstrated that LPS-induced sepsis increases oxidative stress in testicular, lung, and kidney tissues in animal models, and furthermore, cell culture studies have shown that LPS treatment induces DNA damage by increasing ROS production. These findings are consistent with the increased oxidative stress observed in the sepsis group of the present study (Aydin et al., 2013; Bati et al., 2025; Raheem et al., 2025). The TBARS (Thiobarbituric Acid Reactive Substances) method is widely used as an indicator of lipid peroxidation. However, this method has a well-known limitation: Thiobarbituric acid (TBA) reacts not only with MDA (malondialdehyde) but also with other aldehydes and by-products of lipid peroxidation. For this reason, the measured value should be expressed as "TBARS" (thiobarbituric acid reactive substances) rather than "MDA." This situation limits the specificity of the method (Aguilar Diaz De Leon & Borges, 2020).

Sepsis is a complex pathophysiological process that leads to oxidative stress through an imbalance between ROS production and antioxidant defense mechanisms. During sepsis, immune cells such as neutrophils and macrophages become activated in response to microbial invasion and generate large amounts of ROS, including superoxide anion, hydrogen peroxide, and hydroxyl radicals. Although this ROS production is essential for pathogen clearance, excessive and dysregulated production results in

widespread cellular and tissue damage. Furthermore, inflammatory mediators and cytokines stimulate iNOS activation, leading to excessive nitric oxide production. Nitric oxide subsequently reacts with superoxide to form peroxynitrite, a highly reactive molecule. Oxidative stress-induced activation of NF- κ B further amplifies pro-inflammatory cytokine production, thereby creating a vicious cycle. At the mitochondrial level, sepsis-induced electron transport chain dysfunction leads to electron leakage and disproportionate ROS formation, resulting in collapse of the mitochondrial membrane potential, impaired ATP synthesis, and activation of apoptosis through mitochondrial permeability transition pore opening and subsequent cytochrome c release.

At the systemic level, oxidative stress contributes to vascular and endothelial dysfunction by promoting capillary leakage, microcirculatory disturbances, and thrombosis. These effects impair oxygen and nutrient delivery to tissues, triggering hypoxia, metabolic acidosis, and organ failure. In conclusion, sepsis-induced oxidative stress is increased through multiple mechanisms including immune cell activation, iNOS-mediated nitric oxide production, mitochondrial dysfunction, and activation of redox-sensitive transcription factors together with the depletion of antioxidant defenses, this exacerbates tissue damage and predisposes to multiple organ failure (Galley, 2011; Lin et al., 2025; Sahoo et al., 2024).

In the present study, a marked increase in pro-inflammatory cytokine levels (IL-6, TNF- α , IL-1 β , TGF- β 1) was detected in the testicular tissue of rats with LPS-induced sepsis. This finding indicates that the systemic inflammatory response triggered by sepsis is also strongly induced in testicular tissue. In other studies, increased levels of inflammatory cytokines have been reported in testicular, cardiac, and renal tissues in animal models of LPS-induced sepsis, and similarly, pro-inflammatory responses have been shown to be triggered in cell culture studies. These findings are consistent with the increased inflammation observed in the sepsis group of the present study (El-Sherbiny et al., 2025; Guo et al., 2025; Üstündağ et al., 2023).

Both pro-inflammatory (IL-6, IL-8, IL-18, TNF- α) and anti-inflammatory (IL-10) cytokines play critical roles in the development of sepsis. Marked elevations in the circulating

levels of these molecules have been detected in patients diagnosed with sepsis. In particular, IL-8 and MCP-1 levels have been directly correlated with SOFA scores, which grade organ dysfunction, as well as with mortality rates. In the early stages of sepsis research, the underlying mechanism was believed to be an uncontrolled inflammatory process known as a cytokine storm. Accordingly, anti-inflammatory agents such as TNF inhibitors, IL-1 blockers, and corticosteroids were tested in clinical trials. However, these approaches failed to demonstrate the expected benefits and, in some cases, even led to increased mortality rates. Subsequent investigations revealed that the inflammatory response in septic patients is far more complex than initially assumed. Postmortem analyses of splenic tissue showed a marked suppression of cytokine production, a finding attributed to functional exhaustion of T cells. While some septic individuals exhibit rapid synthesis of both pro- and anti-inflammatory cytokines, others display an imbalance favoring anti-inflammatory cytokines or a generalized suppression of cytokine production. Furthermore, sepsis triggers intense inflammatory responses that induce epigenetic alterations, including DNA methylation, histone modifications, and chromatin remodeling. These changes disrupt the expression of genes responsible for regulating immune responses, thereby rendering the host more susceptible to infections. Deficiencies in micronutrients such as vitamins and minerals adversely affect both innate and adaptive immune mechanisms, increasing the risk of infection. Antioxidant vitamins and trace elements play a decisive role in maintaining proper immune function. Oxidative stress triggers inflammatory processes by activating NF- κ B and elevates the circulating levels of inflammatory mediators in septic patients. Although reactive oxygen species are essential for immune cells to carry out their functions, excessive levels cause tissue damage. Therefore, maintaining adequate antioxidant defense systems may represent an effective strategy for reducing tissue injury and immunosuppression (Paul et al., 2026; Sahoo et al., 2024).

Testosterone hormone synthesized from Leydig cells is produced from cholesterol. Four enzymes are very important in this production because they are involved in catalysis. These enzymes are: cytochrome P450 cholesterol side-chain cleavage enzyme, 3 β -HSD isoform, cytochrome P450 17 α -hydroxylase/17-20 lyase, and 17 β -HSD3. Among

these, the dehydrogenases (3 β -HSD and 17 β -HSD) are localized in the smooth endoplasmic reticulum (Hu et al., 2010). Hydroxysteroid dehydrogenases (HSDs) constitute a group of oxidoreductase enzymes that depend on NADPH or NAD⁺ as cofactors. They facilitate oxidation-reduction reactions at distinct sites (3 α , 3 β , 11 β , 17 β , 20 α , and 20 β) on steroidal substrates. These enzymes are essential for both the synthesis and the degradation of steroid hormones. In addition, within target tissues, they interconvert inactive steroids and their active counterparts, thereby enabling pre-receptor modulation of steroid hormone activity. For this reason, HSDs are often described as molecular switches. Humans and certain primates are distinct among animal species because their adrenal glands secrete substantial quantities of inactive steroid precursors, such as DHEA. These precursors do not bind directly to steroid receptors; instead, they must first be converted into active hormones within target tissues before exerting androgenic or estrogenic effects. Disruption of the balance between androgens and estrogens is implicated in the pathogenesis of several serious conditions, including breast cancer, prostate cancer, polycystic ovary syndrome (PCOS), and endometriosis (Atanassova & Koeva 2012).

StAR (Steroidogenic Acute Regulatory Protein) is a critical protein that controls the rate-limiting step of steroid hormone synthesis by actively transporting cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane, thereby enabling CYP11A (P450_{scc}) to access cholesterol; without this transport, steroid synthesis cannot proceed. The protein level of the StAR gene is regulated by Steroidogenic Factor-1 (SF-1), which is expressed from early fetal life and binds to the promoter regions of both the StAR gene and all CYP genes to activate their expression. The essential requirement of StAR in steroidogenesis is demonstrated by the cessation of steroidogenesis in StAR knockout mice and by human StAR mutations that cause a potentially fatal condition known as congenital lipoid adrenal hyperplasia. Similarly, 46XY individuals with SF-1 mutations exhibit impaired fetal testosterone biosynthesis and XY sex reversal, while mice with Leydig cell-specific SF-1 knockout lose CYP11A and StAR expression, resulting in adrenal and gonadal agenesis. Furthermore, the activity of the CYP11A enzyme is regulated by the mitochondrial environment, and

homozygous mutations of this enzyme are lethal due to the inability of the placenta to produce progesterone, while 46XY genetic males with partial CYP11A inactivation exhibit significant deficiencies in masculinization (Atanassova & Koeva 2012). In this study, a significant decrease in the protein level levels of StAR, 3 β -HSD, and 17 β -HSD was detected in the testicular tissue of rats with LPS-induced sepsis. Sepsis-induced oxidative stress and inflammatory response are thought to suppress the steroidogenic acute regulatory protein and dehydrogenase enzymes involved in steroid synthesis, thereby adversely affecting testosterone biosynthesis. These findings demonstrate the suppressive effect of sepsis on testicular steroidogenesis. No study has been found in the literature that directly examines the protein level of StAR, 3 β -HSD, and 17 β -HSD together in testicular tissue during sepsis. However, existing studies indicate that sepsis-induced oxidative stress and inflammation may have suppressive effects on steroidogenesis. The present study is one of the first to reveal the relationship between sepsis and testicular steroidogenic enzymes. Although not directly in sepsis models, decreased activities of StAR, 3 β -HSD, and 17 β -HSD have been reported in various testicular injury models. These findings are consistent with the suppression of the same enzymes observed in sepsis-induced testicular injury in our study. Therefore, the results of the present study support that testicular steroidogenesis may be similarly affected across different injury mechanisms (Ali et al., 2024; Hafez et al., 2025; Jana et al., 2006; Yildirim et al., 2024).

In this study, increased oxidative stress and inflammation were observed in the testicular tissue of rats with LPS-induced sepsis, while the expression levels of StAR, 3 β -HSD, and 17 β -HSD were suppressed. AAO protective significantly reduced oxidative stress and inflammation while increasing the expression of steroidogenic enzymes. These findings suggest that AAO may protect testicular steroidogenesis through its antioxidant and anti-inflammatory properties. It was concluded that AAO partially improved the decrease in StAR, 3 β -HSD, and 17 β -HSD enzymes by suppressing sepsis-induced oxidative damage and inflammatory response, thereby positively affecting testosterone synthesis.

In the literature, AAO (H₂O₈) has been reported as a potent

biocidal agent, demonstrating 99.999% efficacy against SARS-CoV-2 within 15 seconds. The same study reported that anionic oxygen administered orally and via inhalation to rats showed no toxic effects on the heart, liver, kidneys, and stomach; however, dose-dependent peribronchial lymphoid hyperplasia and emphysematous changes were observed. In our study, AAO reduced oxidative stress and inflammation, while increasing the protein level of StAR, 3 β -HSD, and 17 β -HSD enzymes in sepsis-induced testicular injury. These findings suggest that AAO may exhibit protective effects in different tissues and disease models (Gerek et al., 2021). In the current literature review, although a limited number of studies on AAO were found, no research directly investigating the antioxidant and anti-inflammatory effects of this compound was identified. Existing studies have primarily focused on the biocidal and antiviral properties of AAO. In the present study, AAO protective demonstrated antioxidant effects by reducing oxidative stress markers, exhibited anti-inflammatory properties by suppressing pro-inflammatory cytokine levels, and protected steroidogenesis by increasing the protein level of StAR, 3 β -HSD, and 17 β -HSD enzymes in sepsis-induced testicular injury. These findings are significant as they provide the first experimental data revealing the antioxidant and anti-inflammatory potential of AAO. Serum testosterone levels and specific antioxidant mechanisms (e.g., the Nrf2/HO-1 pathway) were not directly assessed. Therefore, our mechanistic interpretations remain limited, and further studies are warranted to confirm these findings.

Conclusion

To the best of our knowledge, this is the first experimental study to investigate the effects of AAO administration on sepsis-induced testicular injury. The findings demonstrate that AAO administration was associated with improvement in selected oxidative stress and inflammatory parameters, as well as partial improvement in the levels of proteins related to steroidogenesis (StAR, 3 β -HSD, and 17 β -HSD). Based on these findings, AAO may be considered a potential supportive agent for sepsis-induced testicular injury, likely attributed to its antioxidant and anti-inflammatory properties. However, further molecular and pharmacological studies are required to elucidate the underlying mechanisms of action, determine the appropriate dose and administration protocol, and evaluate its clinical applicability.

Ethics Committee Approval: Ethical approval for this study was obtained from the Animal Experiments Local Ethics Committee of Kafkas University (Date: May 8, 2026, No: KAÜ-HADYEK/2026-102).

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References

- Aebi, H. (1974). Catalase. *Methods of Enzymatic Analysis*, 2, 673–684. <https://doi.org/10.1016/B978-0-12-091302-2.50032-3>
- Aguilar Diaz de Leon, J., & Borges, C. R. (2020). Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances (TBARS) assay. *Journal of Visualized Experiments*, (159), e61122. <https://doi.org/10.3791/61122>
- Aksak Karamese, S., Gelen, V., Yildiz, G. N., Albayrak, K., Gedikli, S., Kara, A., & Karamese, M. (2025). Investigation of the anti-inflammatory, anti-oxidant and anti-apoptotic activity of 18 β - glycyrrhetic-acid on the model of LPS-induced lung injury in rats. *Molecular Immunology*, 181, 93–101. <https://doi.org/10.1016/j.molimm.2025.03.009>
- Ali, W., Khatyan, U., Sun, J., Alasmari, A., Alshahrani, M. Y., Qazi, I. H., Wang, T., Liu, Z., & Zou, H. (2024). Mitigating effect of pomegranate peel extract against the furan induced testicular injury by apoptosis, steroidogenic enzymes and oxidative stress. *Chemosphere*, 358, 142086. <https://doi.org/10.1016/j.chemosphere.2024.142086>
- Atanassova, N., & Koeva, Y. (2012). Hydrohysteroid dehydrogenases - Biological role and clinical importance - Review. In R. A. Canuto (Eds.), *Dehydrogenases*. IntechOpen. <https://doi.org/10.5772/54149>
- Aydin, S., Bacanlı, M., Taner, G., Şahin, T., Başaran, A. A., & Başaran, N. (2013). Protective effects of resveratrol on sepsis-induced DNA damage in the lymphocytes of rats. *Human & Experimental Toxicology*, 32(10), 1048–1057. <https://doi.org/10.1177/0960327112467047>
- Bati, Y. U., Sezer, M., Yilmaz, A., Baser, L., Guraslan, A., Bayram, P., & Karamese, M. (2025). The protective effects of dose-dependent umbelliferone application on clp-induced acute lung injury (ALI) model. *Journal of Biochemical and Molecular Toxicology*, 39(10), e70549. <https://doi.org/10.1002/jbt.70549>
- Beutler, E., Duron, O., & Kelly, B. M. (1963). Improved method for the determination of blood glutathione. *The Journal of Laboratory and Clinical Medicine*, 61, 882–888.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)

- Cecconi, M., Evans, L., Levy, M., & Rhodes, A. (2018). Sepsis and septic shock. *The Lancet*, 392(10141), 75–87. [https://doi.org/10.1016/S0140-6736\(18\)30696-2](https://doi.org/10.1016/S0140-6736(18)30696-2)
- El-Sherbiny, M., Elkattawy, H. A., Hadhoud, S., Nasr, A. N., Ibrahim, A. M., Ameer, O. Z., Alsaleebi, N., Asfari, J., Zakari, M. O., Mojaddidi, M. A., Ali, E. K., Almohaimeed, H. M., Abdeen, A., Ali, S. K., & Eldesoqui, M. (2025). Oxytocin ameliorates lipopolysaccharide-induced acute orchitis model: interplay of oxidative stress and inflammatory pathways. *Frontiers in Pharmacology*, 15, 1506777. <https://doi.org/10.3389/fphar.2024.1506777>
- Galley, H. F. (2011). Oxidative stress and mitochondrial dysfunction in sepsis. *British Journal of Anaesthesia*, 107(1), 57–64. <https://doi.org/10.1093/BJA/AER093>
- Gerek, M., Erdem, O., Aşık, M. B., Demiriz, M., Alkoclar, E. C., Diler, A. S., Kusbaygi, H., & Bursalı, U. (2021). A biocidal active anionic h2o8 oxygen solution with bronchodilative & oxygenizing features for the treatment of coronaviral infection related hypoxia - GATA animal trials abstract. *Zenodo*. <https://doi.org/10.5281/zenodo.5501039>
- Guo, M., Zhang, Y., Yang, H., & Liu, Q. (2025). On anxiolytic and antidepressant effects of curcumin in sepsis-induced post-intensive care syndrome: Antioxidant and anti-inflammatory effects. *Neuropsychobiology*, 84(5–6), 231–247. <https://doi.org/10.1159/000547791>
- Hafez, M. H., Gad, S. B., & El-Sayed, Y. S. (2025). Quercetin-mediated restoration of high-fat diet-induced male reproductive dysfunction through modifying spermatogenesis and unraveling 3β-HSD, 17β-HSD, and StAR pathways. *BMC Pharmacology and Toxicology*, 26(1), 90. <https://doi.org/10.1186/S40360-025-00918-y>
- Hu, G. X., Zhao, B. H., Chu, Y. H., Zhou, H. Y., Akingbemi, B. T., Zheng, Z. Q., & Ge, R. S. (2010). Effects of genistein and equol on human and rat testicular 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase 3 activities. *Asian Journal of Andrology*, 12(4), 519–526. <https://doi.org/10.1038/aja.2010.18>
- Jana, K., Jana, S., & Samanta, P. K. (2006). Effects of chronic exposure to sodium arsenite on hypothalamo-pituitary-testicular activities in adult rats: possible an estrogenic mode of action. *Reproductive Biology and Endocrinology*, 4(1), 9. <https://doi.org/10.1186/1477-7827-4-9>
- Jarczák, D., Kluge, S., & Nierhaus, A. (2021). Sepsis-pathophysiology and therapeutic concepts. *Frontiers in Medicine*, 8, 628302. <https://doi.org/10.3389/fmed.2021.628302>
- Kükürt, A., Gelen, V., Başer, Ö. F., Deveci, H. A., & Karapehlivan, M. (2021). Thiols: role in oxidative stress-related disorders. In P. Atukeren (Eds.), *Accenting lipid peroxidation*. IntechOpen. <https://doi.org/10.5772/intechopen.96682>
- Lin, Y., Alhaskawi, A., Chen, L., & Moqbel, S. A. A. (2025). Recent advances in understanding oxidative stress in sepsis: pathogenic roles and antioxidant therapeutic prospects - a narrative review. *Frontiers in Pharmacology*, 16, 1695992. <https://doi.org/10.3389/fphar.2025.1695992>
- Paul, S. N., Nessel, I., Puthucherry, Z., & Henson, S. M. (2026). Sepsis and the immunometabolic inflammatory response. *Npj Metabolic Health and Disease*, 4(1), 4. <https://doi.org/10.1038/s44324-025-00095-w>
- Raheem, A. K., Abu-Raghif, A. R., Abbas, A. H., Ridha-Salman, H., & Oubaid, E. N. (2025). Quercetin mitigates sepsis-induced renal injury via inhibiting inflammatory and oxidative pathways in mice. *Journal of Molecular Histology*, 56(3), 184. <https://doi.org/10.1007/S10735-025-10442-2>
- Remick, D. G., Newcomb, D. E., Bolgos, G. L., & Call, D. R. (2000). Comparison of the mortality and inflammatory response of two models of sepsis: lipopolysaccharide vs. cecal ligation and puncture. *Shock*, 13(2), 110–116. <https://doi.org/10.1097/00024382-200013020-00004>
- Rudd, K. E., Johnson, S. C., Agesa, K. M., Shackelford, K. A., Tsoi, D., Kievlan, D. R., Colombara, D. V., Ikuta, K. S., Kisson, N., Finfer, S., Fleischmann-Struzek, C., Machado, F. R., Reinhart, K. K., Rowan, K., Seymour, C. W., Watson, R. S., West, T. E., Marinho, F., Hay, S. I., ... Naghavi, M. (2020). Global, regional, and national sepsis incidence and mortality, 1990–2017: Analysis for the global burden of disease study. *Lancet*, 395(10219), 200–211. [https://doi.org/10.1016/S0140-6736\(19\)32989-7](https://doi.org/10.1016/S0140-6736(19)32989-7)
- Sahoo, D. K., Wong, D., Patani, A., Paital, B., Yadav, V. K., Patel, A., & Jergens, A. E. (2024). Exploring the role of antioxidants in sepsis-associated oxidative stress: A comprehensive review. *Frontiers in Cellular and Infection Microbiology*, 14, 1348713. <https://doi.org/10.3389/fcimb.2024.1348713>
- Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., Bellomo, R., Bernard, G. R., Chiche, J. D., Cooper-Smith, C. M., Hotchkiss, R. S., Levy, M. M., Marshall, J. C., Martin, G. S., Opal, S. M., Rubenfeld, G. D., van der Poll, T., Vincent, J. L., & Angus, D. C. (2016). The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA*, 315(8), 801–810. <https://doi.org/10.1001/jama.2016.0287>
- Sun, Y., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical Chemistry*, 34(3), 497–500. <https://doi.org/10.1093/clinchem/34.3.497>
- Üstündağ, H., Doğanay, S., Kalındemirtaş, F. D., Demir, Ö., Huyut, M. T., Kurt, N., Özgeriş, F. B., & Akbaba, Ö. (2023). A new treatment approach: Melatonin and ascorbic acid synergy shields against sepsis-induced heart and kidney damage in male rats. *Life Sciences*, 329, 121875. <https://doi.org/10.1016/J.LFS.2023.121875>
- Yildirim, S., Sengul, E., Aksu, E. H., Cinar, İ., Gelen, V., Tekin, S., & Dag, Y. (2024). Selenium reduces acrylamide-induced testicular toxicity in rats by regulating HSD17B1, StAR, and CYP17A1 expression, oxidative stress, inflammation, apoptosis, autophagy, and DNA damage. *Environmental Toxicology*, 39(3), 1402–1414. <https://doi.org/10.1002/tox.23996>
- Yoshioka, T., Kawada, K., Shimada, T., & Mori, M. (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. *American Journal of Obstetrics and Gynecology*, 135(3), 372–376. [https://doi.org/10.1016/0002-9378\(79\)90708-7](https://doi.org/10.1016/0002-9378(79)90708-7)