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Ebselen Alleviates Sepsis-Induced Acute Kidney Injury by Regulating Endoplasmic Reticulum Stress, Apoptosis, and Oxidative Stress

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ABSTRACT

Acute kidney injury (AKI) is one of the most serious complications of sepsis, with substantial morbidity and mortality, and no effective treatment exists. Ebselen is of pharmacological significance in the treatment and prevention of a variety of human diseases, such as cancer and cardiovascular disorders. Nevertheless, the role of Ebselen in the pathogenesis of sepsis-induced AKI remains unknown. Therefore, we aimed to elucidate the impact of Ebselen, an active seleno-organic compound, on AKI induced by lipopolysaccharide (LPS) and the associated molecular mechanisms, including endoplasmic reticulum (ER) stress, apoptosis, and oxidative stress. We established the sepsis-induced AKI rat model by injecting 5 mg/kg of LPS intraperitoneally. The rats were given Ebselen (5 and 10 mg/kg, orally) before receiving the LPS injection. Ebselen treatment alleviated renal tubular injury and reduced the levels of blood urea nitrogen (BUN) and creatinine (CREA) in LPS-induced sepsis model. Immunohistochemical and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) analyses revealed that Ebselen reduced caspase-3 expressions and apoptotic cells triggered by LPS in kidney tissues. LPS-induced sepsis caused ER stress, and Ebselen treatment alleviated the ER stress by regulating eukaryotic translation initiation factor 2-alpha kinase 3 (EIF2AK3) and GRP78 in kidney tissue, as well as activating transcription factor 4 (ATF4) and activating transcription factor 6 (ATF6) in serum. Ebselen decreased malondialdehyde (MDA) levels induced by LPS. Ebselen alleviated LPS-induced oxidative stress by modulating MDA and superoxide dismutase (SOD) levels in kidney tissues and SOD, glutathione peroxidase (GPx) and serum total antioxidant status (TAS) levels in serum. In conclusion, we report for the time that Ebselen might alleviate sepsis-induced AKI through the regulation of ER stress apoptosis and oxidative stress.

Abbreviations: AKI, acute kidney injury; ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; BUN, blood urea nitrogen; CAT, catalase; CREA, creatinine; EBS, Ebselen; EIF2AK3, eukaryotic translation initiation factor 2-alpha kinase 3; ER, endoplasmic reticulum; GPx, glutathione peroxidase; GRP78, glucose-regulated protein 78; LPS, lipopolysaccharide; MDA, malondialdehyde; NGAL, neutrophil gelatinase-associated lipocalin; OSI, oxidative stress index; SOD, superoxide dismutase; TAS, total antioxidant status; TOS, total oxidant status; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling.

1 | Introduction

Sepsis is a global health concern that has a significant impact on millions of individuals, resulting in an increase in the incidence of illness and mortality (Poston and Koyner 2019). Acute kidney injury (AKI) is a prevalent and possibly lethal complication of sepsis, impacting approximately 60% of individuals with severe sepsis (Poston and Koyner 2019; Peerapornratana et al. 2019). Sepsis triggers an excessive inflammatory response, causing the body to produce and release inflammatory mediators, chemokines, and cytokines (Poston and Koyner 2019). The eventual result is the destruction of renal tubular epithelial cells due to the dysregulation of multiple cellular mechanisms, such as endoplasmic reticulum (ER) stress, oxidative stress, inflammation, and autophagy (Kaymak et al. 2022). Despite notable progress in scientific research regarding the pathophysiology of AKI, our understanding of the molecular processes involved in sepsis-induced AKI remains unclear, and there is currently no effective therapy available to enhance the clinical outcomes of AKI (Li et al. 2019). Hence, additional research is required to mitigate the acute and long-term clinical consequences of AKI.

The ER is an essential organelle involved in various cellular processes, including protein synthesis, folding, and assembly. Additionally, it plays a key role in post-translational modifications and ensures protein quality control, thereby maintaining cellular homeostasis (Oakes and Papa 2015). The accuracy of the complex molecular pathways expressed here can be disrupted in many physiological states or by external stimuli (Roth et al. 2008). ER stress, a cellular defensive mechanism, is actively involved in renal injury (Han et al. 2008). During periods of stress and inflammation, the ER experiences a disruption in its normal balance, leading to the build-up of proteins that are either unfolded or misfolded; as a result, ER stress might arise. During ER stress, the unfolded protein response (UPR) is initiated to return ER function to normal balance. When the level of stress surpasses or continues beyond the ability of the UPR to compensate, apoptosis will be activated, leading to cellular damage or possibly cell death. As a result, ER stress and UPR have been linked to a wide range of pathogenic and inflammatory disorders. Furthermore, ER stress is closely linked to apoptosis, oxidative stress and cell damage events in sepsis (Di Conza and Ho 2020; Wu et al. 2021). Activating transcription factor 4 (ATF4) regulates several cellular mechanisms, including ER stress, inflammation, and autophagy. ATF4 plays a crucial role in renal fibrosis in diabetic kidney disease by modulating autophagy (Liang et al. 2021). Activating transcription factor 6 (ATF6), a regulatory protein found in the ER, is involved in tissue homeostasis, normal development and the ER stress response under pathological conditions (Ye et al. 2000). The build-up of misfolded proteins in the ER induces the activation of the ATF6, which then activates the genes responsible for the ER stress response (Ye et al. 2000; Hillary and FitzGerald 2018). Eukaryotic translation initiation factor 2-alpha kinase 3 (EIF2AK3) acts as a regulator of ER stress, and its inhibitor may have therapeutic benefits on diseases such as diabetes and cancer (Alasiri et al. 2020; Gupta et al. 2010). Glucose-regulated protein 78 (GRP78), one of the ER stress-related proteins, is a regulator of a variety of cellular processes, such as protein folding, assembly, and trafficking. Moreover, GRP78 plays a critical role in renal injury (Teng et al. 2018). Apoptosis is a vital cellular process that plays a role in the pathophysiology of sepsis-induced AKI by inducing renal tubular cell destruction and resulting in renal dysfunction (Sun et al. 2024). Previous research has highlighted that increased caspase-3 levels and TUNEL-positive cells indicate cell death in sepsis-induced AKI (Sun et al. 2024). A further element contributing to the pathogenesis of sepsis-induced AKI is oxidative stress, characterized by an imbalance between the generation of reactive oxygen species (ROS) and the protective mechanisms of antioxidants. This stress induces cellular damage in renal tissue by increasing malondialdehyde (MDA) levels and reducing defensive enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) (Zhu et al. 2024).

Ebselen is a multifunctional seleno-organic compound that plays a role in various molecular processes, such as oxidative stress, apoptosis and inflammation, potentially offering beneficial effects (Azad and Tomar 2014; Fulco et al. 2020). Furthermore, Ebselen functions as an antioxidant enzyme, playing a crucial role in numerous essential reactions that protect cellular components from damage caused by free radicals (Antony and Bayse 2011). Recent research has emphasized that Ebselen may possess many therapeutic potentials, such as anti-inflammatory, antioxidant and apoptotic effects, in the management and prevention of a variety of diseases, including cancer, osteoarthritis, and cardiovascular disorders (Okuyan et al. 2023; Thabet and Moustafa 2017; Davis and Bartfay 2004).

The aforementioned studies indicate that ER stress, apoptosis and oxidative stress are critical therapeutic mechanisms that can be targeted in sepsis-induced AKI (Teng et al. 2018; Taniguchi and Yoshida 2015). Furthermore, no study has been conducted to date to examine the potential protective role of Ebselen in sepsis-induced AKI or its relationship with ER stress, apoptosis, and oxidative stress. Therefore, we aimed to investigate whether Ebselen may have therapeutic effects on lipopolysaccharide (LPS)-induced renal injury and dysfunction and whether it performs its function in sepsis-induced AKI by regulating ER stress, apoptosis and oxidative stress.

2 | Materials and Methods

2.1 | Experimental Design

Following ethical approval, the animal trials were conducted at Sakarya University's Experimental Medicine Applications and Research Centre. The study included 32 *Wistar albino* male rats weighing between 150 and 250 g, who were unrestrictedly provided with food and drink. The rats were provided with a commercial complete diet containing 65% carbs, 24% protein and 11% fat, which had a metabolizable energy value (Altromin 1324, Lage, Germany). All lab animals were kept in a controlled environment with 12 h of darkness and 12 h of light, with a room temperature of 21°C ± 2°C. We ensured that the rats were acclimatized to the environment for a week prior to beginning the experiments. All rats were divided into four groups of seven rats each: 1. control group, 2. LPS group, 3. LPS + Ebselen group (5 mg/kg) and 4. LPS + Ebselen group (10 mg/kg). We

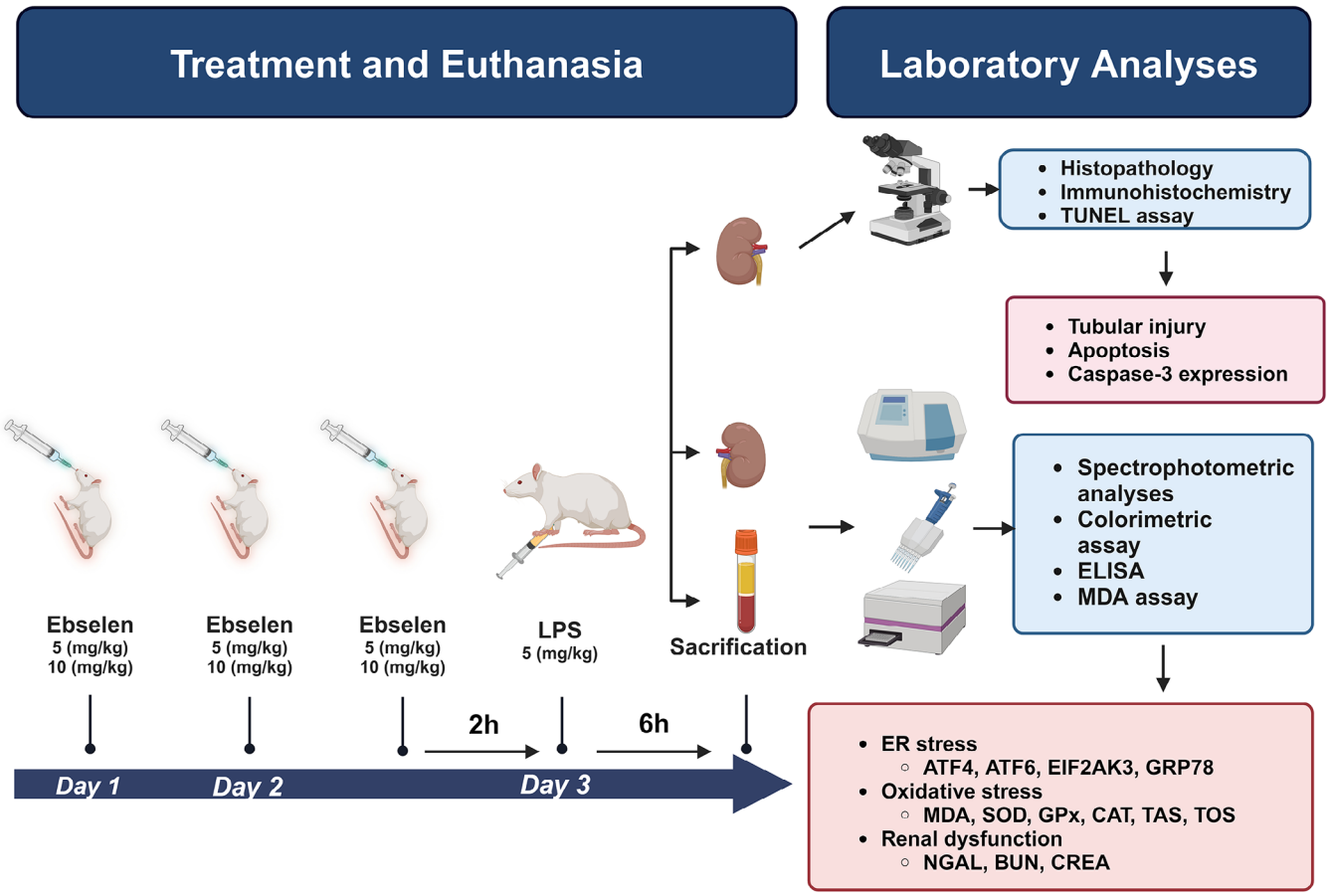

FIGURE 1 | Experimental design of the animal study and laboratory measurements. LPS and Ebselen were administered to the rats. After treatment, biochemical investigations were performed on the left kidney and serum samples, whereas histological and immunohistochemical analyses were performed on the right kidney.

acquired LPS (L2630) and Ebselen (LLC HY-13750) from Sigma-Aldrich (USA) and Medchemexpress (USA), respectively. In order to establish a moderate AKI rat model, a single intraperitoneal dosage of 5 mg/kg LPS was administered, as previously described (Wang et al. 2016; Liu et al. 2017). (1) Control (sham) group: For 3 consecutive days, rats in this group were administered the same volume of orogastric Ebselen solvent as those in the Ebselen group. Furthermore, the same volume of sterile saline was administered with LPS on the same day as the other groups. (2) LPS group: For 3 consecutive days, rats in this group were administered the same volume of Ebselen solvent orally as those in the Ebselen group. Rats in this group were intraperitoneally administered 5 mg/kg LPS on the third day of the study. (3) LPS + Ebselen group (5 mg/kg): Rats in this group were administered 5 mg/kg Ebselen orally for 3 consecutive days. On the third day of the experiments, 2 h after Ebselen administration and 6 h before sacrifice, an intraperitoneal dose of 5 mg/kg of LPS was administered to this group. (4) LPS + Ebselen group (10 mg/kg): Rats in this group were administered 10 mg/kg Ebselen orally for 3 consecutive days. On the third day of the experiments, 2 h after Ebselen administration and 6 h before sacrifice, an intraperitoneal dose of 5 mg/kg of LPS was administered to this group. All rats were promptly anaesthetized with ketamine/xylazine 6 h following the final LPS injection in order to obtain blood and kidney samples. Histological and immunohistochemical analyses were conducted on right kidney tissues, whereas left kidney tissues were employed for other biochemical analyses. Figure 1 presents the study's comprehensive design.

2.2 | Histological Analyses of Kidney Tissues

Routine histological techniques and the hematoxylin–eosin (H&E) staining method were employed to evaluate the structure of right kidney tissues. The tissues were fixed with 4% neutral formalin at room temperature for 48 h. After fixation, the tissues were rinsed under tap water overnight and dehydrated by passing through a rising alcohol series (70%–80%–90%–96%–100%) and cleared with xylene. The tissues with soft and hard paraffin inclusions were blocked and prepared for sectioning, and the embedding procedure was conducted using a paraffin dispenser (Leica HistoCore Arcadia). The sections derived from the prepared blocks were utilized for both histology and immunohistochemical caspase-3 staining. The paraffin blocks were sliced using a semi-motorized microtome (Leica, RM2245) at a thickness of 5 μm. The resulting slices were then placed onto adhesive slides. Then, H&E staining was carried out. Using an Olympus BX43 research microscope with a camera attached, photographs of the sections were taken. Tubular damage analyses in kidney tissue were performed as previously described (Ban et al. 2022). Accordingly, 0, no damage; 1, damaged area 1%–10%; 2, damaged

area 11%–25%; 3, damaged area 26%–75%; and 4, damaged area 75% or more (Ban et al. 2022).

2.3 | Analyses of Caspase-3 Expressions in Kidney Tissues

Caspase-3 immunoreactivity in right kidney tissues was analyzed using the indirect immunohistochemical method. The sections that were kept in the oven overnight were deparaffinized by waiting in xylene and then passed through a decreasing alcohol series and immersed in distilled water. A pool was formed by demarcating sections with a pappen pen after they were rinsed with PBS (phosphate-buffered saline). The sections were boiled in a citrate buffer in a microwave oven to conduct antigen retrieval. Endogenous peroxide suppression was achieved through the use of hydrogen peroxide (3%). The sections were incubated with the primary antibody after blocking was conducted by applying block serum to them for 1 h at room temperature. Subsequently, secondary antibodies containing biotin and streptavidin were employed to label the samples for 30 min. AEC (3-amino-9-ethylcarbazole) was employed as the chromogen, and Mayers haematoxylin was employed for background staining. The immunoreactivity of caspase-3 in the prepared samples was assessed as previously reported by Carter et al. (2004). Staining distribution: <25%; 0.1%; 26%–50%; 0.6%; 51%–75%; 0.9%; 76%–100%); staining intensity: 0, no staining; 0.5, very weak staining; 1, weak staining; 2, moderate staining; 3, very strong staining. Immunohistochemical staining score = distribution × intensity was calculated (Carter et al. 2004).

2.4 | Analyses of Apoptosis

The effects of Ebselen on LPS-induced apoptosis in kidney tissues were assessed using the terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay, following the kit's method (Merck Millipore, Apoptag Peroxidase In Situ Apoptosis Detection Kit, Darmstadt, Germany). A microscope was utilized to visualize the images. TUNEL-positive cells were quantified in renal tissue as the number of stained cells/mm^2, as previously described (Wang et al. 2021).

2.5 | Tissue Preparation for Biochemical Analyses

The left kidney tissues of each rat were weighed and rinsed with PBS at a pH of 7.4. They were then homogenized in precooled PBS, and the kidney homogenates were collected as suspensions. Quantification of protein content in the tissues of the left kidney was conducted using the Bradford protein assay kit.

2.6 | Assessment of Kidney Function

Blood samples from rats were allowed to clot at room temperature for 30 min before being centrifuged at 1500 × *g* for 10 min at +4°C and the supernatant was transferred to new microcentrifuge tubes. Then, we employed standard laboratory procedures to analyze kidney function-related parameters, such as blood urea nitrogen (BUN) and creatinine (CREA). Additionally, we used an enzyme-linked immunosorbent assay (ELISA) to examine the expressions of neutrophil gelatinase-associated lipocalin (NGAL), a marker of kidney injury, in serum and kidney tissues. The rat NGAL measurement was conducted using a commercial kit (BT LAB, E2269Ra).

2.7 | Analyses of ER Stress-Related Markers

The left renal tissues of each rat were homogenized in precooled saline, and the kidney homogenates were collected as suspensions. ER stress-related proteins, such as ATF4, ATF6, EIF2AK3, and GRP78, were evaluated in serum and kidney tissues using commercially available ELISA kits according to the manufacturer's instructions (Finetest, ER1644, ER1645, ER1647, and ER0562).

2.8 | Assessment of Oxidative Stress-Related Markers

MDA serves as a marker of oxidative stress for cells and tissues, and the levels of MDA were analyzed as previously described (Khalil and Salem 1995). The protein amount of each kidney tissue sample was analyzed. The absorbance value was evaluated using a microplate reader (Thermo Fisher Multiscan Go) following incubation.

2.9 | Assessment of Antioxidant Defences

The levels of SOD, GPx and CAT in serum and kidney tissues were analyzed using commercially available ELISA kits according to the manufacturer's instructions (BT-LAB, E2269Ra, E1242Ra and E0869Ra). Serum total antioxidant status (TAS) was analyzed using a colorimetric kit (Elabscience, E-BC-K801-M), and the results were expressed as mmol Trolox Equiv./L. We measured the serum total oxidant status (TOS) using a colorimetric kit (Elabscience, E-BC-K802-M), and the results were expressed as μmol H_2O_2 Equiv./L. As previously explained (Okuyan et al. 2021), the oxidative stress index (OSI) was determined by dividing the TOS data by the TAS values. OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq/L)/TAS (μmol Trolox Eq/L) × 100.

2.10 | Statistical Analyses

GraphPad Prism (v6.0) was employed to conduct all statistical analyses. The Kolmogorov–Smirnov test was used to verify the normal distribution of the data, and the Kruskal–Wallis and Mann–Whitney *U* tests were employed to compare the groups. The results were presented as the mean ± standard deviation, and a *p*-value of less than 0.05 was considered significant for the statistical analyses.

3 | Results

3.1 | Ebselen Relieves LPS-Induced Tubular Damage and Renal Dysfunction

We evaluated the possible therapeutic effect of Ebselen on renal tubular injury in the LPS-induced AKI model and presented

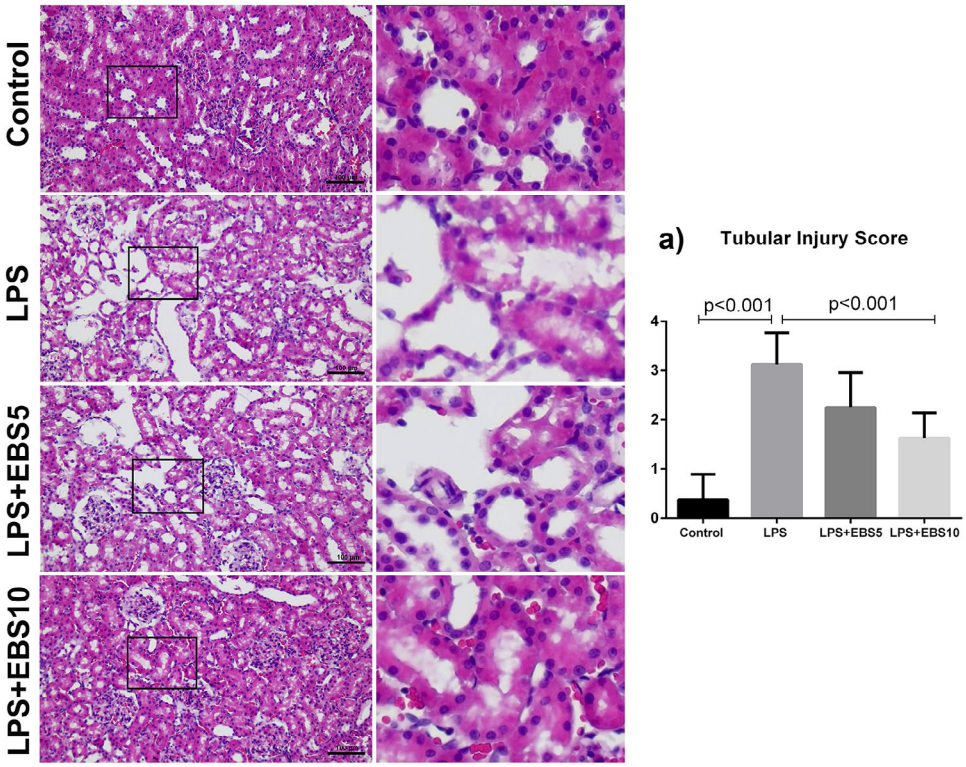

FIGURE 2 | The effect of Ebselen on renal tubular injury in an LPS-induced AKI rat model. A higher dose of Ebselen alleviated sepsis-induced tubular injury (a). The data are presented as the mean ± SD. AKI, acute kidney injury; EBS, Ebselen; EBS10, Ebselen (10 mg/kg); EBS5, Ebselen (5 mg/kg); LPS, lipopolysaccharide.

the histological findings of kidney tissue stained with H&E and tubular injury scores in Figure 2. The renal tissue structure of the control group was observed to be normal (Figure 2a). Renal injury was specifically detected in the epithelial cells of both the proximal and distal tubules in the LPS group (Figure 2b). Ebselen pre-treatment ameliorated tubular damage caused by LPS (Figure 2d). Our statistical analyses showed that tubular injury scores elevated in LPS group compared to the control (Figure 1e) ($p < 0.001$). A higher dose of Ebselen ameliorated the tubular damage in the LPS plus Ebselen group compared to the LPS group (Figure 2e) ($p < 0.001$). We examined the NGAL, BUN and CREA levels in a sepsis-induced AKI rat model to evaluate the impact of Ebselen and LPS on renal function and presented the results in Figure 3. Our data showed that the administration of LPS resulted in an increase in NGAL expression in kidney tissue as compared to the control group (Figure 3a) ($p < 0.05$). Moreover, we did not observe any difference in serum NGAL levels among the groups (Figure 3b) ($p > 0.05$). After LPS injection, serum BUN and CREA levels were significantly elevated in comparison to the control group (Figure 3c,d) ($p = 0.0006$ and $p < 0.05$, respectively). The BUN and CREA increases caused by LPS in the AKI rat model were alleviated by a higher dose of Ebselen treatment (Figure 3c,d) ($p < 0.05$).

3.2 | Ebselen Reduced Caspase-3 Expressions and Apoptotic Cells Triggered by LPS in Kidney Tissues

We utilized the TUNEL and immunohistochemistry methods to investigate the potential role of Ebselen in LPS-stimulated apoptosis in kidney tissues in the AKI rat model. The findings are displayed in Figure 4. Our TUNEL assay analyses revealed that although the control group contained only a few apoptotic cells, the LPS markedly stimulated apoptosis in kidney tissues compared to the control (Figure 4a) ($p < 0.001$). Both doses of Ebselen pre-treatment ameliorated LPS-induced apoptotic cell counts in kidney tissues (Figure 4a) ($p < 0.001$). Furthermore, we conducted an analysis of the expression of caspase-3, an apoptosis-related protein, in kidney tissues and presented our findings in Figure 4. We found that caspase-3 expressions were considerably elevated in kidney tissues after LPS injection (Figure 4b) ($p < 0.001$). Both doses of Ebselen pre-treatment reduced LPS-induced caspase-3 expression in kidney tissues (Figure 4b) ($p < 0.005$).

3.3 | Ebselen Treatment Alleviated the Sepsis-Induced ER Stress

To clarify the molecular mechanism of sepsis in relation to ER stress, we evaluated the ER stress proteins, including ATF4, ATF6, EIF2AK3 and GRP78, using ELISA and presented the results in Figure 5. Our data presented here revealed that LPS-induced sepsis dysregulated some ER stress proteins in kidney tissues and serum, suggesting that ER stress is relationship with pathogenesis of sepsis. We showed that in kidney tissues, LPS increased ATF6 expressions, whereas it lowered EIF2AK3 and GRP78 expressions (Figure 5b–d). Moreover, an increased dosage of Ebselen therapy alleviated the downregulation of EIF2AK3 and GRP78 expressions caused by LPS (Figure 5c,d).

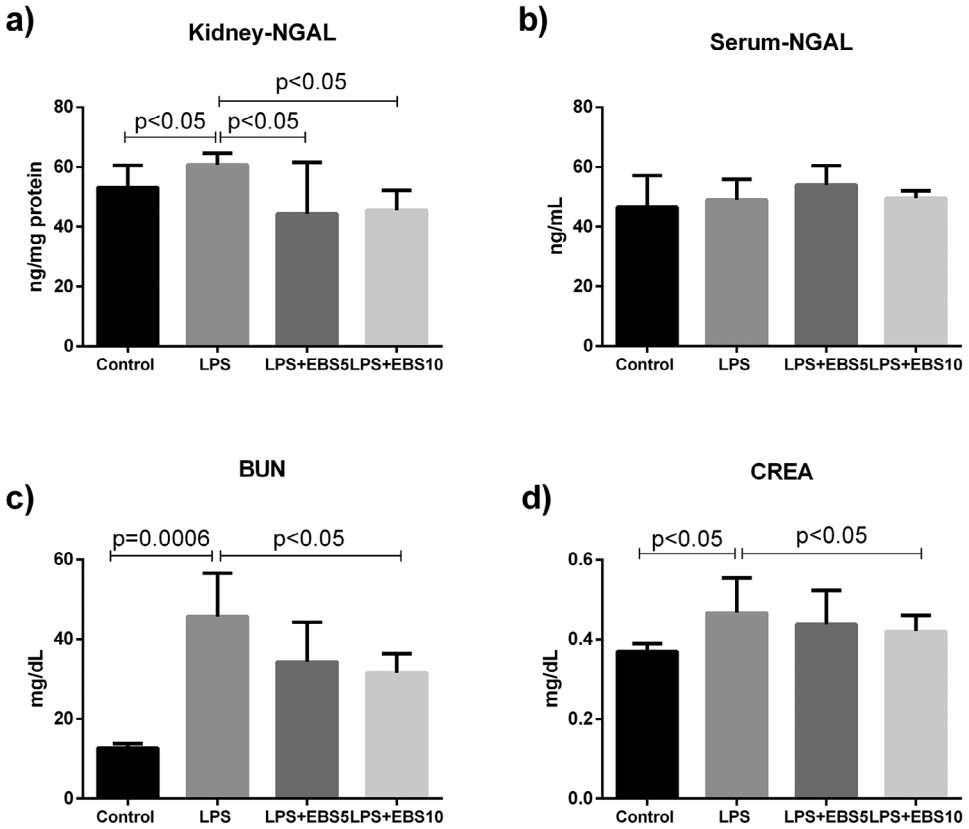

FIGURE 3 | The effect of Ebselen on sepsis-induced renal dysfunction. LPS increased NGAL expression, which is a biomarker that reflects kidney injury (a). Both doses of Ebs treatment decreased LPS-induced NGAL expression. (b) There was no significant difference in serum NGAL levels among groups. (c and d) LPS increased serum BUN and CREA levels and a higher dose of Ebselen treatment reduced LPS-induced BUN and CREA levels. The data are presented as the mean ± SD. BUN, blood urea nitrogen; CREA, creatinine; Ebs, Ebselen; EBS10, Ebselen (10 mg/kg); EBS5, Ebselen (5 mg/kg); LPS, lipopolysaccharide; NGAL, neutrophil gelatinase-associated lipocalin.

We did not find any difference in ATF4 expressions in kidney tissues among groups (Figure 5a) ($p > 0.05$). To determine the impact of LPS-induced sepsis and Ebselen on ER stress at systemic levels, we analyzed the ER stress proteins in serum samples of rats. We unveiled that LPS-induced sepsis increased the serum ATF4 and ATF6 expressions (Figure 5e,f) ($p < 0.005$). Additionally, Ebselen treatment reduced the sepsis-induced serum ATF4 and ATF6 expressions (Figure 5e,f) ($p < 0.005$). There were no significant differences in the expressions of serum EIF2AK3 and GRP78 between the groups ($p > 0.05$).

3.4 | Ebselen Alleviated LPS-Induced Oxidative Stress

In order to elucidate the impact of Ebselen on oxidative stress in the sepsis-induced AKI rat model, we conducted an analysis of oxidative stress-related markers in both serum and kidney tissues and presented the results in Figure 6. We measured the levels of MDA in kidney tissues, which serves as a marker of oxidative stress. Our findings indicated that the levels of MDA in kidney tissues were elevated in the presence of LPS-induced sepsis in comparison to the control group (Figure 6a) ($p < 0.005$). Further, both doses of Ebselen therapy reduced sepsis-induced MDA levels in kidney tissues (Figure 6a) ($p < 0.05$). We analyzed the levels of SOD, which is one of the most significant antioxidant enzymes, in serum and kidney tissues (Figure 6b,e). The findings of our study indicated that sepsis generated by LPS resulted in a significant reduction in the levels of SOD in kidney tissues, as compared to the control group (Figure 6b) ($p < 0.05$). Both doses of Ebselen treatment effectively enhanced the sepsis-induced reduction in SOD levels in kidney tissues (Figure 6b) ($p < 0.01$). We did not observe any substantial variation in the levels of GPx and CAT in kidney tissues (Figure 6c,d) ($p > 0.05$). Moreover, a higher dose of Ebselen treatment improved serum SOD levels compared to the LPS-induced sepsis (Figure 6e) ($p < 0.05$). The levels of GPx, a crucial antioxidant enzyme, exhibited a considerable rise in both the EBS5 and EBS10 groups as compared to the LPS-induced sepsis group (Figure 6f) ($p < 0.05$). No significant difference in serum CAT levels was observed between the groups (Figure 6g) ($p > 0.05$). In addition, serum TAS levels were decreased by LPS-induced sepsis; however, Ebselen pre-treatment alleviated the effect of sepsis on serum TAS levels (Figure 6h) ($p < 0.05$). OSI values were significantly increased in LPS-induced sepsis group compared to the control ($p < 0.01$). The impact of sepsis on OSI values was mitigated by administering a higher dose of Ebselen (Figure 6j) ($p < 0.05$). There is no significant difference in serum TOS levels among groups ($p > 0.05$).

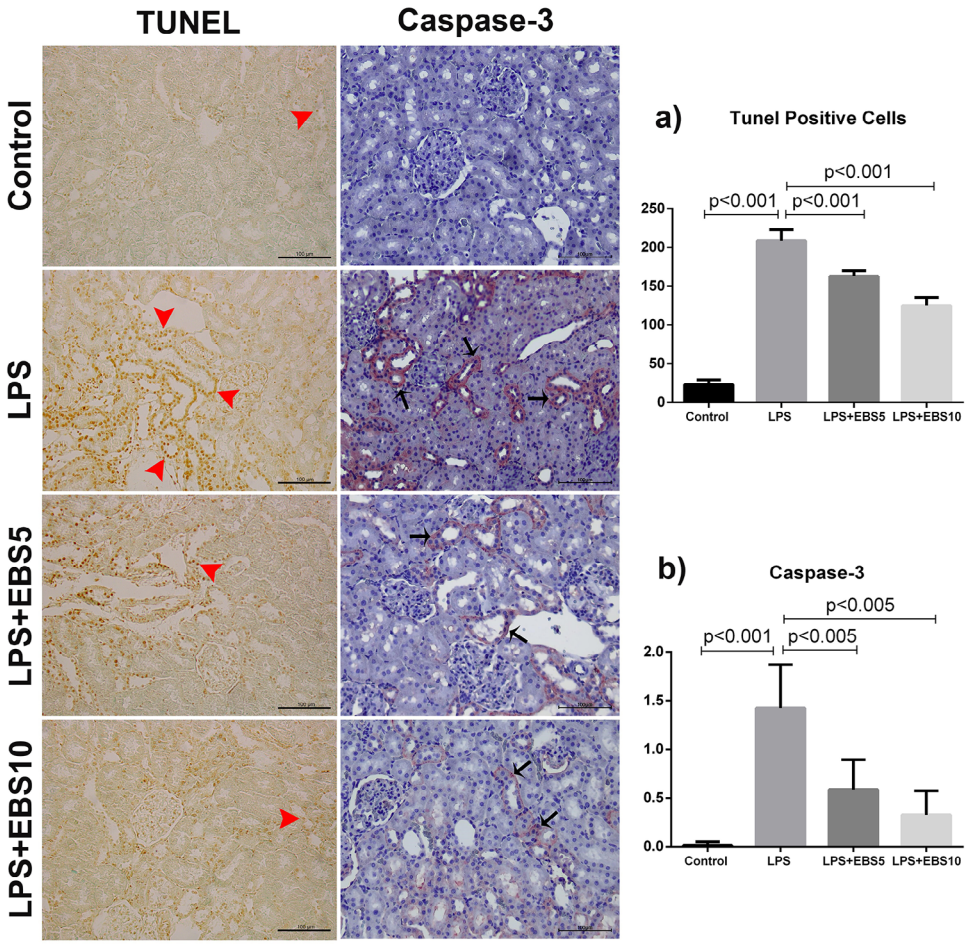

FIGURE 4 | The effect of Ebselen on caspase-3 expression and apoptotic cells in LPS-induced AKI rat model. Ebselen reduced apoptotic cells (a) and caspase-3 expressions (b) triggered by LPS in kidney tissues. The data are presented as the mean ± SD. EBS, Ebselen; EBS10, Ebselen (10 mg/kg); EBS5, Ebselen (5 mg/kg); LPS, lipopolysaccharide; TUNEL, terminaldeoxynucleotidyl transferase dUTP nick end labelling.

4 | Discussion

Acute kidney injury is a severe complication of sepsis, the primary cause of mortality in critically ill patients, and there is currently no effective treatment (Poston and Koyner 2019). Therefore, it is crucial to find novel therapeutic approaches that can effectively halt or decelerate the progression of AKI. We utilized LPS, a powerful trigger of sepsis, to establish a model of renal injury (Li et al. 2019). Sepsis-induced inflammation at the systemic level may result in injury to multiple organs, including the kidneys (Li et al. 2019). Previous studies have shown that renal impairment in AKI induced by sepsis may be reversible (Hsing et al. 2011; Gupta et al. 2007). Although Ebselen is seen as a promising therapeutic molecule for some diseases owing to its anti-inflammatory and antioxidant properties, there is no study investigating its role in sepsis-induced AKI. Hence, we investigated whether Ebselen pre-treatment has an alleviating effect on LPS-induced renal injury and its relationship with molecular pathogenesis of AKI.

Previous research has shown that intraperitoneal injection of LPS may cause AKI for 4 h or more and markers (NGAL, BUN and CREA) of kidney damage and dysfunction are markedly elevated after injection of LPS (Chen et al. 2019; Zhao et al. 2020). In addition to these alterations, an LPS injection causes tubular damage and disrupts the integrity of the tubular structure (Zhao et al. 2020). All these changes indicate that LPS establishes an AKI model. In our study, we observed a rise in the tubular injury score in kidney tissues and an increase in renal dysfunction markers like BUN, CREA and NGAL following LPS treatment.

ER stress is regarded as a critical cellular mechanism that plays a role in the development of sepsis-induced AKI (Teng et al. 2018; Cao et al. 2024). Nevertheless, its precise function of the pathogenesis of AKI remains unclear. Although the ER stress mechanism has recently caught the attention of researchers, there is a limited amount of research available on this particular subject. ATF4 is a key regulatory protein of ER stress that contributes to the development of kidney diseases. Tang et al. (2023) reported in a recent study that an ischaemia-reperfusion kidney injury upregulated ATF4 expressions. Their results emphasized that targeting ATF4 could offer a promising approach for preventing and treating AKI (Tang et al. 2023). Our data revealed that the administration of LPS increased ATF4 levels at a systemic level, whereas Ebselen treatment decreased ATF4 expressions induced by sepsis. ATF6 initiates the UPR in the presence of ER stress (Hetz and Papa 2018). In the present study, our data showed that administration of LPS triggered ATF6 expressions at both tissue and systemic levels. Ebselen pre-treatment regulates

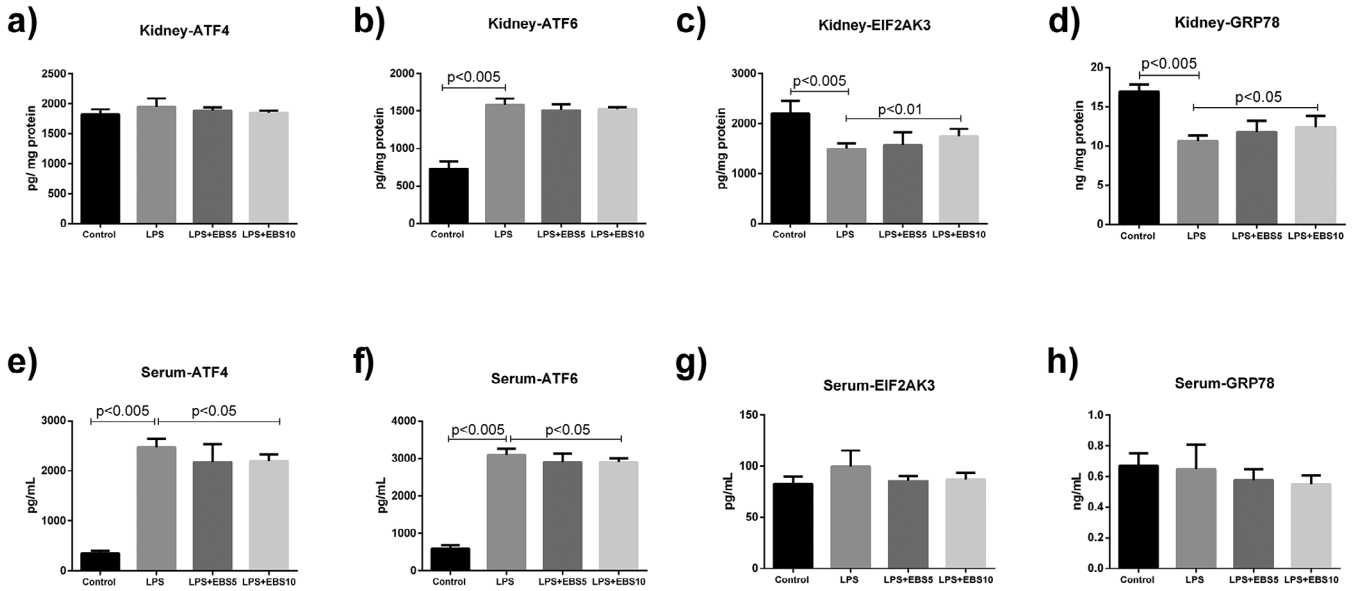

FIGURE 5 | The effect of Ebselen on sepsis-induced endoplasmic reticulum stress. LPS induced ER stress through dysregulation of ATF6 (b), EIF2AK3 (c) and GRP78 (d) in kidney tissue and ATF4 (e) and ATF6 (f) in serum in AKI rat model. Ebselen therapy ameliorates sepsis-induced ER stress by modulating EIF2AK3 and GRP78 in kidney tissue and ATF4 and ATF6 in serum. There was no statistically significant difference in the levels of kidney ATF4 (a), serum EIF2AK3 (g), and GRP78 (h) between the groups. The data are presented as the mean ± SD. AKI, acute kidney injury; ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; Ebs, Ebselen; EBS10, Ebselen (10 mg/kg); EBS5, Ebselen (5 mg/kg); Eif2ak3, eukaryotic translation initiation factor 2-alpha kinase 3; ER, endoplasmic reticulum; GRP78, glucose-regulated protein 78; LPS, lipopolysaccharide.

ER stress levels by decreasing ATF6 expression at the systemic level. EIF2AK3 is an essential molecule in the regulation of the UPR during ER stress. Saptarshi et al. (2022) showed that the downregulated EIF2AK3 expressions cause elevated ER stress levels and disrupt ER stress-stimulated apoptosis and response to oxidative stress. Our findings showed that EIF2AK3 expressions in kidney tissues were downregulated after LPS treatment, whereas Ebselen pre-treatment upregulated the sepsis-reduced EIF2AK3 expressions. Here, we propose that the EIF2AK3 may be downregulated in LPS-induced AKI at an early stage to safeguard cells against apoptosis triggered by the escalation of ER stress. On the other hand, we may speculate that Ebselen pre-treatment could have a decreasing effect on LPS-generated ER stress and an enhancing effect on response to oxidative stress.

GRP78 is a regulatory protein involved in ER stress. Teng et al. (2018) investigated the role of GRP78 in LPS-induced AKI using a renal epithelial cell culture model. Their results showed that downregulation of GRP78 ameliorates sepsis-induced AKI by regulating apoptosis, oxidative stress, and inflammatory response (Teng et al. 2018). In contrast, previous research has revealed that the downregulated GRP78 is tightly linked to higher oxidative stress because of its important function in protein folding, ER stress response, control of antioxidant defences and elevated ROS production (Teng et al. 2018; Wang et al. 2024; Dauer et al. 2019). In another study, Gong et al. (2020) reported that the downregulation of GRP78 is associated with stimulation of apoptosis and suppression of proliferation and migration. Additionally, GRP78 downregulation has been linked to increased susceptibility to apoptosis induced by oxidative stress. We observed that GRP78 expression was downregulated in kidney tissues 6 h after LPS administration. These data suggest that GRP78 plays a critical role in pathogenesis of kidney injury by regulating ER stress and modulating oxidative stress. The expression levels of the ER stress proteins may vary with exposure or duration. Considering existing data and our results, we may speculate that the role of GRP78 is context-specific and may have either protective or pathogenic consequences depending on the particular cellular milieu and stress circumstances. The dual function of GRP78 in renal damage and prevention highlights a critical aspect of cellular stress responses and its context-specific role. Depending on certain circumstances, it may worsen or lessen damage in renal tissues. However, to clarify the possible involvement of GRP78 in renal disorders, more research is necessary. Furthermore, it has been reported that the upregulation of GRP78 is linked to inflammation, acting as both a protective mechanism against acute stress and a contributor to chronic inflammatory conditions. More importantly, Repges et al. discovered that GRP78 upregulation may have protective effects by reducing vascular inflammation and oxidative stress in human coronary endothelial cells. Their data imply that upregulated GRP78 could have anti-inflammatory effects (Repges et al. 2021). We may speculate that a higher dose of Ebselen may have therapeutic effects by increasing the expression of GRP78 in kidney tissues, which has been reduced by sepsis. Overall, the association between GRP78 and oxidative stress as well as inflammation represents a notable subject of investigation, especially in understanding cellular stress responses and inflammatory diseases.

Oxidative stress is a key molecular mechanism that plays a role in the development of sepsis-induced AKI. The progression of sepsis-induced AKI is characterized by elevated levels of oxidative stress and a decrease in antioxidant capacity. Our analysis of oxidative stress highlights the imbalance of oxidants and antioxidants capacity in AKI. Moreover, it is evident that the excessive build-up of ROS is a significant factor contributing

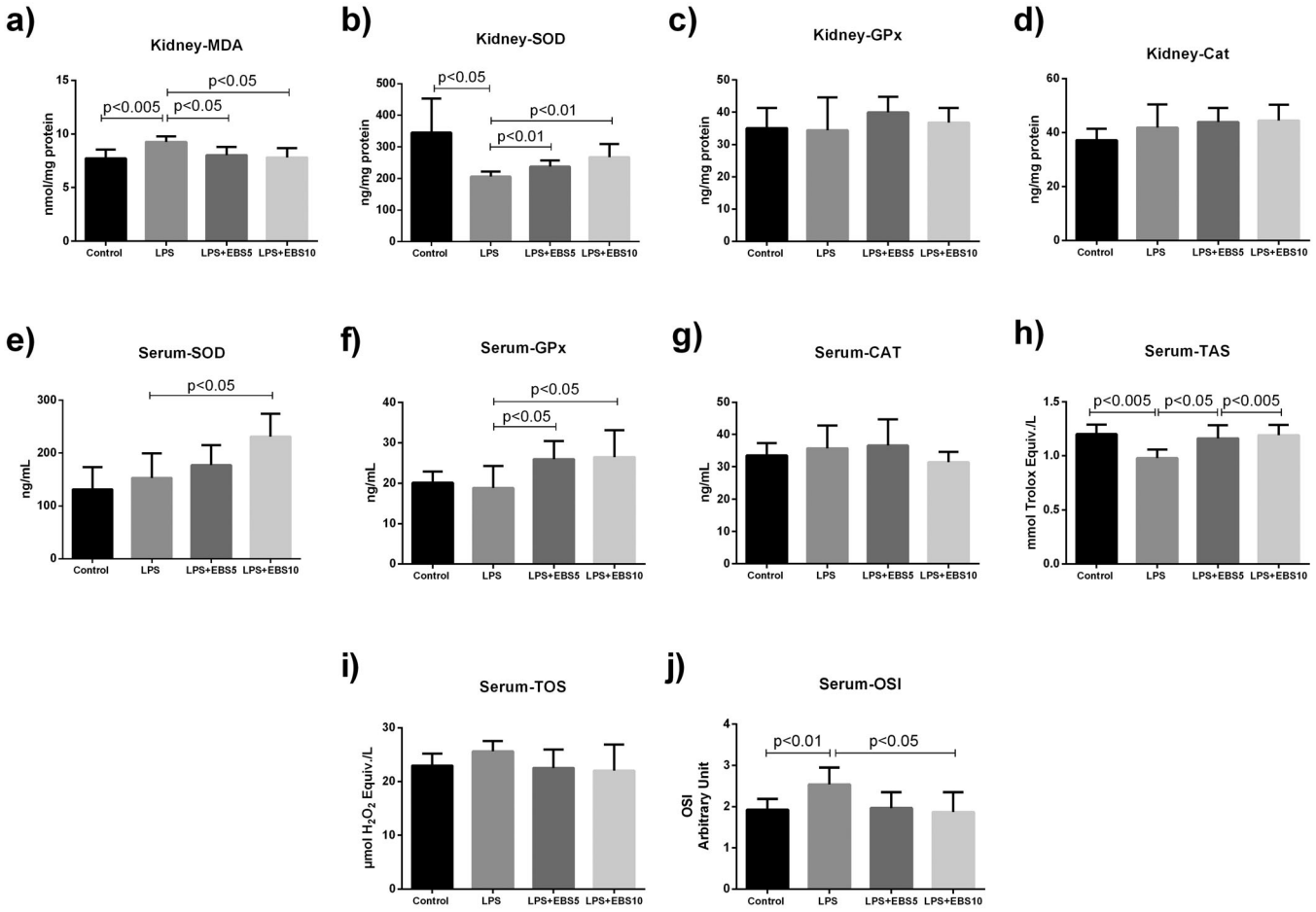

FIGURE 6 | The effect of Ebselen on sepsis-induced oxidative stress. LPS triggered oxidative stress in LPS-induced AKI model. LPS increased MDA levels (a), whereas it decreased SOD (b) levels in kidney tissue and TAS (h) levels in serum. Ebselen treatment alleviated sepsis-induced oxidative stress by regulating MDA (a) and SOD (b) levels in kidney tissues and SOD (e), GPx (f), TAS (h) levels in serum. Higher doses of Ebselen diminished LPS-induced OSI (j) levels. There was no statistically significant difference in the levels of kidney GPx (c) and CAT (d), serum CAT (g), and TOS (i) between the groups. The data are presented as the mean ± standard deviation. AKI, acute kidney injury; Cat, catalase; Ebs, Ebselen; EBS10, Ebselen (10 mg/kg); EBS5, Ebselen (5 mg/kg); GPx, glutathione peroxidase; LPS, lipopolysaccharide; MDA, malondialdehyde; OSI, oxidative stress index; SOD, superoxide dismutase; TAS, total antioxidant status; TOS, total oxidant status.

to AKI (Plotnikov et al. 2018). The excessive accumulation of ROS disrupts cellular components, including DNA, proteins and lipids, leading to the induction of apoptosis, ultimately resulting in renal injury. MDA, generated during ROS-induced lipid peroxidation, is a biomarker of oxidative stress, which is redox disequilibrium (Orrenius et al. 2007). Our findings demonstrated that the administration of LPS led to an elevation in MDA levels in the kidney tissues, whereas pre-treatment with Ebselen resulted in a decrease in MDA levels. SOD is an essential antioxidant enzyme that is involved in the defence against oxidative damage induced by ROS (Li et al. 2018). Here, we showed that SOD levels were reduced in kidney tissues after LPS treatment, whereas Ebselen pre-treatment elevated SOD levels in sepsis-induced AKI. Moreover, we examined that the level of GPx is an antioxidant enzyme that plays a pivotal role in defence against oxidative stress (Wu et al. 2022). Our data showed that Ebselen pre-treatment promotes the antioxidant capacity in sepsis-induced rats by increasing GPx levels. LPS reduces the levels of TAS in the AKI model generated by sepsis, whereas Ebselen enhances the levels of TAS in the serum. Overall, LPS causes kidney damage by increasing ROS generation and lowering antioxidant capability, whereas Ebselen exhibits protective antioxidant activity by blocking ROS-mediated pathways.

Apoptosis is a biological process that is strongly linked to oxidative stress and plays a crucial role in the progression of kidney damage. If the level of oxidative stress exceeds the ability of cells to cope, apoptosis is triggered. Caspase-3, found downstream in the cascade, is a protein that is involved in execution phase of cell death, which is crucial for cellular homeostasis (Zhu et al. 2024). The studies highlight that oxidative stress triggers the activation of caspase-3, which in turn stimulates cell death. For example, increased ROS levels can harm mitochondria in kidney cells; damaged mitochondria release cytochrome c and then activate caspase-3, thereby promoting apoptosis (Yang et al. 2020). In our study, the administration of LPS resulted in an upregulation of caspase-3 levels and an increase in the number of apoptotic cells in sepsis-induced AKI, whereas the pre-treatment of Ebselen reduced the caspase-3 expressions and apoptotic cells induced by sepsis. All in all, our study provides significant evidence about the molecular pathogenesis of LPS-induced AKI, as well as the therapeutic potential of Ebselen in AKI. Ebselen may have

therapeutic benefits for sepsis-induced AKI because it modulates ER stress-related proteins, oxidative stress, and apoptosis.

The reversibility of sepsis-induced AKI has substantial therapeutic consequences, especially in enhancing patient outcomes and mitigating the risk of long-term renal problems. The utilization of innovative agents, such as Ebselen, which target molecular mechanisms, such as ER stress, apoptosis, and oxidative stress, may improve renal recovery and avert the advancement to chronic kidney disease. Some studies have suggested that sepsis-induced AKI may be reversible with prompt and suitable intervention. However, if neglected, it may result in irreversible kidney damage. Timely and assertive measures to maintain renal function during sepsis treatment markedly improve the chances of recovery and diminish the danger of advancing to organ failure (Ferenbach and Bonventre 2016). This study sought to examine the reversibility of Ebselen's potential impact on sepsis-induced AKI and underscore the importance of treatment techniques that improve renal function. Future clinical investigations targeting treatment strategies that enhance this reversibility may provide significant advantages for sepsis therapy.

Although our study provides valuable insights into the role of Ebselen in ER stress, apoptosis, and oxidative stress in LPS-induced AKI, it has certain limitations. The LPS model was selected for its repeatability and capacity to elicit a regulated systemic inflammatory response, consistent with our research aims (Aydin and Bekmez 2023). Nonetheless, numerous sepsis models are utilized in experimental research, and the cecal ligation and puncture model is thought to be the most reliable, as it better captures the intricate pathophysiology of clinical sepsis (Üstündağ et al. 2024; Osuchowski et al. 2018; Üstündağ et al. 2023). The use of male rats in our study is another limitation, as it fails to account for putative sex-specific differences in sepsis-induced AKI. Although this decision was made to reduce hormonal variability that could potentially impact immune and oxidative stress responses, it restricts the generalizability of our findings to female populations. In order to gain a more comprehensive comprehension of the sex differences in sepsis-induced kidney injury, it is imperative to conduct future studies that include female rodents, given the established effects of estrogen on cytokine balance and sepsis susceptibility. We used a moderate LPS dosage (5 mg/kg) to induce AKI without causing severe systemic failure. This dose may not adequately reflect the severity and variability of clinical sepsis-associated AKI. Many studies have utilized intraperitoneal LPS treatment at dosages of 5 mg/kg or less in AKI animal models (Zhu et al. 2018; Kapoor et al. 2015). On the other hand, a greater dosage (10 mg/kg) may have resulted in increased mortality, hence confounding the evaluation of Ebselen's protective effects.

5 | Conclusion

Our data presented here for the first time report that Ebselen alleviates sepsis-induced AKI by modulating ER stress, apoptosis, and oxidative stress. Moreover, we revealed that the mechanism of LPS-induced ER stress differs at the systemic and tissue levels due to the differential expression of several proteins. LPS increased caspase-3 expression and apoptotic cell numbers in renal tissues. Sepsis affects the levels of oxidative stress and the capacity of antioxidants in LPS-induced AKI. Further, we unveiled that Ebselen pre-treatment mitigated the kidney damage and renal dysfunction caused by LPS. Ebselen functions as a modulator in various cellular processes that contribute to kidney damage, such as ER stress, apoptosis, and oxidative stress. The effectiveness of Ebselen in therapy is thought to be due to its probable involvement in regulating molecular pathways. These results emphasize the significance of incorporating Ebselen into clinical practice to prevent and treat AKI caused by sepsis. The exact relationship between the protective impact of Ebselen and sepsis-induced AKI requires validation by animal experiments involving a greater number of subjects as well as sex, various doses, and durations.

Author Contributions

Hamza Malik Okuyan, İhsan Karaboğa and Serdar Doğan designed the study. Hamza Malik Okuyan, Şeyda Öznur Ayçiçek and Hüseyin Çakıroğlu performed animal experiments. İhsan Karaboğa, Hamza Malik Okuyan, and Serdar Doğan performed laboratory analyses. İhsan Karaboğa, Hamza Malik Okuyan, Serdar Doğan, Hüseyin Çakıroğlu and Şeyda Öznur Ayçiçek conducted analyses, prepared graphs/figures and revised the manuscript. All authors of the article wrote/drafted/edited the manuscript and interpreted the results.

Ethics Statement

The present study was conducted in compliance with the Institutional Animal Care and Use Committee Guidelines, and all experimental protocols were approved by Sakarya University's local Animal Ethics Committee (Permission number: 05/2023-13).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Alasiri, G., Y. Jiramongkol, S. Trakansuebkul, et al. 2020. "Reciprocal Regulation Between GCN2 (eIF2AK4) and PERK (eIF2AK3) Through the JNK-FOXO3 Axis to Modulate Cancer Drug Resistance and Clonal Survival." *Molecular and Cellular Endocrinology* 515: 110932.

Antony, S., and C. Bayse. 2011. "Modeling the Mechanism of the Glutathione Peroxidase Mimic Ebselen." *Inorganic Chemistry* 50, no. 23: 12075–12084.

Aydin, P., and H. Bekmez. 2023. "Experimental Sepsis Models: Advantages and Limitations." *Eurasian Journal of Medicine* 55, no. 1: 120–124.

Azad, G., and R. Tomar. 2014. "Ebselen, a Promising Antioxidant Drug: Mechanisms of Action and Targets of Biological Pathways." *Molecular Biology Reports* 41, no. 8: 4865–4879.

Ban, K., G. Y. Nam, D. Kim, Y. S. Oh, and H. S. Jun. 2022. "Prevention of LPS-Induced Acute Kidney Injury in Mice by Bavachin and Its Potential Mechanisms." *Antioxidants (Basel)* 11, no. 11: 2096.

Cao, Y., X. Chen, Z. Zhu, et al. 2024. "STING Contributes to Lipopolysaccharide-Induced Tubular Cell Inflammation and Pyroptosis by Activating Endoplasmic Reticulum Stress in Acute Kidney Injury." *Cell Death & Disease* 15, no. 3: 217.

Carter, J. H., L. E. Douglass, J. A. Deddens, et al. 2004. "Pak-1 Expression Increases With Progression of Colorectal Carcinomas to Metastasis." *Clinical Cancer Research* 10, no. 10: 3448–3456.

Chen, Y., L. Luan, C. Wang, et al. 2019. "Dexmedetomidine Protects Against Lipopolysaccharide-Induced Early Acute Kidney Injury by Inhibiting the iNOS/NO Signaling Pathway in Rats." *Nitric Oxide* 85: 1–9.

Dauer, P., N. S. Sharma, V. K. Gupta, et al. 2019. "ER Stress Sensor, Glucose Regulatory Protein 78 (GRP78) Regulates Redox Status in Pancreatic Cancer Thereby Maintaining "Stemness"." *Cell Death & Disease* 10, no. 2: 132.

Davis, M., and W. Bartfay. 2004. "Ebselen Decreases Oxygen Free Radical Production and Iron Concentrations in the Hearts of Chronically Iron-Overloaded Mice." *Biological Research for Nursing* 6, no. 1: 37–45.

Di Conza, G., and P. Ho. 2020. "ER Stress Responses: An Emerging Modulator for Innate Immunity." *Cells* 9, no. 3: 695.

Ferenbach, D., and J. Bonventre. 2016. "Acute Kidney Injury and Chronic Kidney Disease: From the Laboratory to the Clinic." *Néphrologie & Thérapeutique* 12, no. S1: S41–S48.

Fulco, B. C. W., J. T. K. Jung, P. M. Chagas, S. G. Rosa, V. C. Prado, and C. W. Nogueira. 2020. "Diphenyl Diselenide Is as Effective as Ebselen in a Juvenile Rat Model of Cisplatin-Induced Nephrotoxicity." *Journal of Trace Elements in Medicine and Biology* 60: 126482.

Gong, C., X. Hu, Y. Xu, et al. 2020. "Berberine Inhibits Proliferation and Migration of Colorectal Cancer Cells by Downregulation of GRP78." *Anti-Cancer Drugs* 31, no. 2: 141–149.

Gupta, A., G. J. Rhodes, D. T. Berg, B. Gerlitz, B. A. Molitoris, and B. W. Grinnell. 2007. "Activated Protein C Ameliorates LPS-Induced Acute Kidney Injury and Downregulates Renal INOS and Angiotensin 2." *American Journal of Physiology. Renal Physiology* 293, no. 1: F245–F254.

Gupta, S., B. McGrath, and D. Cavener. 2010. "PERK (EIF2AK3) Regulates Proinsulin Trafficking and Quality Control in the Secretory Pathway." *Diabetes* 59, no. 8: 1937–1947.

Han, S. W., C. Li, K. O. Ahn, et al. 2008. "Prolonged Endoplasmic Reticulum Stress Induces Apoptotic Cell Death in an Experimental Model of Chronic Cyclosporine Nephropathy." *American Journal of Nephrology* 28, no. 5: 707–714.

Hetz, C., and F. Papa. 2018. "The Unfolded Protein Response and Cell Fate Control." *Molecular Cell* 69, no. 2: 169–181.

Hillary, R., and U. FitzGerald. 2018. "A Lifetime of Stress: ATF6 in Development and Homeostasis." *Journal of Biomedical Science* 25, no. 1: 48.

Hsing, C., W. Chou, J. Wang, H. Chen, and C. Yeh. 2011. "Propofol Increases Bone Morphogenetic Protein-7 and Decreases Oxidative Stress in Sepsis-Induced Acute Kidney Injury." *Nephrology, Dialysis, Transplantation* 26, no. 4: 1162–1172.

Kapoor, K., E. Singla, B. Sahu, and A. S. Naura. 2015. "PARP Inhibitor, Olaparib Ameliorates Acute Lung and Kidney Injury Upon Intratracheal Administration of LPS in Mice." *Molecular and Cellular Biochemistry* 400, no. 1–2: 153–162.

Kaymak, E., E. Öztürk, A. T. Akİn, D. Karabulut, and B. Yakan. 2022. "Thymoquinone Alleviates Doxorubicin Induced Acute Kidney Injury by Decreasing Endoplasmic Reticulum Stress, Inflammation and Apoptosis." *Biotechnic & Histochemistry* 97, no. 8: 622–634.

Khalil, M. M., and A. Salem. 1995. "Lipid Peroxidation Products in Pleural Fluid for Separation of Transudates and Exudates." *Clinical Chemistry* 41, no. 9: 1314–1315.

Li, B., C. Liu, K. Tang, et al. 2019. "Aquaporin-1 Attenuates Macrophage-Mediated Inflammatory Responses by Inhibiting p38 Mitogen-Activated Protein Kinase Activation in Lipopolysaccharide-Induced Acute Kidney Injury." *Inflammation Research* 68, no. 12: 1035–1047.

Li, H., X. Meng, C. Huang, L. Zhang, X. Lv, and J. Li. 2019. "Application of Herbal Traditional Chinese Medicine in the Treatment of Acute Kidney Injury." *Frontiers in Pharmacology* 10: 376.

Li, R., X. Zhou, D. Liu, and W. Feng. 2018. "Enhancing the Activity and Stability of Mn-Superoxide Dismutase by One-by-One Ligation to Catalase." *Free Radical Biology and Medicine* 129: 138–145.

Liang, Q., T. Liu, T. Guo, et al. 2021. "ATF4 Promotes Renal Tubulointerstitial Fibrosis by Suppressing Autophagy in Diabetic Nephropathy." *Life Sciences* 264: 118686.

Liu, Q., X. Zhang, X. Hu, et al. 2017. "Emerging Roles of circRNA Related to the Mechanical Stress in Human Cartilage Degradation of Osteoarthritis." *Molecular Therapy Nucleic Acids* 7: 223–230.

Oakes, S., and F. Papa. 2015. "The Role of Endoplasmic Reticulum Stress in Human Pathology." *Annual Review of Pathology* 10: 173–194.

Okuyan, H. M., S. Dogan, M. Y. Terzi, M. A. Begen, and F. H. Turgut. 2021. "Association of Serum lncRNA H19 Expression With Inflammatory and Oxidative Stress Markers and Routine Biochemical Parameters in Chronic Kidney Disease." *Clinical and Experimental Nephrology* 25, no. 5: 522–530.

Okuyan, H. M., Z. Yurtal, I. Karaboğa, F. Kaçmaz, and A. Kalacı. 2023. "Ebselen, an Active Seleno-Organic Compound, Alleviates Articular Cartilage Degeneration in a Rat Model of Knee Osteoarthritis." *Biological Trace Element Research* 201, no. 8: 3919–3927.

Orrenius, S., V. Gogvadze, and B. Zhivotovsky. 2007. "Mitochondrial Oxidative Stress: Implications for Cell Death." *Annual Review of Pharmacology and Toxicology* 47: 143–183.

Osuchowski, M. F., A. Ayala, S. Bahrami, et al. 2018. "Minimum Quality Threshold in Pre-Clinical Sepsis Studies (MQTiPSS): An International Expert Consensus Initiative for Improvement of Animal Modeling in Sepsis." *Shock (Augusta, Ga.)* 50, no. 4: 377–380.

Peerapornratana, S., C. L. Manrique-Caballero, H. Gómez, and J. A. Kellum. 2019. "Acute Kidney Injury From Sepsis: Current Concepts, Epidemiology, Pathophysiology, Prevention and Treatment." *Kidney International* 96, no. 5: 1083–1099.

Plotnikov, E., A. A. Brezgunova, I. B. Pevzner, et al. 2018. "Mechanisms of LPS-Induced Acute Kidney Injury in Neonatal and Adult Rats." *Antioxidants (Basel)* 7, no. 8: 105.

Poston, J., and J. Koyner. 2019. "Sepsis Associated Acute Kidney Injury." *BMJ* 364: k4891.

Repges, E., M. Al Zaidi, S. Sommer-Weisel, et al. 2021. "Circulating Chaperone GRP78 Is Elevated in Patients With Coronary Artery Disease and Reduces Vascular Inflammation in Human Coronary Endothelial Cells." *European Heart Journal* 42: 1092.

Roth, J., G. H. Yam, J. Fan, et al. 2008. "Protein Quality Control: The Who's Who, the Where's and Therapeutic Escapes." *Histochemistry and Cell Biology* 129, no. 2: 163–177.

Saptarshi, N., L. Porter, and L. Paraoan. 2022. "PERK/EIF2AK3 Integrates Endoplasmic Reticulum Stress-Induced Apoptosis, Oxidative Stress and Autophagy Responses in Immortalised Retinal Pigment Epithelial Cells." *Scientific Reports* 12, no. 1: 13324.

Sun, J., L. Niu, Y. Wang, et al. 2024. "MicroRNA-17-5p Alleviates Sepsis-Related Acute Kidney Injury in Mice by Modulating Inflammation and Apoptosis." *Molecular Medicine Reports* 30, no. 2: 139.

Tang, S., X. Wu, Q. Dai, et al. 2023. "Vitamin D Receptor Attenuate Ischemia-Reperfusion Kidney Injury via Inhibiting ATF4." *Cell Death Discovery* 9, no. 1: 158.

Taniguchi, M., and H. Yoshida. 2015. "Endoplasmic Reticulum Stress in Kidney Function and Disease." *Current Opinion in Nephrology and Hypertension* 24, no. 4: 345–350.

Teng, J., M. Liu, Y. Su, et al. 2018. "Down-Regulation of GRP78 Alleviates Lipopolysaccharide-Induced Acute Kidney Injury." *International Urology and Nephrology* 50, no. 11: 2099–2107.

Thabet, N., and E. Moustafa. 2017. "Synergistic Effect of Ebselen and Gamma Radiation on Breast Cancer Cells." *International Journal of Radiation Biology* 93, no. 8: 784–792.

Üstündağ, H., Ö. Demir, M. T. Huyut, and N. Yüce. 2024. "Investigating the Individual and Combined Effects of Coenzyme Q10 and Vitamin C on CLP-Induced Cardiac Injury in Rats." *Scientific Reports* 14, no. 1: 3098.

Üstündağ, H., S. Doğanay, F. D. Kalındemirtaş, et al. 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.

Wang, B., X. He, J. Zhang, and Y. Zhang. 2024. "Cell Surface GRP78: A Potential Therapeutic Target for High Glucose-Induced Endothelial Injury." *Biochemical and Biophysical Research Communications* 692: 149347.

Wang, J., P. Jiao, Y. Zhou, and Q. Liu. 2016. "Protective Effect of Dihydromyricetin Against Lipopolysaccharide-Induced Acute Kidney Injury in a Rat Model." *Medical Science Monitor* 22: 454–459.

Wang, Y., J. Zhu, Z. Liu, et al. 2021. "The PINK1/PARK2/Optineurin Pathway of Mitophagy Is Activated for Protection in Septic Acute Kidney Injury." *Redox Biology* 38: 101767.

Wu, Y., H. Shi, Y. Xu, et al. 2022. "Ebselen Ameliorates Renal Ischemia-Reperfusion Injury via Enhancing Autophagy in Rats." *Molecular and Cellular Biochemistry* 477, no. 6: 1873–1885.

Wu, Y., M. Zhao, and Z. Lin. 2021. "Pyrroloquinoline Quinone (PQQ) Alleviated Sepsis-Induced Acute Liver Injury, Inflammation, Oxidative Stress and Cell Apoptosis by Downregulating CUL3 Expression." *Bioengineered* 12, no. 1: 2459–2468.

Yang, R., P. Guo, Z. Ma, et al. 2020. "[Corrigendum] Effects of Simvastatin on iNOS and Caspase-3 Levels and Oxidative Stress Following Smoke Inhalation Injury." *Molecular Medicine Reports* 22, no. 6: 5479.

Ye, J., R. B. Rawson, R. Komuro, et al. 2000. "ER Stress Induces Cleavage of Membrane-Bound ATF6 by the Same Proteases That Process SREBPs." *Molecular Cell* 6, no. 6: 1355–1364.

Zhao, Y., X. Feng, B. Li, et al. 2020. "Dexmedetomidine Protects Against Lipopolysaccharide-Induced Acute Kidney Injury by Enhancing Autophagy Through Inhibition of the PI3K/AKT/mTOR Pathway." *Frontiers in Pharmacology* 11: 128.

Zhu, C., F. Fan, C. Li, Y. Xiong, and X. Liu. 2024. "Caspase-3 Promotes Oncogene-Induced Malignant Transformation via EndoG-Dependent Src-STAT3 Phosphorylation." *Cell Death & Disease* 15, no. 7: 486.

Zhu, J., S. Xu, J. Li, et al. 2018. "Farnesoid X Receptor Agonist Obeticholic Acid Inhibits Renal Inflammation and Oxidative Stress During Lipopolysaccharide-Induced Acute Kidney Injury." *European Journal of Pharmacology* 838: 60–68.

Zhu, W., Y. Ou, C. Wang, et al. 2024. "A Neutrophil Elastase Inhibitor, Sivelestat, Attenuates Sepsis-Induced Acute Kidney Injury by Inhibiting Oxidative Stress." *Heliyon* 10, no. 8: e29366.