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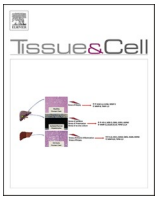

Combating oxidative stress and inflammation in gentamicin-induced nephrotoxicity using hydrogen-rich water

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ABSTRACT

Gentamicin-induced nephrotoxicity primarily results from renal inflammatory cascades and increased oxidative stress. This study aims to examine the effects of hydrogen-rich water (HRW) on gentamicin-induced renal damage in rats. Thirty-two rats were equally divided into four groups, including control (no treatment), hydrogen, gentamicin, and gentamicin+hydrogen. At the end of one week, all animals were euthanized following ethical rules, and blood and tissue samples were analyzed for examining Malondialdehyde (MDA), glutathione (GSH), Tumor Necrosis Factor-Alfa (TNF-α), Tumor Necrosis Factor-Beta (TNF-β), Interleukin 6 (IL-6), endoglin, endocan, urea, creatinine, Na^+, and K^+ parameters. Levels of 8-Hydroxyguanosine (8-OHdG), MDA, and Bax were immunohistochemically analyzed. Data showed that while MDA (control $P<0.0001$, H_2 $P<0.0001$, ↱Genta+H_2 $P<0.0007$), TNF-α (control $P<0.0002$, H_2 $P<0.0040$, ↱Genta+H_2 $P<0.0381$), IL-6 (control $P<0.0044$, H_2 $P<0.0070$, ↱Genta+H_2 $P<0.0109$), endocan (control $P<0.0460$, H_2 $P<0.0286$, ↱Genta+H_2 $P<0.0452$), and endoglin (control $P<0.0131$, H_2 $P<0.0164$, ↱Genta+H_2 $P<0.0397$), urea (control $P<0.0024$, H_2 $P<0.0001$, ↱Genta+H_2 $P<0.0180$), and creatinine parameters (control $P<0.0017$, H_2 $P<0.0178$, ↱Genta+H_2 $P<0.0011$) increased in the gentamicin group compared to the other groups, a decrease in these parameters was observed in the gentamicin+hydrogen group compared to the gentamicin group. The Genta group had greater levels of TNF-β than the control ($P<0.0042$) and H2 groups ($P<0.0268$). GSH content was higher in the hydrogen group compared to the gentamicin group. Immunohistochemically, 8-OHdG, MDA, and Bax expressions increased in the gentamicin group compared to the control group, whereas they decreased in the gentamicin+hydrogen group compared to the gentamicin group. Hydrogen may be an alternative treatment for oxidative stress-induced nephrotoxicity.

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1. Introduction

An aminoglycoside antibiotic called gentamicin is frequently used to treat Gram-negative bacteria that harm the body (Hodiamont et al., 2022). When used for an extended period, the most prominent side effect of gentamicins is nephrotoxicity (Akyüz et al., 2021; Erdem et al., 2000). Nephrotoxicity forms one of the most frequent causes of acute kidney injury (Akbaş et al., 2023). Approximately 30 % of patients may develop symptoms of nephrotoxicity after approximately seven days of gentamicin use (Paterson et al., 1998). Renal inflammatory cascades, increased renal oxidative stress, and elevations in related pathogenic signaling systems are the primary causes of gentamicin-induced nephrotoxicity (Erseçkin et al., 2022; Kandemir et al., 2015). The generation of free radicals in the renal cortex is one of the primary causes of the pathophysiology of gentamicin nephrotoxicity. Although reactive oxygen species (ROS) are crucial to physiology in numerous systems, excessive ROS generation can cause cellular macromolecule damage, ultimately resulting in cell death. Lipid peroxidation can result from free radicals created during oxidative metabolism damaging phospholipids in cell membranes. Lipid peroxidation produces malondialdehyde (MDA), a marker commonly used to quantify oxidative stress. Similarly, measurement of the endogenous antioxidant glutathione (GSH) also helps determine the degree of oxidative stress in the body (Dolanbay et al., 2021). As a result, MDA and GSH levels are regarded as crucial indicators for assessing the oxidative/antioxidant status (Akyüz et al., 2021; Alwazeer, 2024; Makav et al., 2023, 2021; Ohsawa et al., 2007; Ölmez et al., 2020; Yıldız et al., 2022).

The gas known as molecular hydrogen (H2) is tasteless, colorless, and non-toxic. H_2 possesses other physical and chemical properties, making it unique among other gases and elements (Makav et al., 2021). Despite the chemical neutrality of H_2, many *in vitro*, *in vivo*, and animal and clinical studies have revealed the multi-biological activities of H_2, especially its selective antioxidant activity (Alwazeer, 2024; Alwazeer et al., 2021). H_2 showed its scavenging activity against the most potent oxidants, i.e., hydroxyl radical ($^{\bullet}OH$) and peroxynitrite ($ONOO^-$) (Ohta, 2012). In various health studies, H_2 has exhibited more than 37 biological activities (Alwazeer, 2024). From these properties, H_2 can help regulate redox homeostasis, including the GSH/Glutathione disulfide (GSSG) ratio, cellular enzymatic antioxidants, as well as the innate antioxidants' gene expressions (glutathione peroxidase, catalase, and superoxide dismutase). In addition, H2 demonstrated anti-inflammatory, anti-apoptotic, anti-cancer, anti-disease, and anti-stress biological characteristics. Furthermore, because of its small size, high diffusion rate, and apolar characteristics, H2 can pass through a variety of biological tissue barriers and biomembranes (Alwazeer, 2024; Alwazeer et al., 2021). H_2 can be administered in different ways, including hydrogen-rich water (HRW), hydrogen-rich saline (HRS), and inhalation (Alwazeer et al., 2024). HRW is formed by infusing normal water with external hydrogen molecules (H_2) in the dissolved form. HRW possesses many unique physicochemical and biological properties due to dissolved H_2 (Alwazeer, 2024). In animal and human studies, HRW intake has shown many health benefits (Kayabaş et al., 2023; Xie and Song, 2024; Yıldız et al., 2022). Since Gentamicin-induced nephrotoxicity is mainly caused by renal inflammatory cascades and elevated renal oxidation stress (Erseçkin et al., 2022; Kandemir et al., 2015), this lets us assume that hydrogen may positively affect the Gentamicin-induced nephrotoxicity. The purpose of this study was to look at how HRW affected rats' acute renal damage caused by gentamicin.

2. Materials and methods

The study was started after obtaining permission from the local ethics committee of Kafkas University Experimental Animals (KAU-HADYEK/2023–118). The National Institutes of Health (NIH) criteria for the use of experimental animals were followed for conducting animal experiments (Care and Animals, 1986; Council, 2011). Thirty-two male Wistar albino rats (body mass = 200–250 g, 3-month-old) were divided into four groups. Rats were fed *ad libitum* with regular rat pellet feed and drinking water throughout the study. During to study, the rats were housed in typical rat cages with a 12-hour dark/light cycle, a standard temperature of 23 ± 2°C, and a humidity level of 45–50 %. Gentamicin was administered intraperitoneally to the treatment groups once daily for a week at a dose of 80 mg/kg to cause acute renal damage. The rat groups were as follows:

1. Control Group (control): 5 mL of drug-free, non-hydrogen water/kg was intraperitoneally provided daily for one week.
2. Hydrogen Group (H_2): 5 mL HRW/kg (Makav et al., 2023; Yuan et al., 2018) was intraperitoneally provided daily for one week.
3. Gentamicin Group (Genta): 80 mg gentamicin/kg (Akbaş et al., 2023) was intraperitoneally given daily for one week.
4. Gentamicin+Hydrogen Group (Genta+H_2): 80 mg gentamicin+5 mL HRW/kg was intraperitoneally given daily for one week.

Following ethical guidelines (cervical dislocation), all animals were slaughtered at the conclusion of the seven-day trial period while under anesthesia from ketamine hydrochloride (75 mg/kg) and xylazine (15 mg/kg) administered intramuscularly. Blood samples (Cardiac puncture) were collected from the rats in blood tubes for biochemical measurements. Prior to analysis, the separated serum samples were kept at −20°C after the blood samples were centrifuged at 3000 rpm. For histopathological analysis, tissue sampling was performed, and the tissues were stored in a 10 % buffered formaldehyde solution.

HRW was prepared using an oxyhydrogen machine (HB-33 Epoch, Taiwan) by bubbling oxyhydrogen into 2 L pure water for 30 min at 1.25 L/min (Kuru et al., 2024). The concentration of H_2 in HRW was estimated to be approximately 1 mg/L per Henry's Law using a redox probe (SP60X, Consort) (Kayabaş et al., 2023).

2.1. Biochemical methods

The serum contents of MDA, GSH, urea, K^+, creatinine, Na^+, Tumor Necrosis Factor-Alfa (TNF-α), Tumor Necrosis Factor-Beta (TNF-β), Interleukin 6 (IL-6), endocan, and endoglin were analyzed. MDA analysis was performed (duplicate) using the method of Yoshioka et al., (1979). GSH analysis was analyzed (duplicate) spectrophotometrically at 412 nm according to the method of Beutler et al. (1963). A Beckman-Coulter AU5800 autoanalyzer (Beckman Coulter®, U.S.) was used to perform spectrophotometric analyses of urea, creatinine, K+, and Na+. Using commercial Enzyme-Linked Immuno Sorbent Assay (ELISA) kits (Elabscience®, U.S.), the levels of TNF-α, TNF-β, IL-6, endocan, and endoglin were measured in accordance with the kit protocol.

2.2. Histopathologic methods

The kidney tissues were preserved in a 10 % buffered formaldehyde solution for microscopic inspection following the systemic necropsy of the animals in the groups. Hematoxylin and eosin (H&E) staining was performed on paraffin blocks following standard tissue follow-up procedures. Sections were inspected and captured on camera using an Olympus Bx53 light microscope. Kidney tissues were qualitatively examined and photographed for hyperemia and inflammatory, degenerative, and necrotic changes.

2.3. Immunohistochemical methods

Poly-L-lysine-coated adhesive slides were used to segment paraffin blocks at a thickness of 3 μm. In order to reveal the antigenic receptors, the sections were subjected to microwave treatment (citrate buffer solution pH 6 for 10 min). Subsequently, the sections were treated with primary antibodies (MDA: ABCAM - ab6463, 8-OHdG: Bioss Antibodies,

bs-1278R, Bax: 6A7-MA5–14003/1:100 Bcl) diluted in phosphate-buffered saline (PBS) and incubated overnight in the refrigerator (4℃). The sections were then treated with biotinized secondary antibody (Thermo Scientific Histostain IHC Kit, HRP, broad spectrum, REF: TP-125-HL) and peroxidase-linked Strep Avidin (Thermo Scientific Histostain IHC Kit, HRP, broad spectrum, REF: TP-125-HL) for 10 min at room temperature. AEC was used as chromogen, ground staining was performed with Harris hematoxylin, and the preparations were examined under a light microscope (Beytut et al., 2024).

In the immunohistochemical staining method using 8-OHdG, MDA, and Bax primary antibody, three different areas of each kidney tissue were semi-quantitatively analyzed at 40 magnification. Positive reactions were evaluated as 0, no expression; 1, weak; 2, moderate; 3, severe, based on the staining intensity and the width of the positive areas (Mohammed et al., 2019).

2.4. Statistical analysis

The data were analyzed using GraphPad version 8.1.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was determined using a two-tailed t-test (or appropriate statistical test) with a significance threshold set at $P< 0.05$. Results with *P*-values less than 0.05 were considered statistically significant. All data are presented as mean ± standard deviation unless otherwise specified.

3. Results

3.1. Biochemical analysis

Data on MDA and GSH levels are displayed in Fig. 1. Results showed that the Genta group had significantly increased MDA levels compared to the other groups (control $P<0.0001$, H_2 $P<0.0001$, ↱Genta+H_2 $P<0.0007$). However, GSH levels were significantly higher in the H_2 group compared to the Genta group ($P<0.0487$).

Data for urea, creatinine, K^+, and Na^+ are given in Fig. 2. The levels of urea were significantly higher in the Genta group compared to the other groups (control $P<0.0024$, H_2 $P<0.0001$, ↱Genta+H_2 $P<0.0180$). The levels of creatinine were significantly higher in the Genta group compared to the other groups (control $P<0.0017$, H_2 $P<0.0178$, ↱Genta+H_2 $P<0.0011$). However, urea and creatinine levels decreased when H_2 was added to gentamicin, i.e., the Genta+H2 group. However, there was no significant difference between all the groups regarding Na^+ and K^+ parameters (*P>0.05*↱).

Data of TNF-α TNF-β, IL-6, endocan, and endoglin are given in Fig. 3. Genta group samples showed the highest levels of TNF-α (control $P<0.0002$, H_2 $P<0.0040$, ↱Genta+H_2 $P<0.0381$), IL-6 (control $P<0.0044$, H_2 $P<0.0070$, ↱Genta+H_2 $P<0.0109$), endocan (control $P<0.0460$, H_2 $P<0.0286$, ↱Genta+H_2 $P<0.0452$), and endoglin (control $P<0.0131$, H_2 $P<0.0164$, ↱Genta+H_2 $P<0.0397$). However, when H_2 was included with gentamicin, i.e., Genta+H_2 group, TNF-α, IL-6, endocan, and endoglin levels were decreased. Regarding TNF-β, Genta group samples showed higher levels than the control ($P<0.0042$↱) and H_2 groups ($P<0.0268$↱).

3.2. Histopathologic examination analysis

As a result of the examinations of the kidney tissues, both control and hydrogen groups preserved their typical histologic structures (Fig. 4A-4B). On the other hand, samples from the Genta group exhibited significant necrosis and degenerative and necrotic alterations in the tubules of the cortical region. In addition, dilatation in some tubules and the presence of hyaline cylinders in their lumens and atrophic glomeruli were observed (Fig. 4C). In the Genta + H_2 group, single-cell necrosis, hydropic degeneration, hyaline cylinders, and hyperemia were noticed in the epithelial cells of the tubules in the cortical region of the kidney tissue. Furthermore, a notable reduction in the lesions' severity was noted (Fig. 4D).

3.3. Immunohistochemical staining analysis

The immunohistochemical staining results using 8-OHdG primary antibody showed no positive reaction in tissue samples of the control and hydrogen groups (Fig. 5A-5B). In the Genta group, intracytoplasmic immunopositive reactions were detected mainly in the tubulous epithelium, which underwent degeneration and necrosis in the cortex. Immunopositive reactions were also observed in some glomeruli (Fig. 5C). In the Genta+H_2 group, the immune-positive responses found in the tubular epithelium were similar to those in the Genta group. However, their severity and intensity were lower than the Genta group (Fig. 5D).

In the staining analysis using the MDA primary antibody, positive immune reactions were observed in the glomeruli of kidney tissues of the control and hydrogen groups (Fig. 6A-6B). In the Genta group, intracytoplasmic immunopositive reactions were observed in the degenerated tubule epithelium in the cortex, glomeruli, and epithelial cells of the effluent ducts in the medulla. Additionally, immunopositive

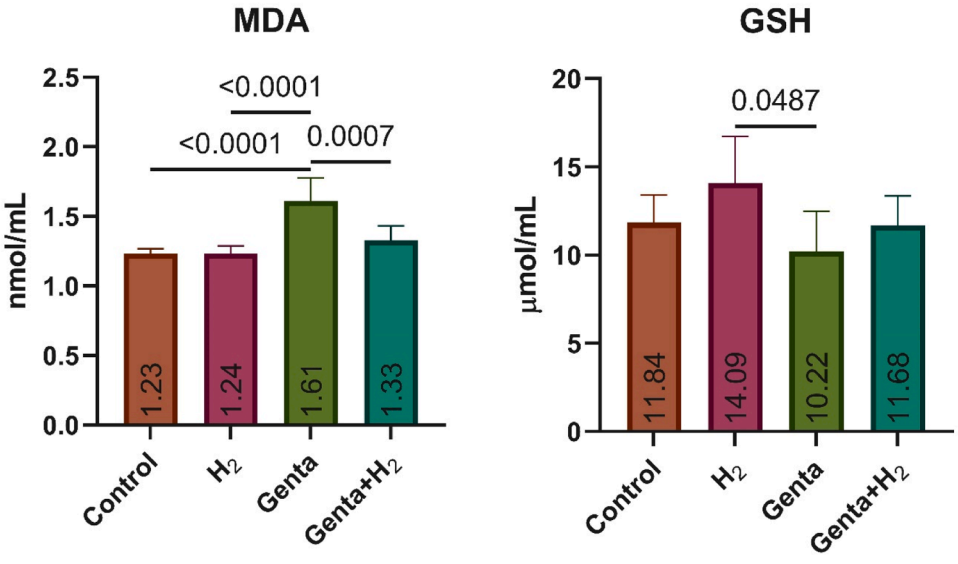

Fig. 1. Graphical display of MDA and GSH parameters. Means and standard deviation of the four groups for MDA and GSH.

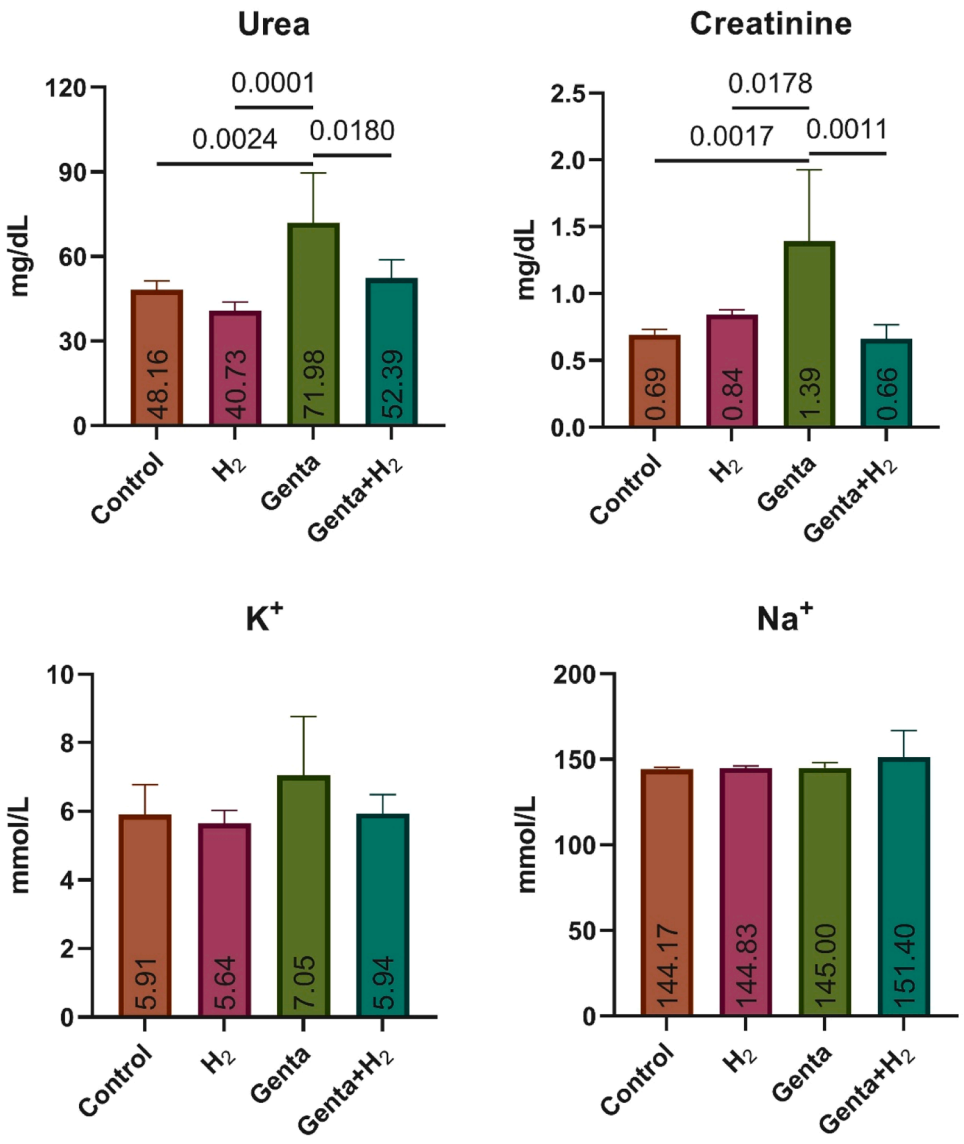

Fig. 2. Graphical display of urea, creatinine, K^+, and Na^+ parameters. Means and standard deviation of the four groups for urea, creatinine, K^+, and Na^+.

reactions were noticed in the fluid channels in the medulla (Fig. 6C). In the Genta+H_2 group, intracytoplasmic immunopositive reactions were seen in renal tubule epithelia (Fig. 6D).

Immunohistochemical staining analysis using Bax primary antibody revealed no immunopositive reaction in the control and H_2 groups (Fig. 7A-7B). In the Genta group, intracytoplasmic immunopositive responses were observed in the renal tubule epithelium in the cortical region. Both intracytoplasmic and intranuclear immunopositive reactions were noticed in the epithelium of the fluid channel in the medulla (Fig. 7C). In the Genta+H_2 group, immunopositive reactions were found to be intracytoplasmic in the renal tubule epithelium and the fluidic ducts in the medulla (Fig. 7D).

Moreover, 8-OHdG, MDA, and Bax immunopositive reactions scores were evaluated (Fig. 8). In this context, the Genta group showed the highest 8-OHdG score compared to the other groups (control $P<0.0001$, H_2 $P<0.0001$, ↱Genta+H_2 $P<0.0257$). The Genta+H2 group also showed a statistical increase compared to the control ($P<0.0001$) and H2 group ($P<0.0001$). When compared to the other groups (control $P<0.0001$, H_2 $P<0.0001$, ↱Genta+H_2 $P<0.0201$), the Genta group had the highest MDA score. The Genta+H2 group also showed a statistical increase compared to the control ($P<0.0008$) and H2 group ($P<0.0002$). When the control ($P<0.0001$) and H2 ($P<0.0001$) groups were compared with the Genta group, the Genta group showed the highest Bax score. When control ($P<0.0003$) and H2 ($P<0.0001$) groups were compared with the Genta+H2 group, the Bax score increased in the Genta+H2 group. The Bax score shows no statistical difference between the genta group and the genta+H2 group ($P>0.05$). When the data of 8-OHdG, MDA, and Bax immunopositive reactions were analyzed, one can observe a decrease in the severity of the reaction in the Genta+H_2 group compared to the Genta group.

4. Discussion

The results indicated that while the gentamicin group had higher levels of MDA, TNF-α, IL-6, endocan, endoglin, urea, and creatinine parameters than the other groups, the gentamicin+hydrogen group showed lower levels of these parameters than the gentamicin group. The Genta group had greater levels of TNF-β than the control and H2 groups.

Nephrotoxicity is the term used to describe the rapid deterioration of renal function brought on by a variety of sources, including industrial chemicals, environmental toxins, and most notably, medications (Laorodphun et al., 2022). Proximal tubule epithelial cells in the renal cortex may sustain irreversible damage from toxic dosages of gentamicin. The precise origin of toxicity is uncertain, however it is believed

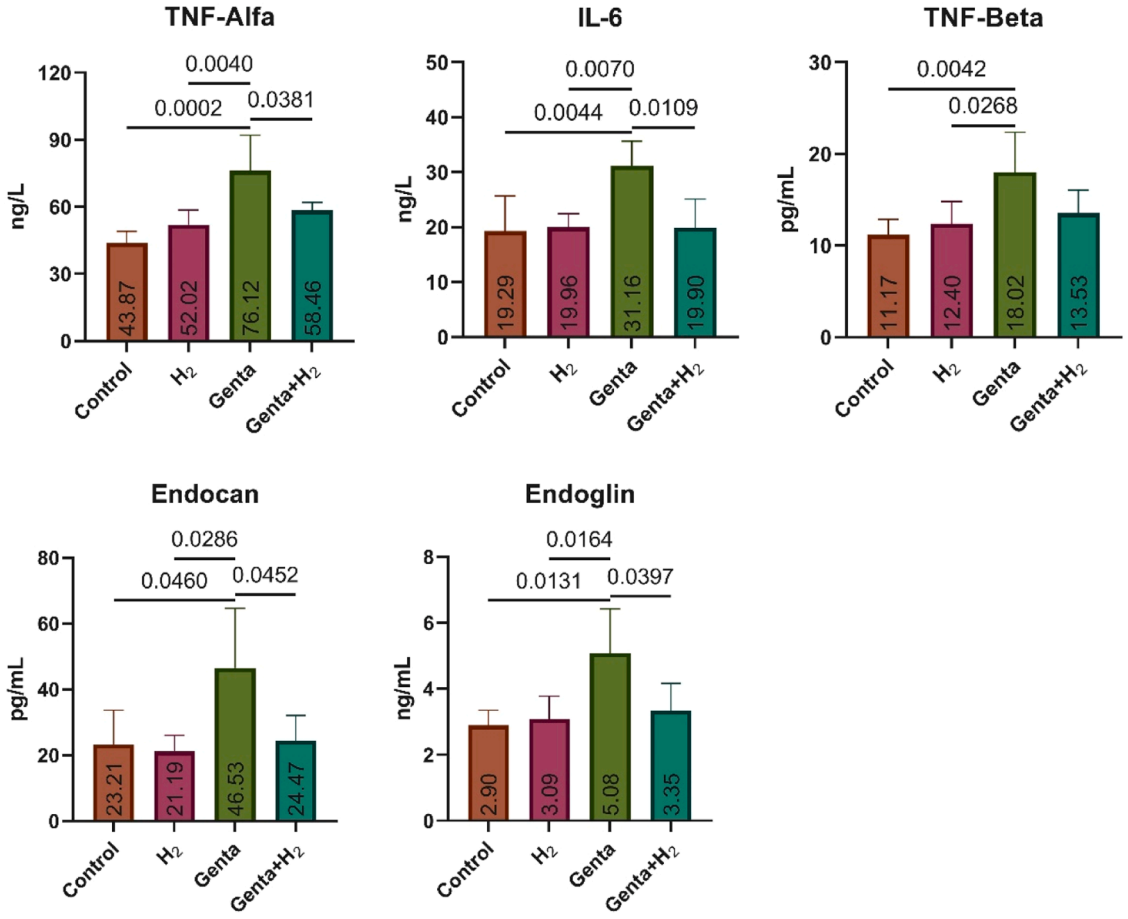

Fig. 3. Graphical display of TNF-α, IL-6, TNF-β, Endocan, and Endoglin parameters. Means and standard deviation of the four groups for TNF-α, IL-6, TNF-β, Endocan, and Endoglin.

to be mostly caused by necrosis, ROS buildup, mitochondrial respiratory chain failure, and apoptotic pathway activation (Mahmoud et al., 2021). Free radicals can cause oxidative stress, which can lead to cell death. These free radicals have the ability to harm cell membrane phospholipids through the process of lipid peroxidation.

Furthermore, lipid peroxidation causes a drop in GSH levels and an increase in MDA levels (Dolanbay et al., 2021; Yıldız et al., 2022). It is well known that the H_2 in HRW exerts antioxidant activity, especially towards the most potent free oxidants in cells, i.e., $^{\bullet}OH$ and $ONOO^{-}$, allowing protection of cells from oxidative damage (Alwazeer et al., 2021; Yıldız et al., 2022). In the present study, the decrease in MDA levels of serum and immunohistochemical gentamicin toxicity analyses in the Genta+H_2 group samples can be interpreted by the antioxidant activity of hydrogen molecules. In terms of the GSH data, there was no statistically significant difference between the groups; however, the H2 group did indicate an increase. In contrast, an apparent numerical increase can be noticed for the Genta+H_2 group.

By reducing the levels of pro-inflammatory chemicals that lead to inflammation, like cytokines, HRW reduces inflammation (Alwazeer et al., 2021; Alwazeer and Çiçek, 2021). Gentamycin's capacity to maximally decrease the level of the Nuclear Factor Kappa B (NF-κB) inhibitor (IkB-α) protein can result in an increase in nuclear NF-κB-DNA binding activity and renal cytoplasmic protein expression of NF-κB. These factors can then trigger the production of inflammatory cytokines like TNF-α and IL-6 (Ansari et al., 2016; Katary and Salahuddin, 2017). Renal mesangial and epithelial cells release TNF-α that is elevated in response to gentamycin therapy and increased macrophage infiltration (Sahu et al., 2013). IL-6, which controls immunological and inflammatory responses, is secreted by macrophages when stimulated by gentamycin (Fielding et al., 2008).

Wang et al. (2017) reported that TNF-α deficiency prevents inflammation occurring in kidney injury. They also noted that oxidative stress and apoptosis were increased when TNF-α levels were increased. The administration of HRW in the current study resulted in a decrease in TNF-α, which may have been caused by H2's anti-inflammatory and antioxidant qualities. HRW also decreased the apoptosis marker Bax, which was detected histologically. This finding suggests that the administration of HRW could prevent apoptosis that increases with the increase in TNF-α levels.

In inflammatory processes, cytokines such as TNF and IL-6 have been reported to induce ROS formation (Stenvinkel et al., 2005). Similarly, these cytokines were increased after toxication, as shown in the present study.

Tumor necrosis factor-beta (TNF-β) is a protein encoded by the lymphotoxin alpha gene, also known as lymphotoxin alpha (LTA). Studies have shown that TNF-β expression increases in case of inflammation (MAKAV and KURU, 2021; Nedwin et al., 1985). In the present study, an increase in TNF-β was observed in the toxication group (Genta). The decrease in TNF-β levels observed in the Genta+H_2 group demonstrates the anti-inflammatory property of H_2 in HRW.

The cells of the vascular endothelium secrete a soluble proteoglycan called endocan, or specific molecule 1 (ESM-1). It is essential for inflammation, lymphocyte function, endothelial cytoskeleton reorganization, and the upregulation of several adhesion molecules (Gunay and Mertoğlu, 2019). In addition, inflammation and endothelial damage have been emphasized in the pathogenesis of acute kidney injury. It has also been reported that endothelial and smooth muscle cell disruption is critical in kidney injury and that endocan levels increase with

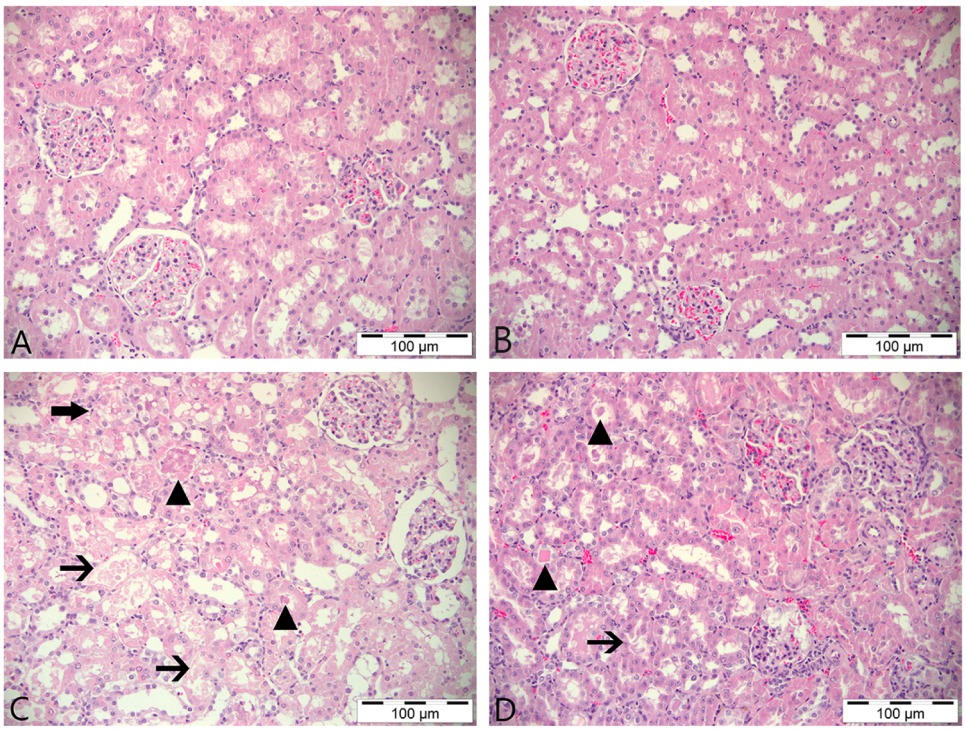

Fig. 4. Histopathologic evaluation of kidney tissue in experimental groups; A: Control group (Normal histologic structure), B: H_2 group (Normal histologic structure), C: Genta group, D: Genta+H_2 group, triangle: hyaline cylinders in tubule lumens, thick arrow: hydropic degeneration of tubules, thin arrow: degeneration, necrosis of tubule epithelium. H&E, 100 µm.

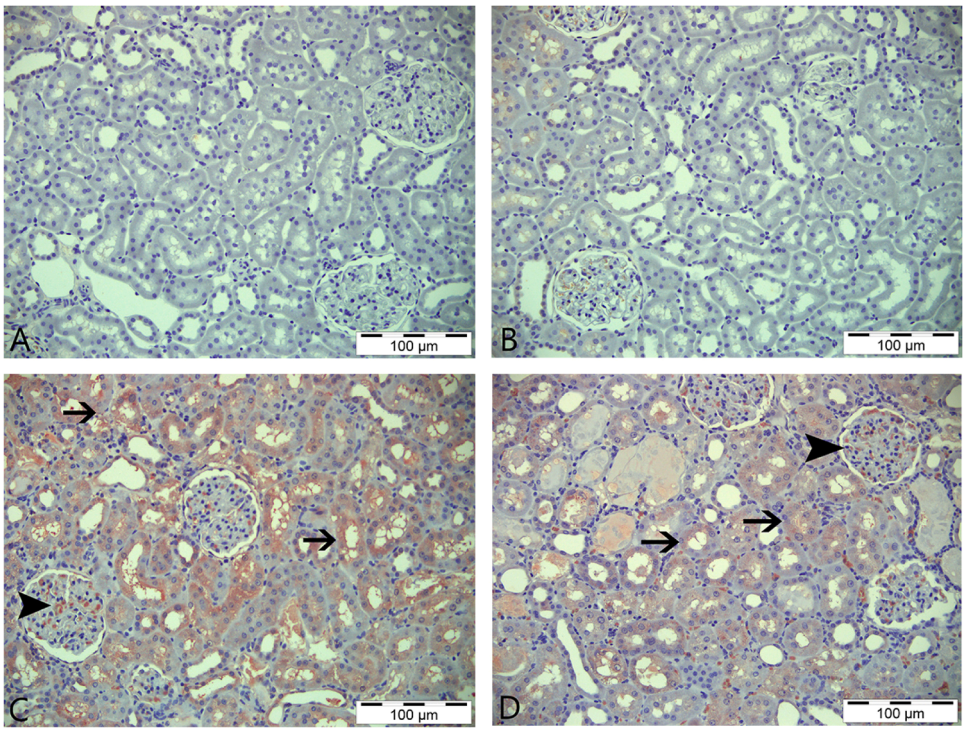

Fig. 5. Immunohistochemical reactivity of 8-OHdG in tubules (arrow) and glomeruli (arrowhead) in kidney tissue; A: Control group (negative immunostaining), B: Hydrogen group (negative immunostaining), C: Gentamicin group, D: Genta+H_2 group, IHC, 100 µm.

endothelial damage (Bonventre and Yang, 2011). Yilmaz et al. (2014) reported increased plasma endocan levels in renal patients with low GFR. In the present study, an increase in endocan levels was observed in the group with renal toxicity (i.e., Genta). The decrease in endocan levels of the Genta+H_2 group also indicates an anti-inflammatory effect of HRW.

A TGF-β co-receptor, endoglin regulates the responses of various cell types to transforming growth factor-beta (TGF-β). TGF-β-induced

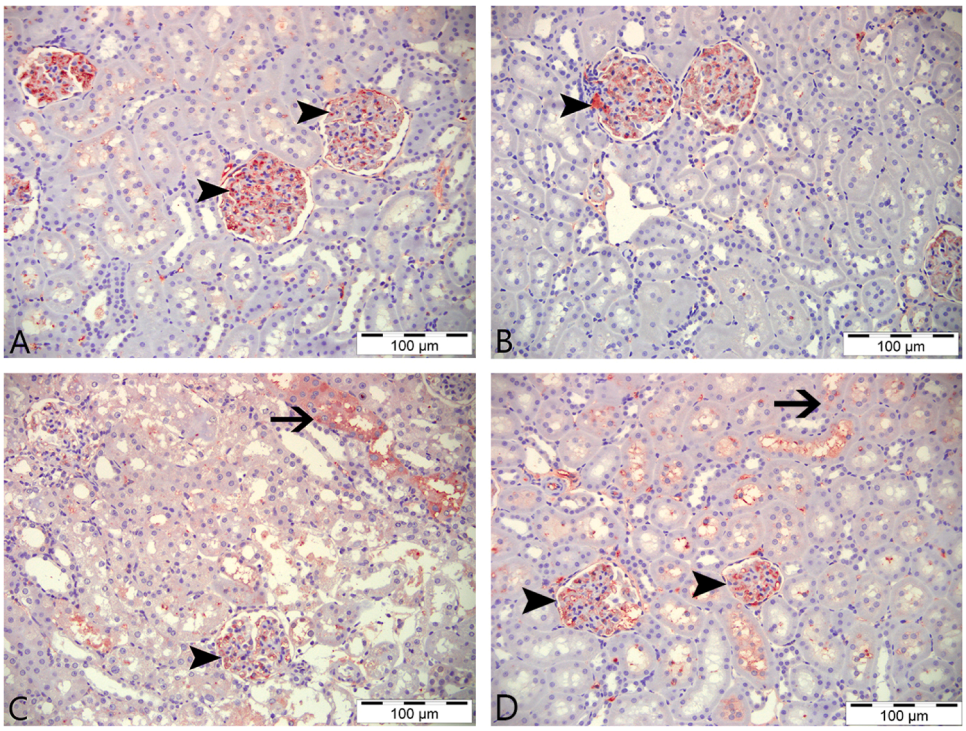

Fig. 6. Immunohistochemical reactivity of MDA in tubules (arrow) and glomeruli (arrowhead) of kidney tissue; A: Control group, B: Hydrogen group, C: Gentamicin group, D: Genta+H_2 group, IHC, 100 µm.

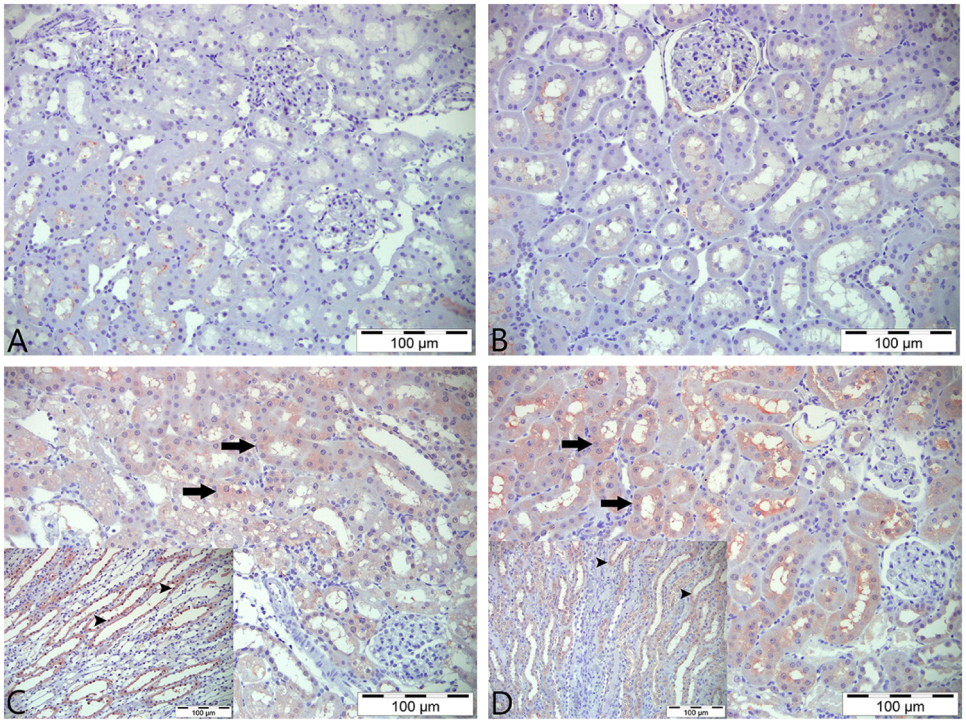

Fig. 7. Immunohistochemical reactivity of Bax in the tubulous (arrow) and medullary inflow channels (arrowhead) of kidney tissue; A: Control group (negative immunostaining), B: Hydrogen group (negative immunostaining), C: Gentamicin group, D: Gentamicin+Hydrogen group, IHC, 100 µm.

production of extracellular matrix (ECM) proteins is negatively regulated by endoglin in a variety of cell types, including mesangial cells and myoblasts. Nevertheless, it has recently been demonstrated that endoglin stimulates fibrotic reactions in vivo (Muñoz-Felix et al., 2014), and In kidney and other tissue experimental models of renal fibrosis, its endoglin expression was elevated (Muñoz-Felix et al., 2014). In the current investigation, the group with kidney toxicity (Genta) showed elevated endoglin levels. However, in the Genta+H_2 group, a decrease in endoglin levels was noticed. In various stages of renal fibrosis, including myofibroblast activation and proliferation, ECM protein production, and

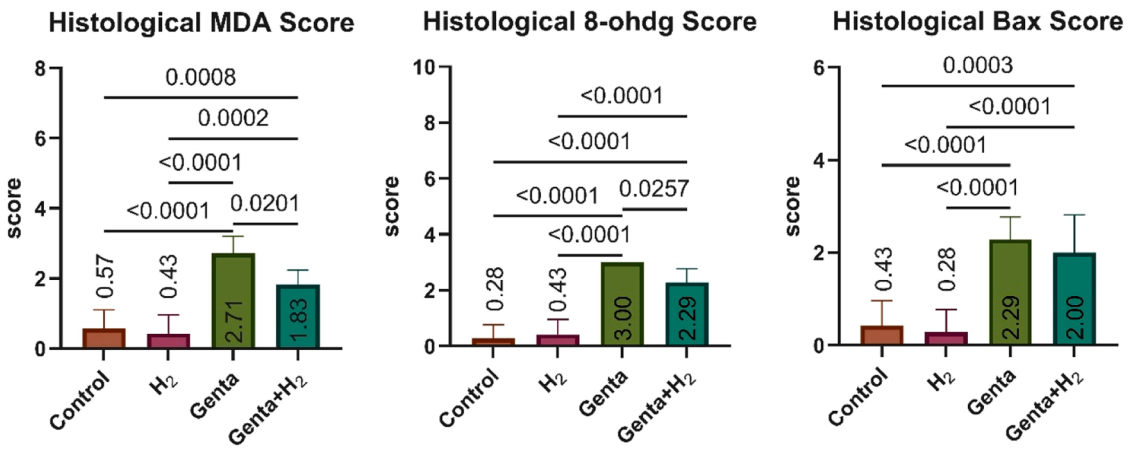

Fig. 8. Graphical display of 8-OHdG, MDA, and Bax immune positive reactions scores. Means and standard deviation of the four groups for MDA immune positive reactions scores.

inflammatory cell infiltration, TGF-β1 is a crucial cytokine (Muñoz-Felix et al., 2014). Accordingly, H_2 may attenuate inflammatory cell infiltration *via* the TGF-β1 pathway.

Urea and creatinine are known to be removed from the body by the kidneys. However, serum urea and creatinine levels increase in the case of kidney diseases. The elevation of these molecules is due to the changes in glomerular filtration in situations of kidney damage. As a result, substances are less efficiently filtered and removed from the blood, leading to elevated levels in the bloodstream (Ahmadvand et al., 2020; Alsharidah et al., 2021; Hashim et al., 2020; Kovalčíková et al., 2020; Liu et al., 2021). In the present study, an increase in urea and creatinine levels in the Genta group agrees with the findings of the literature. The decrease in the urea and creatinine levels shown in the Genta+H_2 group suggests the potential therapeutic effect of hydrogen in cases of kidney damage.

According to previous studies, oxidative stress can lead to the oxidation of amino acids and DNA (Gelen et al., 2022; Şengül et al., 2017). 8-OHdG is one of the indicators used to assess DNA damage brought on by elevated oxidative stress (Gelen et al., 2022). In another study, it has been revealed that hydrogen-rich water could alleviate the Mercury-linked damage of 8-OHdG in earthworms (Köktürk et al., 2022). In the present study, 8-OHdG, an elevated expression, was shown immunohistochemically after gentamicin toxication. However, DNA damage from oxidative stress induced by gentamicin ↱ toxication was decreased by HRW administration.

5. Conclusions

In conclusion, our study highlights the role of oxidative stress in gentamicin-induced kidney injury, as evidenced by the observed inflammation and apoptosis. However, the treatment of hydrogen-rich water (HRW) significantly reduced oxidative stress and inflammation. Restoring urea and creatinine levels to normalcy following HRW administration suggests its potential as an effective therapeutic intervention for ameliorating kidney injury. These findings underscore the promising therapeutic implications of HRW in mitigating oxidative stress-related renal pathologies, warranting further investigation into its clinical application.

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CRediT authorship contribution statement

Mustafa CUMAOGLU: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization. **Mustafa MAKAV:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Serpil DAG:** Visualization, Validation, Resources, Methodology, Data curation. **Ayfer Uysal:** Visualization, Methodology, Formal analysis, Data curation. **Lale Baser:** Writing – original draft, Resources, Formal analysis, Data curation. **Tyler LeBaron:** Writing – review & editing, Investigation. **Duried Alwazeer:** Writing – review & editing, Writing – original draft, Investigation.

Declaration of Competing Interest

There are no conflicting interests, according to the authors.

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Data availability

Data will be made available on request.

References

Ahmadvand, H., Nouryazdan, N., Nasri, M., Adibhesami, G., Babaeenezhad, E., 2020. Renoprotective effects of gallic acid against gentamicin nephrotoxicity through amelioration of oxidative stress in Rats. Braz. Arch. Biol. Technol. 63, e20200131. https://doi.org/10.1590/1678-4324-2020200131.

Akbaş, P., Kaya, E., Makav, M., Yıldız, G., 2023. Investigation of antimicrobial and antioxidant activities of chenopodium album extracts and their effects on gentamicin nephrotoxicity in rats. Food Sci. Nutr. 11, 8121–8130. https://doi.org/10.1002/FSN3.3733.

Akyüz, E., Makav, M., Kuru, M., Bayram, P., 2021. The effect of tarantula cubensis extract on gentamicin-induced acute kidney injury in ovariectomized rats. Kafkas Univ. Vet. Fak. Derg. 27, 559–566. https://doi.org/10.9775/KVFD.2021.25390.

Alsharidah, M., Abdel-Moneim, A.M.H., Alsharidah, A.S., Mobark, M.A., Rahmani, A.H., Shata, A., Abdellatif, A.A.H., El-Readi, M.Z., Mohany, K.M., Al Rugaie, O., 2021. Thymoquinone, but not metformin, protects against gentamicin-induced nephrotoxicity and renal dysfunction in rats, 2021, 11, 3981 Appl. Sci. 11, *3981*. https://doi.org/10.3390/APP11093981.

Alwazeer, D., 2024. Consumption of Hydrogen-Treated Foods Provides Nutritional and Health Benefits. In: Slezak, J., Kura, B. (Eds.), Advances in Biochemistry in Health and Disease, Molecular Hydrogen in Health and Disease. Springer, Cham, pp. 319–337. ISBN 978-3-031-47374-6.
Alwazeer, D., 2024. Use of Hydrogen Extraction in the Food Industry. In Comprehensive Sampling and Sample Preparation. Elsevier. ISBN 978-0-12-409547-2.
Alwazeer, D., Çiçek, S., 2021. Moleküler Hidrojenin Sağlık Alanında Kullanımı. Karya J. Health Sci. 3, 30–34. https://doi.org/10.52831/kjhs.899237.
Alwazeer, D., Hancock, J.T., Russell, G., Stratakos, A.C., Li, L., Çiğdem, A., LeBaron, T. W., 2024. Molecular hydrogen: a sustainable strategy for agricultural and food production challenges. Front. Food Sci. Technol. 4, 1448148.
Alwazeer, D., Liu, F.F.-C., Wu, X.Y., LeBaron, W.T., 2021. Combating oxidative stress and inflammation in COVID-19 by molecular hydrogen ↱therapy: mechanisms and perspectives. Oxid. Med Cell Longev. https://doi.org/10.1155/2021/5513868.
Ansari, M.A., Raish, M., Ahmad, A., Ahmad, S.F., Mudassar, S., Mohsin, K., Shakeel, F., Korashy, H.M., Bakheet, S.A., 2016. Sinapic acid mitigates gentamicin-induced nephrotoxicity and associated oxidative/nitrosative stress, apoptosis, and inflammation in rats. Life Sci. 165, 1–8. https://doi.org/10.1016/j.lfs.2016.09.014.
Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. J. Lab Clin. Med 61, 882–888.
Beytut, E., Coskun, N., Dag, S., Nuhoglu, H., Karakurt, E., Yilmaz, V., Yildiz, A., 2024. Pathological and molecular investigations in the aborted Fetal Lamb due to the Poxvirus. J. Hell. Vet. Med. Soc. 75 (1), 7185–7190.
Bonventre, J.V., Yang, L., 2011. Cellular pathophysiology of ischemic acute kidney injury. J. Clin. Invest 121, 4210–4221. https://doi.org/10.1172/JCI45161.
Dolanbay, T., Makav, M., Gul, H.F., Karakurt, E., 2021. The effect of diclofenac sodium intoxication on the cardiovascular system in rats. Am. J. Emerg. Med. 46, 560–566. https://doi.org/10.1016/j.ajem.2020.11.022.
Yuan, J., Wang, D., Liu, Y., Chen, X., Zhang, H., Shen, F., Fu, J., 2018. Hydrogen-rich water attenuates oxidative stress in rats with traumatic brain injury via Nrf2 pathway. J. Surg. Res. 228, 238–246.
Erdem, A.; Gündogan, N.; … A.U.-N.D; 2000, undefined The Protective Effect of Taurine against Gentamicin-induced Acute Tubular Necrosis in Rats. academic.oup.comA Erdem, NÜ Gündogan, A Usubütün, K Kılınç, ŞR Erdem, A Kara, A BozkurtNephrology Dialysis Transplantation, 2000●academic.oup.com 2000.
Erseçkin, V., Mert, H., İrak, K., Yildirim, S., Mert, N., 2022. Nephroprotective effect of ferulic acid on gentamicin-induced nephrotoxicity in female rats. Drug Chem. Toxicol. 45, 663–669. https://doi.org/10.1080/01480545.2020.1759620.
Fielding, C.; McLoughlin, R.; … L.M.-T journal of; 2008, undefined IL-6 Regulates Neutrophil Trafficking during Acute Inflammation via STAT3. journals.aai.orgCA Fielding, RM McLoughlin, L McLeod, CS Colmont, M Najdovska, D Grail, M ErnstThe journal of immunology, 2008●journals.aai.org 2008, doi:10.4049/jimmunol.181.3.2189.
Gelen, V., Yildirim, S., Şengül, E., Çinar, A., Çelebi, F., Küçükkalem, M., Gök, M., 2022. Naringin attenuates oxidative stress, inflammation, apoptosis, and oxidative DNA damage in acrylamide-induced nephrotoxicity in rats. Asian Pac. J. Trop. Biomed. 12, 223–232. https://doi.org/10.4103/2221-1691.343390.
Gunay, M., Mertoğlu, C., 2019. Increase of endocan, a new marker for inflammation and endothelial dysfunction, in acute kidney injury. Northern Clin. Istanb. 6 (2).
Hodiamont, C.J., van den Broek, A.K., de Vroom, S.L., Prins, J.M., Mathôt, R.A.A., van Hest, R.M., 2022. Clinical pharmacokinetics of gentamicin in various patient populations and consequences for optimal dosing for gram-negative infections: an updated review. Clin. Pharmacokinet. 61 (8), 1075–1094. https://doi.org/10.1007/S40262-022-01143-0.
Kandemir, F.M., Ozkaraca, M., Yildirim, B.A., Hanedan, B., Kirbas, A., Kilic, K., Aktas, E., Benzer, F., 2015. Rutin attenuates gentamicin-induced renal damage by reducing oxidative stress, inflammation, apoptosis, and autophagy in rats. Ren. Fail. 37 (3), 518–525.
Katary, M., Salahuddin, A., 2017. Ameliorative effect of gossypin against gentamicin-induced nephrotoxicity in rats. Life Sci. 176, 75–81. https://doi.org/10.1016/J.LFS.2017.03.009.
Kayabaş, M., Şahin, L., Makav, M., Alwazeer, D., Aras, L., Yiğit, S., LeBaron, T.W., 2023. Protective effect of hydrogen-rich saline on spinal cord damage in rats. Pharmaceuticals 16, 527.
Köktürk, M., Yıldırım, S., Eser, G., Bulut, M., Alwazeer, D., 2022. Hydrogen-rich water alleviates the nickel-induced toxic responses (inflammatory responses, oxidative stress, DNA damage) and ameliorates cocoon production in earthworm. Biol. Trace Elem. Res. 1–11.
Kovalčíková, A.G., Pavlov, K., Lipták, R., Hladová, M., Renczés, E., Boor, P., Podracká, Ľ., Šebeková, K., Hodosy, J., Tóthová, Ľ., et al., 2020. Dynamics of salivary markers of kidney functions in acute and chronic kidney diseases, 2020, *10*, 1–12 Sci. Rep. 10 (1). https://doi.org/10.1038/s41598-020-78209-1.
Kuru, M., Makav, M., Kuru, B.B., Bektaşoğlu, F., Demir, M.C., Bulut, M., Alwazeer, D., 2024. Hydrogen-rich water supplementation improves metabolic profile during peripartum period in Gurcu goats and enhances the health and survival of kids. Res. Vet. Sci. 171, 105208.
Laorodphun, P., Cherngwelling, R., Panya, A., Arjinajarn, P., 2022. Curcumin protects rats against gentamicin-induced nephrotoxicity by amelioration of oxidative stress, endoplasmic reticulum stress and apoptosis. Pharm. Biol. 60, 491–500. https://doi.org/10.1080/13880209.2022.2037663.
Liu, Y.F., Zhang, Z., Pan, X.L., Xing, G.L., Zhang, Y., Liu, Z.S., Tu, S.H., 2021. The chronic kidney disease and acute kidney injury involvement in COVID-19 pandemic: a systematic review and meta-analysis. PLoS One 16, e0244779. https://doi.org/10.1371/JOURNAL.PONE.0244779.
Mahmoud, A.M., Abd El-Ghafar, O.A.M., Alzoghaibi, M.A., Hassanein, E.H.M., 2021. Agomelatine prevents gentamicin nephrotoxicity by attenuating oxidative stress and TLR-4 signaling, and upregulating PPARγ and SIRT1. Life Sci. 278, 119600. https://doi.org/10.1016/J.LFS.2021.119600.
Makav, M., Dolanbay, T., Gül, H.F., Karakurt, E., 2021. Determinate of ECG, oxidative stress, and angiogenesis in APAPInduced toxicity in rats. Kafkas Univ. Vet. Fak. Derg. https://doi.org/10.9775/kvfd.2021.25733.
Makav, M., Kuru, M., Aras, Ş.Y., Sarı, E.K., Bulut, M., Alwazeer, D., 2023. The effect of hydrogen-rich water on letrozole-induced polycystic ovary syndrome in rats. Reprod. Biomed. Online 47, 103332. https://doi.org/10.1016/J.RBMO.2023.103332.
Hashim, M.; … S.A.-C.-A. of T.; 2020, undefined Determination the Effect of Ciprofloxacin and Gentamicin Alone and in Combination to Induce Nephrotoxicity in Experimental Rats. academia.eduMU Hashim, SHA Al-Cekal, AJ AL-KhamasAnnals of Tropical Medicine & Public Health, 2020●academia.edu.
Makav, M., Ölmez, M., Eroğlu, H.A., Şahin, T., 2021. The effect of turkish coffee on performance, biochemical, and physiological parameters in rabbits. Harran Üniversitesi Vet. Fak. ültesi Derg. 10, 36–42. https://doi.org/10.31196/HUVFD.832486.
MAKAV, M., KURU, M., 2021. Determination of TNF-beta marker in intrauterine E. coli induced endometritis model in rats. Eurasia J. Toxicol. 3, 16–20. https://doi.org/10.51262/EJTOX.893215.
Mohammed, M.A., Aboulhoda, B.E., Mahmoud, R.H., 2019. Vitamin D Attenuates Gentamicin-Induced Acute Renal Damage via Prevention of Oxidative Stress and DNA Damage. Hum. Exp. Toxicol. 38, 321–335. https://doi.org/10.1177/0960327118812166/ASSET/IMAGES/LARGE/10.1177_0960327118812166-FIG8.JPEG.
Muñoz-Felix, J.M., Oujo, B., Lopez-Novoa, J.M., 2014. The role of endoglin in kidney fibrosis. Expert Rev. Mol. Med 16, e18. https://doi.org/10.1017/ERM.2014.20.
Nedwin, G.E., Naylor, S.L., Sakaguchi, A.Y., Smith, D., Jarrett-Nedwin, J., Pennica, D., Goeddel, D.V., Gray, P.W., 1985. Human lymphotoxin and tumor necrosis factor genes: structure, homology and chromosomal localization. Nucleic Acids Res 13, 6361–6373. https://doi.org/10.1093/NAR/13.17.6361.
Ohsawa, I., Ishikawa, M., Takahashi, K., Watanabe, M., Nishimaki, K., Yamagata, K., Katsura, K., Katayama, Y., Asoh, S., Ohta, S., 2007. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat. Med 13, 688.
Ohta, S., 2012. Molecular hydrogen is a novel antioxidant to efficiently reduce oxidative stress with potential for the improvement of mitochondrial diseases. Biochim. Biophys. Acta (BBA) Gen. Subj. 1820 (5), 586–594.
Ölmez, M., Şahin, T., Makav, M., Karadağoğlu, Ö., 2020. Effect of resveratrol supplemented to japanese quail (Coturnix coturnix Japonica) rations on performance and some biochemical parameters. Kafkas Univ. Vet. Fak. Derg. 26, 807–812. https://doi.org/10.9775/kvfd.2020.24542.
Paterson, D.L., Robson, J.M.B., Wagener, M.M., 1998. Risk factors for toxicity in elderly patients given aminoglycosides once daily. J. Gen. Intern Med 13, 735–739. https://doi.org/10.1046/J.1525-1497.1998.00224.X/METRICS.
Sahu, B.D., Kuncha, M., Sindhura, G.J., Sistla, R., 2013. Hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress, inflammation and DNA damage. Phytomedicine 20, 453–460. https://doi.org/10.1016/j.phymed.2012.12.001.
Şengül, E., Gelen, V., Gedikli, S., Özkanlar, S., Gür, C., Çelebi, F., Çınar, A., 2017. The protective effect of quercetin on cyclophosphamide-induced lung toxicity in rats. Biomed. Pharmacother. 92, 303–307. https://doi.org/10.1016/J.BIOPHA.2017.05.047.
Stenvinkel, P., Ketteler, M., Johnson, R.J., Lindholm, B., Pecoits-Filho, R., Riella, M., Heimbürger, O., Cederholm, T., Girndt, M., 2005. IL-10, IL-6, and TNF-α: central factors in the altered cytokine network of uremia—the good, the bad, and the ugly. Kidney Int 67, 1216–1233. https://doi.org/10.1111/J.1523-1755.2005.00200.X.
Wang, H., Li, J., Gai, Z., Kullak-Ublick, G.A., Liu, Z., 2017. TNF-α deficiency prevents renal inflammation and oxidative stress in obese mice. Kidney Blood Press Res 42, 416–427. https://doi.org/10.1159/000478869.
Xie, Y., Song, G., 2024. The clinical use of hydrogen as a medical treatment. Mol. Hydrog. Health Dis. 93–111.
Yıldız, K., Makav, M., Adalı, Y., Bulut, M., 2022. Therapeutic effects of boric acid in a septic arthritis model induced by escherichia coli in rats. Biol. Trace Elem. Res 200, 4762–4770. https://doi.org/10.1007/s12011-021-03065-7.
Yilmaz, M.I., Siriopol, D., Saglam, M., Kurt, Y.G., Unal, H.U., Eyileten, T., Gok, M., Cetinkaya, H., Oguz, Y., Sari, S., et al., 2014. Plasma endocan levels associate with inflammation, vascular abnormalities, cardiovascular events, and survival in chronic kidney disease. Kidney Int 86, 1213–1220. https://doi.org/10.1038/KI.2014.227.
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. Am. J. Obstet. Gynecol. 135, 372–376. https://doi.org/10.1016/0002-9378(79)90708-7.
Council, N. R. (2011). Guide fo the Care and Use of Laboratory Animals: Eight Edition. The National Academies Press, *Washington, DC.[Google Scholar]*.