



Anti-inflammatory and Antioxidant Effects of Ketotifen Against Gentamicin-Induced Hepatotoxicity Through NF- κ B Pathway Suppression

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Abstract

Background: Gentamicin (GEN) is one of the most effective aminoglycoside antibiotics, and its repeated use leads to impaired liver function. The antioxidant, anti-inflammatory, and anti-apoptotic properties of ketotifen (KTF) as a first-generation antihistamine have been proven in various studies.

Objectives: The present study aimed to evaluate the hepatoprotective effects of KTF against GEN-induced toxicity in mice, focusing on inflammation, oxidative stress, and NF- κ B modulation.

Methods: Thirty-seven male NMRI mice were randomly divided into 5 groups ($n = 6 - 10$), including: Control (normal saline 80 mL/kg, i.p, $n = 6$), GEN (80 mg/kg, i.p, $n = 10$), KTF (3 mg/kg, i.p, $n = 7$), and groups treated with GEN 80 mg/kg + KTF 2 mg/kg ($n = 7$) and GEN 80 mg/kg + KTF 3 mg/kg ($n = 7$). On day 8 of the study, mice were anesthetized, and their liver tissue was removed. Oxidative stress and inflammation factors, NF- κ B protein expression were evaluated, and histological examinations were conducted.

Results: Ketotifen resulted in significant reductions in the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), thiobarbituric acid-reactive substances (TBARS), tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6), interleukin 1 β (IL-1 β), and NF- κ B expression, and significant increases in total thiol and the activities of antioxidant enzymes catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) compared to the GEN group ($P < 0.001$). Histopathology results confirmed this finding.

Conclusions: The results of this study showed that KTF can significantly improve GEN-induced liver injury in mice. The hepatoprotective mechanism of KTF is through inhibiting inflammation, reducing oxidative stress, and modulating the NF- κ B signaling pathway. This research can provide a new approach to the treatment of liver injury and help identify new drugs.

Keywords: Ketotifen, Gentamicin, Hepatotoxicity, Inflammation, NF-Kb Signaling Pathway

1. Background

The liver is a vital organ in the body that performs important functions such as metabolism, digestion, and detoxification. Hepatotoxicity is one of the side effects

of some drugs, which has led to the withdrawal of some of them from the market (1). The occurrence of various metabolic processes in the liver, the metabolism of substances and drugs, and sometimes their conversion into active metabolites, increases the susceptibility of

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the liver to damage. Liver damage includes various problems such as fatty liver, cirrhosis, and vascular disorders, which may appear due to a decrease in detoxification capacity. Free radicals can damage liver cells and lead to dysfunction of this vital organ (2).

Gentamicin (GEN) is a broad-spectrum aminoglycoside antibiotic that is commonly used to combat infections caused by gram-negative bacteria *Pseudomonas* and *Proteus* (3). Gentamicin has the lowest drug resistance rate among antibiotics, which is why it is an effective option in the treatment of infections (4). Gentamicin enters the cell through the phospholipids of the cell membrane wall and accumulates in lysosomes, the endoplasmic reticulum, and the Golgi apparatus, causing phospholipidosis and ultimately toxicity (5-7). Lysosomal phospholipidosis is considered a marker of potential GEN toxicity, indicating the importance of monitoring cellular function in response to phospholipidosis (8). Lysosomal phospholipidosis negatively affects the phosphatidylinositol signaling pathway, which can lead to impaired cellular functions (9), decreased phospholipase activity (10, 11), and specific inhibition of calcium-dependent enzymes (6).

Despite the antibacterial benefits of GEN, its excessive consumption can lead to oxidative stress in liver cells, fibrosis, and liver failure (12, 13). This liver injury has also been shown in previous studies (14-16). Elimination of etiological factors can have a positive effect on the treatment of fibrosis at different stages, helping to improve tissue and reduce complications (17). Oxidative stress refers to the excess of free radicals in the body over antioxidants, which can cause cellular damage. Hydroxyl radicals and superoxide anions are among the most important free radicals that can be generated in the process of oxidative stress (18). Antioxidant supplements are important in the treatment of liver disorders due to their ability to reduce cell damage and inflammation.

Ketotifen (KTF) is a first-generation antihistamine and mast cell stabilizer used to treat allergy symptoms and some respiratory disorders. Ketotifen prevents allergic reactions and reduces allergy symptoms by inhibiting the release of allergic mediators from mast cells and blocking histamine receptors. Mast cells are innate immune cells found in body tissues, especially in the vascular bed and epithelial surfaces. They play an important role in inflammatory and immune responses by migrating into vascular tissues and secreting mediators such as cytokines, chemokines, and lectins (19). Mast cells play a critical role in the immune system and are specifically increased in response to drug-induced liver injury and hepatotoxicity (20). Mast cells

can reduce serum tumor necrosis factor alpha (TNF- α) levels by secreting specific substances, which leads to reduced damage in mouse models. Studies have shown that certain extracts can affect TNF- α levels and mast cell function (21). In various models of liver injury, the antioxidant, anti-inflammatory, and anti-apoptotic properties of KTF have been demonstrated (22).

2. Objectives

Considering the antioxidant and anti-inflammatory effects of KTF as well as the side effects of GEN, in this study, the anti-inflammatory and antioxidant effects of KTF against GEN-induced hepatotoxicity through suppression of the NF- κ B pathway were investigated.

3. Methods

3.1. Chemicals

Ketotifen (purity $\geq 99\%$) and GEN (purity $\geq 99\%$) were purchased from Sigma-Aldrich (USA). To measure alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels, kits from Pars Azmoun Company (Iran) were used. The SOD, CAT, and glutathione peroxidase (GPx) assay kits were obtained from ZellBio (Germany). To measure the levels of TNF- α , interleukin 6 (IL-6), and interleukin 1 β (IL-1 β), kits from Biotech (China) and the ELISA method were used.

3.2. Animals

In this study, male NMRI mice weighing approximately 25 ± 2 g were used. The maintenance conditions were $25 \pm 2^\circ\text{C}$, with 12 hours of light and 12 hours of darkness, and access to sufficient water and food. The ethical principles of work and care of laboratory animals were in accordance with the AJUMS protocol (IR.AJUMS.AEC.1403.057).

3.3. Experimental Design

Thirty-seven male NMRI mice were randomly divided into 5 groups ($n = 6 - 10$), including: Control (normal saline 80 mL/kg, i.p, $n = 6$), GEN (80 mg/kg, i.p, $n = 10$), KTF (3 mg/kg, i.p, $n = 7$), and groups treated with GEN 80 mg/kg + KTF 2 mg/kg ($n = 7$) and GEN 80 mg/kg + KTF 3 mg/kg ($n = 7$). G-Power software was used to determine sample size and power. Doses and duration of KTF (23) and GEN (24) were selected based on recent studies. On the eighth day and in fasting conditions, the animals were anesthetized, blood was taken from the heart, and serum was separated and frozen at -20°C for

biochemical experiments. The liver was removed and one portion was preserved in formalin for histopathological analysis, while the remaining portion was kept at -70°C for the assessment of oxidative and inflammatory markers, as well as NF-κB protein expression.

3.4. Serum Biochemical Markers Determination

Alanine aminotransferase, AST, and ALP levels were measured using a Pars Azmoun kit (Iran) and an autoanalyzer and expressed as U/L.

3.5. Preparation of Homogenate and Determining Protein Content

Liver tissue was homogenized in phosphate buffer with pH = 7.4 and concentration of 10% v/w, using a homogenizer and centrifuged, then supernatant was preserved at -70°C for measurement of oxidative stress and inflammation factors and NF-κB protein expression. The Bradford method was employed to determine the protein concentration in the supernatant (25).

3.6. Total Thiol Determination

The total thiol level measurement was determined using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (26). Accordingly, 40 µl supernatant was added to 40 µl DTNB (0.01 M), and absorption was read at 412 nm and expressed as nmol/mg protein.

3.7. Thiobarbituric Acid Reactive Substances Determination

The Kei method was used to measure TBARS level (27). Accordingly, 0.5 mL supernatant was mixed with 0.5 mL 10% trichloroacetic acid, and centrifuged. Then, 0.5 mL 0.67% thiobarbituric acid was added to the supernatant and incubated. The absorption was read at 532 nm and expressed as nmol/mg protein.

3.8. Antioxidant Enzymes Activity Determination

Antioxidant enzymes activity of SOD, CAT, and GPx were measured with ZellBio (Germany) kits according to the instructions of the manufacturer and reported as U/mg protein.

3.9. Pro-inflammatory Markers Determination

Evaluation of the inflammatory marker's levels TNF-α, IL-6, and IL-1β were performed using commercial enzyme-linked immunosorbent assay (ELISA) kits from Biotech Co (China) according to the instructions of the manufacturer. The absorbances were measured at 450 nm and reported as pg/mg protein.

3.10. Western Blotting

This method was used to measure NF-κB protein expression. Liver samples were homogenized and centrifuged. An amount of 20 µg protein for each group was electrophoresed on a sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. The membranes were blocked with 5% skim milk for 2 hours at 4°C. The membranes were probed with the primary antibodies of NF-κB (1:500, Cat No: 8242) and GAPDH (1:500, Cat No: 5174) for 1 hour. Membranes were treated with mouse anti-rabbit IgG-HRP (1:1000, Cat No: sc-2357) for 1 hour. An electrochemiluminescence (ECL) kit (USA) was used to visualize protein bands, and Image Lab™ Touch software Bio-Rad (USA) was used to quantify the bands. GAPDH was used as a loading control in Western blotting.

3.11. Histopathological Examination

After fixation of liver tissue in 10% formalin solution and embedding in paraffin, sections of 4 - 6 µm were prepared and stained with hematoxylin and eosin (28). For each animal, three microscopy slides were examined for assessment of histopathological changes such as pycnotic and karyolytic changes, lobules disorganized, dilation of the sinusoids, and cellular turgescence. Tissue damage and changes were graded into 4 categories: Normal (0), mild (1), moderate (2), or severe (3), and the averages were considered. If the tissue sections of the liver had a normal tissue structure, a clear and regular lobular arrangement, and regular cell cords and sinusoids had a normal size, and focal and local inflammation was not seen, we gave grade 0. Grade 1 was considered for pycnotic and karyolytic changes, lobules disorganized, dilation of the sinusoids, and cellular turgescence to a small degree. If these changes were moderate in the liver parenchyma, grade 2 was applied, and if lobular disarray and pycnotic and karyolytic changes, dilation of the sinusoids, and cellular turgescence were very high, grade 3 was applied.

3.12. Statistical Analysis

Statistical analysis was performed by GraphPad Prism 9.5.1, and the normality of the data was confirmed using the Kolmogorov-Smirnov test. The mean ± SEM of the data in each group was calculated, and finally, the results were analyzed using one-way ANOVA and Tukey's post hoc test for multiple comparisons. Values of $P < 0.05$ were deemed statistically significant. A schematic diagram of the study design is illustrated in Figure 1.

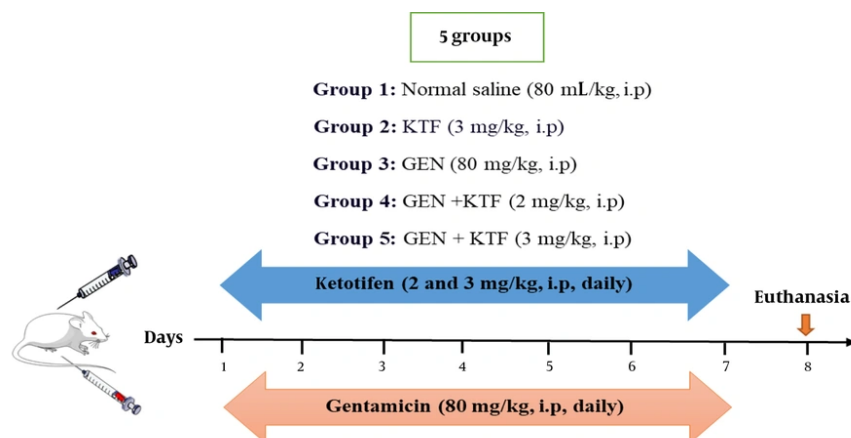


Figure 1. Schematic diagram of the study design for investigating the effect of the ketotifen (KTF) on the hepatotoxicity induced by gentamicin (GEN) in mice. Mice were treated with KTF (2 and 3 mg/kg, i.p, daily), after administration of GEN (80 mg/kg, i.p, daily) for 7 days. On the eighth day of the study, mice were anesthetized.

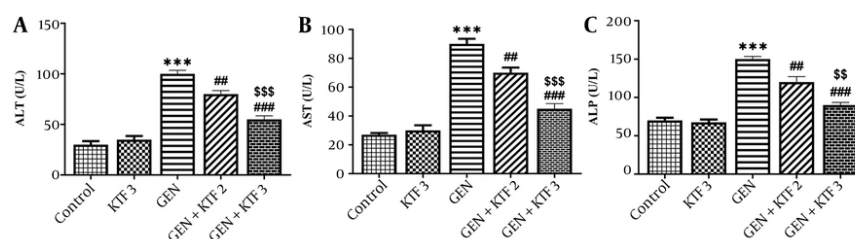


Figure 2. Effect of ketotifen (KTF) on the A, alanine aminotransferase (ALT); B, aspartate aminotransferase (AST); and C, alkaline phosphatase (ALP) in the gentamicin (GEN) hepatotoxicity in mice. Data are expressed as mean $\pm$ SEM (n = 6-10). *** P < 0.001 compared to the control group; ## P < 0.01 and ### P < 0.001 compared to the KTF-treated groups with GEN group; \$\$ P < 0.01 and \$\$\$ P < 0.001 comparison of KTF 2 and KTF 3 groups.

Accordingly, mice were treated with KTF (2 and 3 mg/kg, i.p, daily) for 7 days after administration of GEN (80 mg/kg, i.p, daily). On the eighth day of the study, mice were anesthetized.

4. Results

4.1. Effect of Ketotifen on Liver Function Biomarkers

The levels of liver biomarkers are shown in Figure 2. The ALT, AST, and ALP levels were elevated in the GEN group in comparison to the control group (P < 0.001). Ketotifen treatment reduced these biomarkers in comparison to the GEN group. The group receiving 3 mg/kg KTF showed significantly more effectiveness than the 2 mg/kg KTF group (P < 0.001).

4.2. Effect of Ketotifen on Oxidative Stress Markers

The results related to the antioxidant levels of total thiol, SOD, CAT, and GPx, as well as the oxidant factor TBARS in the liver, are shown in Figure 3. The level of total thiol and the activity of SOD, CAT, and GPx were decreased in the GEN group in comparison to the control group (P < 0.001). Ketotifen improved these markers and increased total thiol level and SOD, CAT, and GPx activities in the treated groups. Moreover, this increase was significant in the 3 mg/kg KTF group. The TBARS level was significantly increased in the GEN group in comparison to the control group (P < 0.001). Ketotifen significantly reduced the TBARS level in the treated groups. The group receiving 3 mg/kg KTF showed

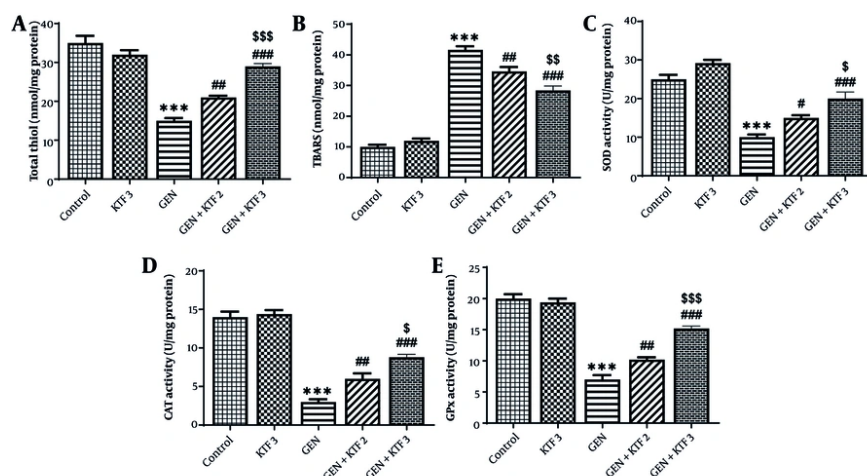


Figure 3. Effect of ketotifen (KTF) on the oxidative stress markers of A, total thiol; B, thiobarbituric acid reactive substances (TBARS); C, superoxide dismutase (SOD) activity; D, catalase (CAT) activity; and E, glutathione peroxidase (GPx) activity in gentamicin (GEN) hepatotoxicity in mice. Data are expressed as mean $\pm$ SEM ($n = 6 - 10$). *** $P < 0.001$ compared to control group; # $P < 0.05$, ## $P < 0.01$, and ### $P < 0.001$ compared to the KTF-treated groups with GEN group; \$ $P < 0.05$, \$\$ $P < 0.01$, and \$\$\$ $P < 0.001$ comparison of KTF 2 and KTF 3 groups.

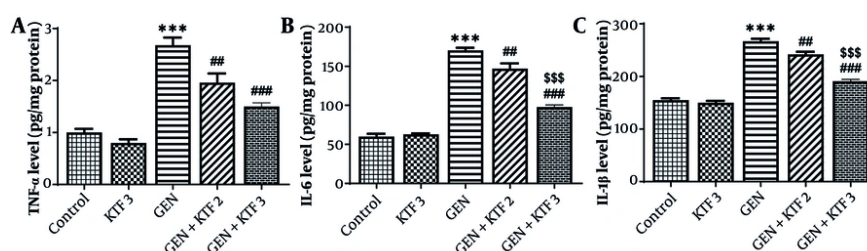


Figure 4. Effect of ketotifen (KTF) on the pro-inflammatory markers of A, tumor necrosis factor- α (TNF- α); B, interleukin 6 (IL-6); and C, interleukin 1 β (IL-1 β) in the gentamicin (GEN) hepatotoxicity in mice. Data are expressed as mean $\pm$ SEM ($n = 6 - 10$). *** $P < 0.001$ compared to control group; ## $P < 0.01$ and ### $P < 0.001$ compared to KTF-treated groups with GEN group; \$\$\$ $P < 0.001$ comparison of KTF 2 and KTF 3 groups.

significantly more effectiveness than the 2 mg/kg KTF group ($P < 0.01$).

4.3. Effect of Ketotifen on Inflammatory Markers

The results related to the inflammatory markers TNF- α , IL-6, and IL-1 β are shown in Figure 4. The levels of these markers after GEN administration were increased in comparison to the control group ($P < 0.001$). Ketotifen improved these markers, causing a significant reduction in their levels in the treated groups in comparison to the GEN group. The group receiving 3 mg/kg KTF showed significantly more effectiveness than the 2 mg/kg KTF group ($P < 0.001$).

4.4. Effect of Ketotifen on NF- κ B Protein Expression

The results related to NF- κ B protein expression are shown in Figure 5. The NF- κ B protein expression in the GEN group increased in comparison to the control group ($P < 0.001$). In the KTF treatment groups, the expression of this protein was significantly reduced in comparison to the GEN group ($P < 0.01$). The effect of 3 mg/kg KTF on reducing NF- κ B protein expression was greater than that of 2 mg/kg KTF ($P < 0.001$).

4.5. Effect of Ketotifen on Histopathological Evaluation

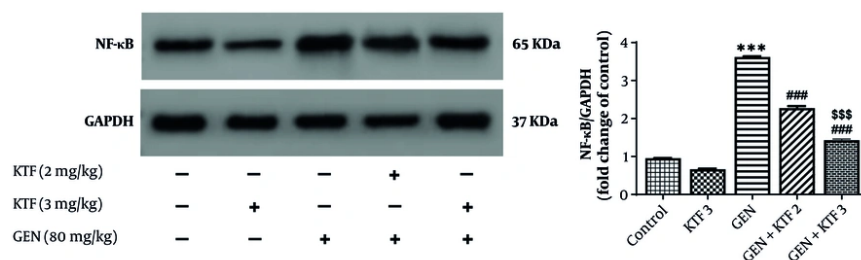


Figure 5. Effect of ketotifen (KTF) on the NF- κ B protein expression in the gentamicin (GEN) hepatotoxicity in mice. Data are expressed as mean $\pm$ SEM (n = 6 - 10). *** P < 0.001 compared to the control group; ### P < 0.001 compared to KTF-treated groups with GEN group; \$\$\$ P < 0.001 comparison of KTF 2 and KTF 3 groups.

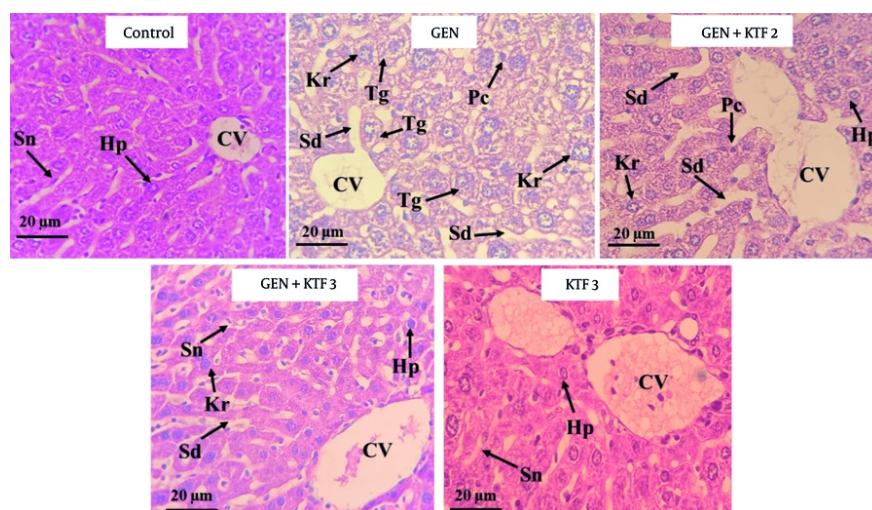


Figure 6. Effect of ketotifen (KTF) on histopathological evaluation of H&E-stained liver tissue. Control group, gentamicin (GEN) group, GEN+KTF 2 group, GEN+KTF 3 group, and KTF 3 group; Magnification: 400x (Abbreviations: Hp, hepatocyte; Sn, sinusoid; CV, centrilobular vein; Tg, turgescence; Sd, sinusoidal dilation; Kr, karyorrhexis; Pc, pycnosis)

The results of H&E staining of liver tissue are shown in Figure 6, and the quantitative results of tissue disorganization are shown in Figure 7. Accordingly, the liver structure was normal in the control and 3 mg/kg KTF groups. In the GEN group, the liver tissue was severely damaged in comparison to the control group, with the liver lobules largely disorganized and the port spaces not identifiable. In this group, the liver cells were turgescence, and the nuclei of some cells were pycnotic and karyolytic. Gentamicin injection in the liver parenchyma induced dilation of the sinusoid spaces. However, in groups treated with KTF, sinusoidal widening, lobular disorganization, pyknotic and

karyolytic nuclei, and cellular turgescence were significantly reduced in comparison to the GEN group.

5. Discussion

Some antibiotics, such as GEN, may decrease liver function and cause hepatotoxicity with chronic use. Early diagnosis and treatment methods can help to clear the liver and prevent further risks. Various models of liver injury have shown that KTF has antioxidant and anti-inflammatory properties (22). Considering the antioxidant and anti-inflammatory effects of KTF as well as the side effects of GEN, in this study, the anti-inflammatory and antioxidant effects of KTF against GEN-induced hepatotoxicity via suppression of the NF-

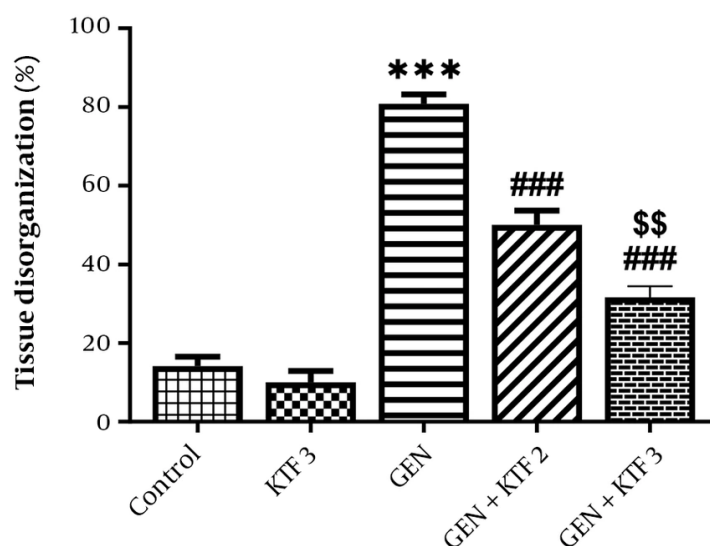


Figure 7. Quantitative results of the effect of Ketotifen (KTF) on the liver tissue irregularity as mean $\pm$ standard deviation. *** $P < 0.001$ compared to control group; ### $P < 0.001$ compared to KTF-treated groups with gentamicin (GEN) group; \$\$ $P < 0.01$ comparison of KTF 2 and KTF 3 groups.

κ B pathway were investigated. Bulboaca et al. showed in their study that administration of GEN at a dose of 80 mg/kg for 7 days led to an increase in liver enzyme levels (24). In this study, GEN injection for 7 days led to increased levels of liver enzymes ALT, AST, and ALP in serum and ultimately liver damage. To modulate GEN hepatotoxicity, KTF was administered at doses of 2 mg/kg and 3 mg/kg, which resulted in a decrease in liver enzyme levels. However, KTF at a dose of 3 mg/kg showed more protective results. Abdelzaher et al. investigated the protective effect of KTF, a mast cell stabilizer, on cyclophosphamide-induced hepatotoxicity. Based on the results, KTF significantly reduced liver enzymes and improved liver dysfunction (29). The results of this study were in line with our results.

Gentamicin can increase the production of reactive oxygen species (ROS) such as superoxide and hydrogen peroxide. Increased ROS production can overwhelm the antioxidant defense system of the liver and lead to oxidative stress. Oxidative stress can damage cellular components such as lipids, proteins, and DNA and contribute to liver dysfunction and toxicity. According to previous studies, the hepatotoxicity of GEN is partly attributed to the production of free radicals and increased oxidative stress. In other words, GEN can increase the level of liver enzymes and reduce the activity of antioxidant enzymes (30). Subsequently, the

antioxidant defense mechanisms of cells are activated to neutralize free radicals (31). Ketotifen can help reduce oxidative stress and maintain liver health due to its antioxidant effects. In the present study, the total thiol level and the activities of SOD, CAT, and GPx enzymes in hepatocytes were decreased after GEN administration, indicating the use of these enzymes to neutralize free radicals. In the study by Khaksari et al., liver and kidney malondialdehyde increased in the GEN group and glutathione decreased (15). Ketotifen reduced GEN-induced oxidative stress by increasing antioxidant enzyme activities and total thiol levels. Ketotifen 3 mg/kg was significantly more effective than KTF 2 mg/kg. Gentamicin administration also increased TBARS level in liver tissue. Ketotifen treatment significantly reduced TBARS level. Refaie et al. investigated the effect of KTF on methotrexate toxicity and the mechanisms involved. In this study, KTF significantly increased the level of reduced glutathione and the activities of antioxidant enzymes CAT and SOD, and decreased the level of malondialdehyde, which was consistent with our study (32).

According to the study of Ali et al., GEN led to the exacerbation of inflammatory reactions and the increase of proinflammatory cytokines in liver tissue through the increase of NF- κ B-p65 and TNF- α expression along with the decrease of IL-10 (14). In this study, GEN

led to the increase of inflammatory factors IL-6, IL-1 β , and TNF- α in liver tissue. In contrast, KTF improved these changes with its anti-inflammatory effects. In the study of Chen et al., KTF at a dose of 0.09 mg/kg was able to significantly reduce the levels of IL-6, TNF- α , and MDA and increase the activity of SOD (33).

The NF- κ B regulatory network is a complex system that controls the activity of NF- κ B transcription factors, which play a critical role in various cellular processes, including inflammation, immune responses, cell survival, and proliferation. This network involves multiple regulatory layers, including interaction with inhibitory proteins (I κ Bs), activation of I κ B kinases (IKKs), and various post-translational modifications. In addition, NF- κ B activity is influenced by other signaling pathways and regulatory molecules, forming complex feedback loops and crosstalk with other cellular networks (34). The NF- κ B transcription factor is a key regulator of inflammatory responses induced by the proinflammatory cytokines IL-6 and TNF- α (35). NF- κ B plays an important role in mediating the cellular response to these cytokines by influencing the expression of genes involved in inflammation. NF- κ B activation is one of the hallmarks of inflammatory liver diseases (36). Research has shown that NF- κ B is activated through both “focal” and “non-focal” pathways. I κ B kinase acts as a key regulator of NF- κ B activity and is involved in liver and GEN-induced inflammation and apoptosis. Gentamicin hepatotoxicity can be reduced by regulating NF- κ B and related immune pathways (37).

In this study, GEN exposure resulted in increased expression of NF- κ B protein in liver tissue. This suggested that GEN could activate an inflammatory pathway or affect cell survival mechanisms in the liver. Increased NF- κ B expression could be a direct result of GEN exposure or a secondary effect mediated by other factors (14). In this study, KTF treatment significantly reduced NF- κ B protein expression in liver tissue. The reduction in protein expression in the 3 mg/kg KTF treatment group was greater than that in the 2 mg/kg KTF group. These results indicate the protective effect of KTF on the liver in inflammatory processes and acquired stress. Zhang et al. showed that KTF significantly improved liver inflammation by reducing oxidative stress and inhibiting NF- κ B associated with the accumulation of mast cells and macrophages (38).

In H&E staining of liver tissue, distinct histological changes were observed in the GEN group compared to the control group, including sinusoidal dilation, asymmetry in hepatic lobules, pyknic and karyolytic nuclei, and cell protrusion. Ketotifen treatment

significantly reduced these histological changes. The results of this study showed that GEN administration led to an increase in the oxidant factor TBARS, a decrease in the antioxidant factors CAT, GPx, and SOD, an increase in the inflammatory markers IL-6 and TNF- α , and an increase in the expression of the NF- κ B protein in the liver. Ketotifen significantly improved these parameters due to its antioxidant and anti-inflammatory properties and mast cell membrane stabilization and showed protective effects against GEN-induced damage.

N-acetylcysteine (NAC) and silymarin are hepatoprotective agents that have been suggested in various studies. The results of the effect of NAC on GEN-induced hepatoprotection indicate that this substance acts by restoring glutathione levels and combating free radicals (39). Silymarin, derived from milk thistle, has shown potential in mitigating GEN-induced liver and kidney damage due to its antioxidant and anti-inflammatory properties. Studies suggest that silymarin can help protect against GEN-induced nephrotoxicity and hepatotoxicity by reducing oxidative stress and inflammation (40). Both KTF and silymarin have been investigated for their potential to mitigate GEN-induced hepatotoxicity in animal models, but with different mechanisms and varying degrees of effectiveness.

In-depth studies of the biochemical pathways of GEN using multi-omics approaches in *in vitro* hepatotoxicity studies, such as transcriptomics, proteomics, and metabolomics, provide a more comprehensive understanding of the effects of xenobiotics at the cellular level. By simultaneously analyzing the levels of multiple types of biomolecules, such as genes, proteins, and metabolites, insights can be gained into the complex interactions between GEN and KTF and the underlying biological processes that are affected. However, further research is needed to mitigate the impact of existing technical limitations that hinder reproducibility, standardization, comparison of data across laboratories, and compatibility with *in vitro* models.

5.1. Conclusions

The results of this study showed that KTF can significantly improve GEN-induced liver injury in mice. The hepatoprotective mechanism of KTF is through inhibiting inflammation, reducing oxidative stress, and modulating the NF- κ B signaling pathway. The present study can provide a new approach to the treatment of liver injury and help identify new drugs.

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Footnotes

Authors' Contribution: Study concept and design: M. S. B., A. H. R., and E. S.; Analysis and interpretation of data: M. S. B., N. Gh., and F. F.; Drafting of the manuscript: M. S. B., A. H. R., M. M., E. S., and F. F.; Critical revision of the manuscript for important intellectual content: M. S. B., M. M., E. S., F. F., and N. Gh.; Statistical analysis: M. S. B., A. H. R., F. F., N. Gh., and M. M.

Conflict of Interests Statement: The authors declare no conflict of interests.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

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