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RESEARCH ARTICLE

Efficacy of Resatorvid and Alpha-Lipoic Acid in Ameliorating Gentamicin-Induced Liver Injury: Insights from Rat Model Study

Durmus Hatipoglu[1*], Mehmet Burak Ates[2], Aysegul Bulut[2], Buse Gezgic[1], and Burak Dik[3]

[1]Department of Physiology, Faculty of Veterinary Medicine, Selcuk University, Konya, Türkiye
[2]Department of Pathology, Faculty of Veterinary Medicine, Selcuk University, Konya, Türkiye
[3]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Selcuk University, Konya, Türkiye
*Corresponding author: drhatip@selcuk.edu.tr

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ABSTRACT

The aim of our study was to investigate the potential effects of alpha-lipoic acid (ALA) and resatorvid (RES) on gentamicin-induced hepatotoxicity. Thirty-four rats were divided into the following groups: Healthy control (n=6), Gentamicin (GNT, 80 mg/kg, n=7), GNT + Sham (10% hydroalcoholic solution, n=7), GNT + RES (5 mg/kg, n=7), and GNT + ALA (100 mg/kg, n=7). In the GNT group, a statistically significant increase in ALT levels and a significant decrease in total protein (TP) and albumin (TA) levels were observed compared to the control group. On the other hand, a significant increase in NRF-2, NF-κB, NR4A2 and CAS-3 expression was detected in the GNT group as compared to the control group. Simultaneous administration of RES or ALA with GNT led to a significant decrease in ALT levels compared to the GNT group. In addition, these treatments caused a significant increase in TP and TA levels. RES administration caused a significant decrease in gene expression compared to the GNT group. On the other hand, simultaneous ALA treatment with GNT caused a significant decrease in NRF-2, CAS-3 and NF-κB expression and partially reduced NR4A2 expression. These findings draw attention to the potential of RES or ALA to alleviate gentamicin-induced hepatotoxicity and to their increased efficacy in the treatment of infections.

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INTRODUCTION

Multiple investigations on drug-induced liver injury (DILI) have shown that antibiotics are the predominant class of drugs responsible for liver injury (Chalasani *et al.,* 2015; Björnsson, 2017). Since the 1940s, gentamicin (GNT), an aminoglycoside antibiotic, has been widely used primarily in the treatment of Gram-negative bacterial infections (Chaves and Tadi, 2022). The use of GNT is known to cause nephrotoxicity and ototoxicity (Khaliq *et al.,* 2015; Dik *et al.,* 2024). While it is acknowledged that GNT can cause hepatotoxicity, understanding the precise mechanisms behind this remains limited due to the complex pathophysiology involved (Arjinajarn *et al.,* 2017). Nevertheless, several researchers have documented that GNT induces the generation of reactive oxygen species (ROS), which leads to the deterioration of many macromolecules, including proteins, lipids, and DNA (Feyzi *et al.,* 2020; Hamdy *et al.,* 2024)

The Nrf2 and NF-κB pathways collaboratively control cellular responses to oxidative stress and inflammation (Gao *et al.,* 2022). Studying gene expression in primary liver cells exposed to different chemicals showed that blocking NF-κB could make liver cells more vulnerable to cell death signals sent by naturally occurring toxic pro-inflammatory cytokines, which could lead to DILI. This aligns with a strong stress response controlled by Nrf2, which might play a substantial role in controlling DILI (Herpers *et al.,* 2016). Moreover, drug-induced liver damage can cause the death of hepatocytes and the development of inflammation, finally resulting in the destruction of the hepatocytes (Iorga *et al.,* 2017; Gungor *et al.,* 2023). However, the function of hepatic apoptosis and its pathophysiological significance in liver damage exhibit significant discrepancies (Wang, 2014). Nuclear receptor subfamily 4 group A (NR4A) receptors are activated by many signals that are unique to different types of cells, such as growth factors, signals related to cell death (apoptotic signals), messages related to inflammation, and hormones (Pei *et al.,* 2006). However, the precise role of NR4As in regulating the expression of genes associated with DILI remains unknown. As a result, additional

research is needed to understand the role of NR4As (especially NR4A2) in the course of DILI and their impact on liver metabolism. In any case, GNT-induced hepatotoxicity is marked by changes in the liver's histology and function, such as serum biochemical indices/parameters (Khaksari *et al.,* 2021).

Today, there is a significant rise in antibiotic resistance, leading to increased mortality rates from infections caused by organ failure due to antibiotic use and the lack of effective new antibacterial drugs. Several additional therapeutic strategies have been created to enhance the effectiveness of therapy while minimizing the adverse effects of hepatotoxic medications, particularly GNT. Lowering oxidative stress in the liver and controlling inflammation to reduce drug-induced tissue damage are two essential first steps in creating future therapy options. Resatorvid (RES) is a notable TLR4 inhibitor that effectively hinders the inflammatory response (Zandi *et al.,* 2020). According to reports, the RES application reduces the production of NF-κB by blocking TLR4 signaling. It also suppresses the activity of MAP kinase/AP-1 and NF-κB and, as well as the expression of some inflammatory cytokines (Zandi *et al.,* 2020). Alpha-lipoic acid (ALA), an antioxidant that helps remove oxidative radicals and oppose the negative effects of oxidative stress, has been shown in a number of experimental studies to successfully lessen tissue damage brought on by hepatotoxic drugs in the pathogenesis of DILI (Abdulrazzaq *et al.,* 2019; Pınar *et al.,* 2020).

Studies conducted to examine the effectiveness of treatment techniques for DILI caused by hepatotoxic drugs such as GNT and to improve treatment protocols by minimizing drug side effects are of great importance in this context. The current research aims to optimize the use of GNT in medical treatment by reducing its adverse effects, as its hepatotoxic activity has gained importance in recent years. Upon reviewing the existing literature, no research has been identified that investigates the impact of RES and ALA on liver damage generated by GNT. Thus, the objective of this work was to examine the possible safeguarding properties of RES and ALA against GNT-induced liver damage.

MATERIALS AND METHODS

Establishment of the Experimental Groups: The Ethics Committee of Selcuk University Animal Experiments approved the research protocol (Approval No. 2024-082). This study utilized 34 Wistar Albino rats, aged 8–12 weeks and weighing around 250 grams each. Prior to commencing the investigation, the overall health condition of the animals was assessed. During the study, the rats were placed in plastic cages, which allowed them to walk freely. The rats were subjected to a 12-hour light and 12-hour dark cycle, with an ambient temperature of 22±2°C and humidity of 55±5%. The rats' body weights were measured and sorted into five groups based on comparable average body weights. After a seven-day acclimation phase, we set up an eight-day experimental period and grouped the animals as shown in Table 1.

The animal experiments concluded on the 8th day. Blood samples were taken from the hearts while under ketamine/xylazine anesthesia (90/10mg/kg, i.p.). Euthanasia was accomplished by decapitation. Liver tissues were kept at -80°C for molecular studies and fixed in 10% formaldehyde for histological examination following saline rinse.

Biochemical Analyses: During the investigation, blood samples were collected into gel tubes and then subjected to centrifugation at a force of 1600 g, resulting in the separation of the serum. The blood and urine samples were collected and analyzed for biochemical parameters including albumin (mg/dl), ALT (U/L), AST (U/L), and total protein (mg/dl) using an autoanalyzer (Abbott Architect c8000 Chemistry Analyzer) (Gumus and Baltaci, 2023).

Quantitative Real Time-PCR (qRT-PCR) and RNA Isolation: Gene expression analysis was carried out using the methods described in a previous work (Ogaly *et al.,* 2015). Total RNA was extracted from snap-frozen brain and sciatic nerve samples with the SanPrep Column microRNA Miniprep Kit (BIO BASIC) according to the manufacturer's instructions. To synthesise cDNA templates, 2 μg of isolated RNA aliquot was reverse transcribed with the OneScript® Plus cDNA Synthesis Kit (ABM) using oligo dT primer. The Nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2), Nuclear Factor kappa B (NF-κB), Nuclear receptor subfamily 4 group A member 2 (NR4A2) and Caspase-3 mRNA levels were measured by fluorescent real-time quantitative PCR (RT-qPCR) using BlasTaq™ 2X qPCR MasterMix (ABM) and the primer sequence summarised in Table 2. PCR reactions were performed in triplicate on a LightCycler® 96 System (Roche, SWITZERLAND). The thermal programme consisted of an initial denaturation at 95 °C for 3 min followed by 40 cycles of denaturation (95 °C/10 s), annealing (56 °C/15 s) and extension (72 °C/10 s). The relative expression of target genes was calculated according to the comparative cycle threshold (2-ΔΔCT) method after normalising target mRNA Ct values to those of ß-actinin as an internal control. A melting curve analysis was performed to ensure that only one PCR product was obtained.

Histopathological Examination: During necropsy, liver samples obtained were fixed in 10% formaldehyde solution for 24 hours. To remove formaldehyde solution from the tissues, the tissues were washed in running water for 24

Table 1: Experimental design

Group	Administration Details
Healthy control (n:6)	Oral administration of 1 ml physiological saline daily.
Gentamicin (GNT, n:7)	Intraperitoneal injection of Gentamicin, 80 mg/kg, for 8 days.
Gentamicin+Sham (GNT+Sham, n:7)	Intraperitoneal injection of Gentamicin, 80 mg/kg and oral administration of 10% hydroalcoholic solution, for 8 days.
Gentamicin + Alpha lipoic acid (GNT+ALA, n:7)	Intraperitoneal injection of Gentamicin, 80 mg/kg, for 8 days, and oral gavage of ALA, 100 mg/kg.
Gentamicin + Resatorvid (GNT+RES, n:7)	Intraperitoneal injection of Gentamicin, 80 mg/kg, and Resatorvid, 5 mg/kg, for 8 days.

Table 2: Sequences of primes

Gene	Primer sequence (5′–3′)
NRF2	F:5′TAGATGACCATGAGTCGCTT3′ R: 5′CTGTAACTCGGGAATGGAAA3′
Caspase3	F: 5′GGACAGCAGTTACAAAATGGATTA3′ R: 5′CGGCAGGCCTGAATGATGAAG3′
NR4A2	F: 5′CCACGTCGACTCCAATCC3′ R: 5′TAGTCAGGGTTTGCCTGGAA3′
NF-κB	F: 5′AGCACCAAGACCGAAGCAA3′ R: 5′TCTCCCGTAACCGCGTAGTC3′
B-Actin	F: 5′CGTGAAAAGATGACCCAGAT3′ R: 5′ATTGCCGATAGTGATGACCT3′

hours. Subsequently, the tissues were processed in a routine tissue processor (Leica TP1050) and embedded in paraffin. Sections of 5 microns thickness were obtained from the paraffin blocks using a microtome (Leica RM2120). Routine Hematoxylin-Eosin staining procedure was applied to the obtained sections, and the sections were examined under a light microscope. In the microscopic examination, parameters such as hydropic degeneration, steatosis, necrosis, bile duct proliferation, dissociation, congestion, megalocytosis, and mononuclear cell infiltration were scored on a scale ranging from 0 to 4 (Bulut *et al.,* 2023).

Histopathological lesions scoring: The grading method was as follows: a score of 0 indicated the absence of a lesion, a score of 1 indicated a mild severity, a score of 2 indicated a moderate severity, a score of 3 indicated a severe severity, and a score of 4 indicated a very severe severity (Bulut *et al.,* 2023).

Statistical Analyses: The data were presented as the mean value ± standard deviation. The data was analyzed using One Way ANOVA in SPSS 25.0, and then a posthoc Duncan test was conducted. The statistical significance was established with a p-value of less than 0.05. Graphs produced using GraphPad Prism 9.5.1.

RESULTS

The Influence of RES and ALA on Biochemical Parameters: Figure 1 presents the impact of Resatorvid (5 mg/kg, intraperitoneal) and Alpha-lipoic acid (100 mg/kg, oral) therapies on certain biochemical parameters in the serum of rats with induced hepatotoxicity caused by GNT application. Following the GNT application, it was observed that the ALT level showed a statistically significant rise compared to the control group. Additionally, the levels of total protein (TP) and albumin (TA) showed a statistically significant drop ($p<0.05$). The study found that the administration of RES and ALA, in combination with GNT, significantly reduced the ALT level compared to the group that received just gentamicin. Additionally, this reduction brought the ALT level closer to that of the control group. The statistical analysis showed a significant difference ($p<0.05$). However, it was shown that administering both RES and ALA along with GNT simultaneously significantly enhanced TP and TA levels compared to the group that received only gentamicin ($p<0.05$).

Response of Gene Expressions to Treatment: Figure 2 shows how resatorvid (5 mg/kg, intraperitoneal) and alpha-lipoic acid (100 mg/kg, oral) therapy affected gene expressions in the livers of rats with GNT-induced hepatotoxicity. The GNT application significantly increased the expression of NF-κB, NRF-2, CAS-3, and NR4A2 compared to the control group ($p<0.05$). RES treatment considerably lowered the expression of these genes compared to the GNT group. ALA treatment significantly decreased the expressions of NRF-2, CAS-3, and NF-κB compared to the GNT group ($p<0.05$) and slightly reduced the expression of NR4A2.

Histopathological changes: The liver assessment findings are succinctly shown in Table 3 and Figure 3. The overall liver lesion score, which rose due to the administration of gentamicin, notably declined with the use of ALA and/or REV ($p<0.05$) (Figure 3). Mild hydropic degeneration and dissociation were seen in the cytoplasm of hepatocytes in the control group. In the gentamicin group, severe hydropic degeneration was observed in the hepatocyte cytoplasm compared to the control group. The severity of steatosis was found to be increased relative to the control group. Dilations in the sinusoids and localized atrophic hepatocytes were observed as a result. Dissociation was notable in the hepatic cords. Hepatocytes with pyknotic nuclei and eosinophilic cytoplasm (single cell necrosis) were present. Mononuclear cell infiltrations were detected around the central veins. Congestion was observed in both the central veins and portal veins.

An increase in Kupffer cell activation was noted. A statistically significant increase in the total liver lesion score was determined compared to the control group ($p<0.05$, Table 3). In the GNT+Sham group, there was a statistically significant increase in hydropic degeneration, steatosis, necrosis, dissociation, mononuclear cell infiltration, and the total liver lesion score compared to the control group ($p<0.05$, Table 3). In the GNT+ALA group, the severity of hydropic degeneration in hepatocyte cytoplasm was found to be reduced compared to the gentamicin group ($p<0.05$). Dilations in the sinusoids were observed. The severity of dissociation was noted to be milder than that in the gentamicin group. Occasional single-cell necrosis was observed. An improvement in the total lesion score was noted compared to the gentamicin

Table 3: Histopathological scores

Groups	Hydropic degeneration	Steatosis	Necrosis	Bile duct proliferation	Dissociation	Congestion	Megalocytosis	Mononuclear cell infiltration	Total Score
Control	0.50±0.16^{a}	0.40±0.10^{a}	0.50±0.15^{a}	0.70±0.25^{a}	0.60±0.19^{a}	0.70±0.12^{a}	0.50±0.16^{ab}	0.20±0.12^{a}	4.00±0.67^{a}
Gentamicin	2.20±0.25^{c}	1.90±0.29^{c}	2.60±0.18^{c}	2.20±0.25^{b}	2.60±0.19^{c}	1.00±0.15^{a}	1.60±0.19^{c}	1.50±0.22^{c}	14.90±0.79^{d}
Gentamicin + Sham	1.90±0.19^{c}	1.90±0.29^{c}	2.80±0.12^{c}	1.00±0.16^{a}	2.70±0.20^{c}	0.60±0.10^{a}	0.50±0.16^{ab}	1.60±0.19^{c}	13.00±0.76^{cd}
Gentamicin + ALA	1.30±0.12^{b}	0.10±0.18^{b}	1.50±0.22^{b}	0.80±0.12^{a}	1.50±0.22^{b}	1.00±0.22^{a}	2.00±0.16^{c}	1.20±0.25^{bc}	10.20±0.51^{c}
Gentamicin + REV	1.00±0.16^{ab}	0.60±0.10^{ab}	0.70±0.12^{a}	1.10±0.19^{a}	0.80±0.12^{a}	0.80±0.12^{a}	0.40±0.10^{a}	1.60±0.19^{c}	6.80±0.25^{b}
Gentamicin + ALA+ REV	0.70±0.12^{a}	0.70±0.12^{ab}	0.90±0.18^{a}	1.20±0.20^{a}	0.90±0.18^{a}	0.80±0.12^{a}	0.90±0.10^{b}	0.70±0.12^{ab}	6.80±0.80^{b}

*Different letters in the same column are statistically significant according to one-way ANOVA and post-hoc Duncan test.

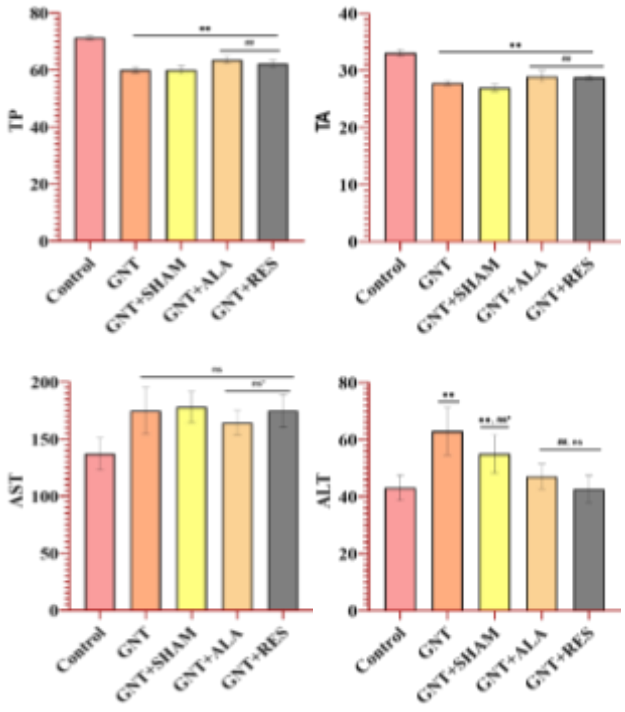

Fig. 1: The findings regarding the serum TA, TP, AST, and ALT levels of RES or ALA due to GNT-induced liver damage are as follows: The '**' symbol represents the control group, whereas '##' indicates a statistically significant difference relative to the GNT group ($p<0.05$). The 'ns' sign represents the comparison with the control group, whereas 'ns*' indicates a statistically insignificant difference with the GNT group ($p>0.05$).

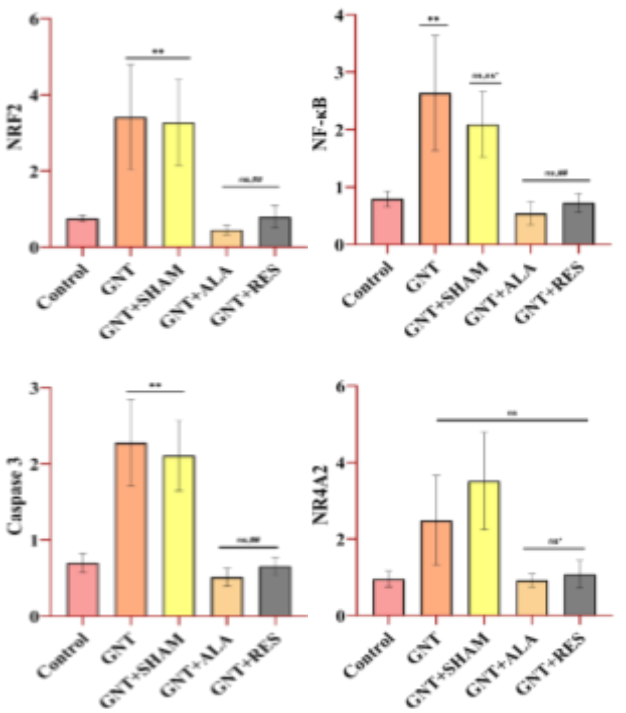

Fig. 2: The gene expressions of Nrf2, NF-κB, NR4A2, and Caspase 3 mRNA transcripts in the livers of rats fed GNT, RES, and ALA showed the following results: A '**' sign indicates a difference from the control group, whereas a '##' shows a statistically significant difference from the GNT group ($p<0.05$). The 'ns' sign compares to the control group, whereas 'ns*' denotes a statistically insignificant difference from the GNT group ($p>0.05$).

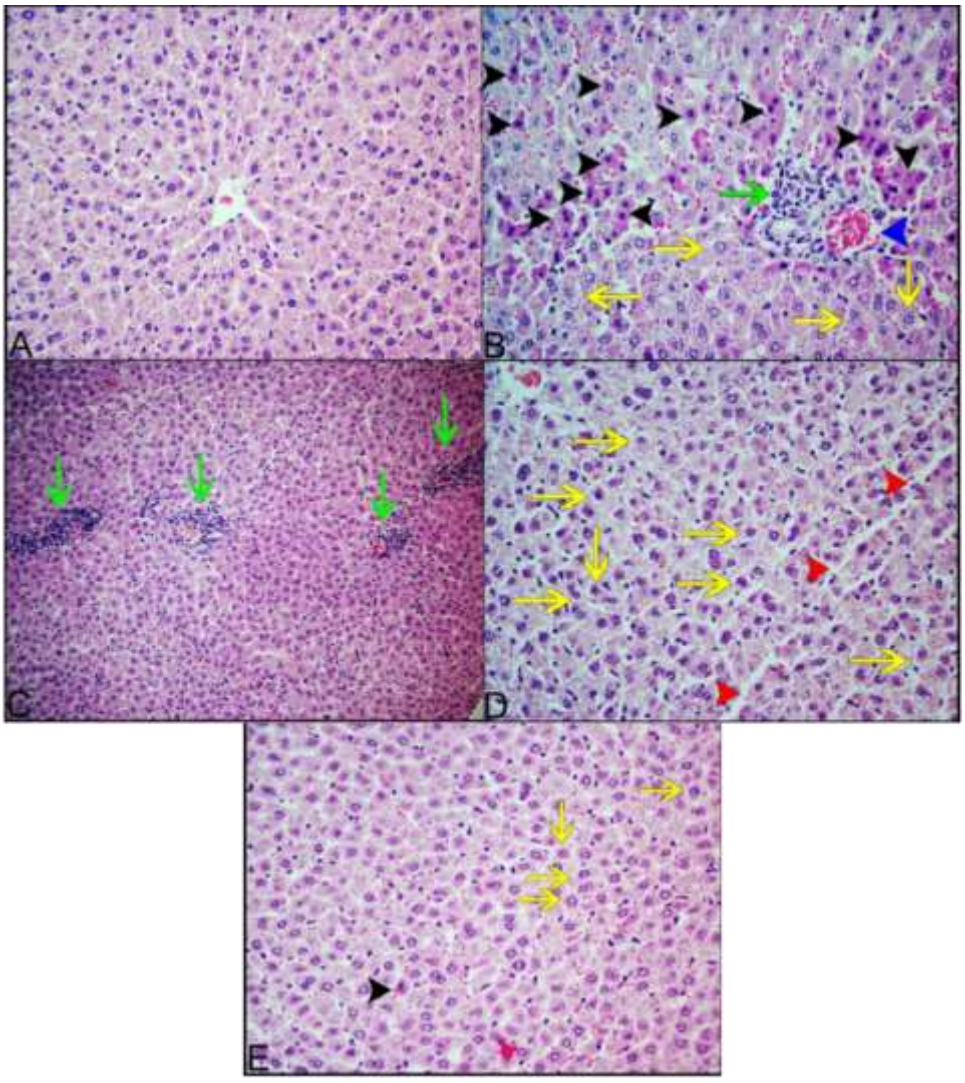

Fig. 3: A. Control group. 40X, HXE. **B.** Gentamicin group. Hepatocytes with pyknotic nuclei and eosinophilic cytoplasm (black arrowheads), mononuclear cell infiltration in the portal area (green arrow), hydropic degeneration of hepatocytes (yellow arrows), and congestion in the portal vein (blue arrowhead), 40X, HXE. **C.** Gentamicin+Sham group. Mononuclear cell infiltrates (green arrows), 20X, HXE. **D.** Gentamicin+ALA group. Hydropic degeneration of hepatocytes (yellow arrows) and the enlargement of sinusoids (red arrowheads), 40X, HXE. **E.** Gentamicin+REV group. Hepatocytes with hydropic degeneration (yellow arrows) and necrosis (black arrow), 40X, HXE.

group ($p<0.05$, Table 3). In the GNT+REV group, there was no significant difference in hydropic degeneration, steatosis, necrosis, bile duct proliferation, dissociation, congestion, megalocytosis, and mononuclear cell infiltration compared to the control group ($p>0.05$). However, a slight increase in the total score was observed ($p<0.05$, Table 3).

DISCUSSION

When evaluating DILI epidemiologically, it was reported to be frequently observed in the community. (Leise *et al.,* 2014). Antibiotics are the drug class most frequently associated with idiosyncratic DILI (Katarey and Verma, 2016). Recently, deleterious effects of GNT on liver tissue have been reported (Ali *et al.,* 2020). Therefore, this study was designed to evaluate the protective capacity and ameliorative effects of ALA and REV against GNT-induced hepatotoxicity. Our findings indicate that GNT-induced hepatotoxicity is associated with elevated oxidative stress, inflammation, and apoptosis. Co-administration of ALA or RES with GNT has demonstrated improvements in liver function

In our study, administration at a dose of 80 mg/kg body weight for 8 consecutive days caused hepatotoxicity in rats. The increase in serum ALT levels and histopathological changes indicates that the synthetic and detoxifying functions of the liver were severely affected. These results were correlated with the observed decreases in TP and albumin levels of liver injury (Galaly *et al.,* 2014), indicating that the protein synthesis and detoxification processes of the liver were affected. Significant histological alterations in the liver tissues of the GNT group, including atrophic hepatocytes, severe degeneration, and enhanced Kupffer cell activation, are also consistent with these findings (Mirazi *et al.,* 2021; Mohamadi Yarijani *et al.,* 2021). Under normal circumstances, Nrf2, which is constantly degraded in the cytosol by the ubiquitin-proteasome in a Keap1-dependent manner (Taguchi *et al.,* 2011), is arrested and translocated to the nucleus, where it activates phase II detoxifying enzymes (Itoh *et al.,* 2004). This research reveals that GNT administration triggers oxidative stress by causing an increase in Nrf2 mRNA transcript levels in the liver. Oxidative stress can cause activation of NF-κB. In the state of stimulation, the NF-κB complex (NF-κB-IκB) dissociates and is transported to the nucleus. This process enhances the transcriptional activation of target genes such as IL-6, iNOS, TNF-α, and COX-2, which are pivotal in cellular damage, oxidative stress reactions, and inflammation prevention (Umesalma and Sudhandiran, 2010). This study shows that GNT also triggers an inflammatory response by increasing NF-κB mRNA transcript levels. On the other hand, apoptosis plays an important role in many liver diseases and drug-induced hepatotoxicity, as well as in the inflammatory process (Wang, 2014). To sustain cell hemostasis in pathological circumstances such as ischemia and some forms of cancer, NR4A2 gene expression is enhanced (Watanabe *et al.,* 2015). Increased oxidative stress, inflammation, and free radicals activate the ROS/NF-κB/NR4A2 signaling pathway, leading to apoptosis (Shi *et al.,* 2017). In this study, liver apoptosis was demonstrated by an increase in caspase-3 mRNA transcript level in GNT. These findings are consistent with recent studies showing that the caspase-dependent apoptotic signaling pathway is associated with gentamicin-induced apoptotic liver injury (Arjinajarn *et al.,* 2017). Increased levels of NF-κB, NR4A2, and caspase-3 in hepatic tissue due to GNT administration are consistent with other findings in the literature, but the hepatic Nrf2 level differs from other studies (Ali *et al.,* 2021). This may be because the activation status of Nrf2, known for its role in activating numerous cytoprotective genes effective against chemical and oxidative stresses, is closely associated with cellular survival processes. The activation state of Nrf2 may be inhibited when toxicity is severe, and cell death may be negatively affected through signaling (Copple *et al.,* 2019). Increased caspase-3 levels in the liver and histopathological changes such as degeneration, atrophy, and single-cell necrosis in the tissue may be responsible for the increase in hepatic Nrf2 in this study. Indeed, Ghanim *et al.* (2021) investigated the factors affecting Nrf2 expression during DILI with different doses of acetaminophen and reported that the expression of genes related to cell survival was regulated in mild acetaminophen hepatotoxicity, but as the dose of acetaminophen increased, hemorrhagic necrosis and impaired genetic transcription of both Nrf2 (18-fold increase) and some other genes were evident (Ghanim *et al.,* 2021).

Based on the above discussion, pathogenic mechanisms such as oxidative stress, inflammation, and apoptosis are identified as important routes of toxicity caused by GNT, not only in the liver but also in other organs of the body. Therefore, focusing on these harmful processes might be seen as a successful approach to reduce and/or address the toxicity caused by GNT.

In response to GNT-induced DILI, the simultaneous administration of RES and ALA treatments significantly improved liver function. A significant reduction in ALT levels and an increase in total protein and albumin levels marked this improvement. The results show that both ALA and RES protect the structural integrity of liver cells by preventing the release of liver enzymes into the bloodstream due to GNT. The research we conducted provides evidence that RES has a positive effect on several forms of liver damage, such as liver ischemia/reperfusion injury and acute and acute-chronic liver failure induced by endotoxins (Shao *et al.,* 2016, Engelmann *et al.,* 2020). Moreover, studies have indicated that RES has hepatoprotective properties against fulminant hepatitis produced by lipolysaccharide/D-galactose (LPS/D-GalN) in mice (Wang *et al.,* 2021). Consistent with our results, previous studies have shown that ALA enhances the stability and functionality of liver cells by increasing the levels of antioxidant defense systems and lowering lipid peroxidation. This is achieved through its ability to scavenge reactive oxygen species, thereby minimizing oxidative damage to the liver (Sadek *et al.,* 2018). ALA may exhibit this function via binding to metals, neutralizing specific radicals, and restoring other antioxidants such as ascorbate, vitamin E, and glutathione (GSH) (Sadek *et al.,* 2018).

Furthermore, our results showed that ALA and RES suppressed elevated levels of inflammatory and apoptotic biomarkers, which was manifested by significantly

decreased hepatic tissue mRNA transcript levels of NF-κB, Nrf2, NR4A2, and caspase-3. This improvement is similar to Tanaka *et al.* (2015) who found that ALA has antioxidant, anti-inflammatory, and anti-apoptotic effects (Tanaka *et al.,* 2015). It is suggested that ALA provides more effective protection against oxidative damage compared to the endogenously reduced or oxidized glutathione system. The main reason behind this is that ALA has a high redox potential (-320 mV) (El-Mancy *et al.,* 2022). ALA has been found to inhibit the activation of hepatic stellate cells and provide protection against liver damage caused by several toxins, particularly in the liver (Deore *et al.,* 2021). The findings provide evidence for the efficacy of ALA as a possible therapeutic or preventive agent against damage caused by oxidative stress. This hypothesis is strengthened by the significant reduction in inflammation and hepatopathologic outcomes on histopathologic examination. Indeed, Pınar *et al.* (2020) suggested that ALA treatment against cisplatin-induced liver injury leads to increased hepatocyte mitosis, decreased perivenular sinusoidal dilatation, and parenchymal inflammation, thereby initiating hepatocyte regeneration. Additionally, Khalaf *et al.* (2017) reported that ALA alleviated histopathological lesions in liver tissue induced by copper nanoparticle (CNP) hepatotoxicity. They observed regression in granular degeneration of hepatocytes and a decrease in the number of necrotic and apoptotic hepatocytes limited to single cells, indicating the effectiveness of ALA against CNP-induced hepatotoxicity.

On the other hand, the improvement due to RES administration may be related to RES being a selective inhibitor of the TLR4 signaling pathway. Because TLR4 is a member of the pattern recognition receptor (PRR), a family that activates the hepatic tissue inflammatory response (Miller *et al.,* 2005). GNT hepatotoxicity may have been shaped by TLR4-induced production of proinflammatory cytokines and RES treatment may have shown efficacy by acting as an attractive target for GNT-induced liver injury by blocking TLR4. Indeed, Samarpita *et al.* (2023) reported that RES inhibited the mobilization of NF-κB to the nucleus in lipopolysaccharide (LPS)-stimulated fibroblast-like synoviocytes in vitro (Samarpita *et al.,* 2020). On the other hand, blockade of TLR4 by RES has been reported to reduce hepatic transaminase serum levels and hepatocyte damage observed in animal models after acetaminophen-induced liver injury and bile duct ligation (Salama *et al.,* 2015). Liu *et al.* (2022) reported that RES alleviated varying degrees of high collagen and fibrous tissue hyperplasia and inflammatory cell infiltration induced by CCl4-induced liver damage. It has also been reported to provide a significant modulation of liver histopathologic changes following I/R injury in a rat model (Yokoi *et al.,* 2018). This is confirmed by the histopathologic evaluation of the present study, which showed that the severity of GNT-induced liver injury was reduced by RES administration. Nevertheless, it is necessary to do more research to explore the impact of ALA and RES on the translocation of these factors to the nucleus.

Although GNT is an important antibiotic for both human and animal health, its harmful effects, such as ototoxicity, nephrotoxicity, and hepatotoxicity, limit the confidence interval. The current study found that the anti-inflammatory properties of RES and the antioxidant properties of ALA reduced the harmful effects of GNT on the liver. This highlights the potential for GNT to target and reduce hepatotoxic side effects in patients with liver failure. Focusing on pathways such as NF-κB, Nrf2, and NR4A2 to minimize the negative effects of GNT in the future may bring about very important results.

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Authors contributions. D.H. and B.D. formulated the experimental design, analyzed the data, and composed the initial and last iterations of the paper. B.D. and B.G. gathered and processed samples, while D.H., M.B.A., and A.B. conducted analyses on the raw data and verified all figures and tables. All writers collaborated in the process of editing and amending the early versions of the paper.

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