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Research Paper

Evaluating the Protective Role of Rutin Against Azithromycin-Induced Hepatorenal Histopathological Changes in Albino Rats

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ABSTRACT

Background: Azithromycin, a widely used macrolide antibiotic, is associated with dose-dependent hepatorenal toxicity primarily mediated by oxidative stress and inflammation. Rutin, a natural flavonoid, possesses potent antioxidant and anti-inflammatory properties that can mitigate organ damage. This study aimed to evaluate the potential of Rutin to ameliorate azithromycin-induced histopathological alterations in the livers and kidneys of albino rats.

Methods: Thirty-six adult male albino rats (180–200 g) were allocated into five groups: a control group (normal saline), groups receiving azithromycin (30 mg/kg) for 7 (T1) or 14 days (T2), and groups receiving concurrent azithromycin and rutin (50 mg/kg) for 7 (T3) or 14 days (T4). After the treatment period, liver and kidney tissues (one kidney per animal) were harvested for histopathological examination using hematoxylin and eosin (H&E) staining.

Results: Azithromycin administration induced a significant time-dependent histopathological damage. The T2 group (14-day azithromycin treatment) exhibited severe hepatic injury, including hepatocellular necrosis, sinusoidal dilatation, and inflammatory infiltration, as well as renal damage characterized by glomerular atrophy and tubular necrosis. Cotreatment with Rutin markedly attenuated these effects. The T4 group (14-day cotreatment) showed near-complete preservation of the hepatic architecture and renal histology, with minimal signs of inflammation and cellular damage.

Conclusion: Co-administration of Rutin confers significant protection against azithromycin-induced hepatorenal damage, attributed to its antioxidative and anti-inflammatory mechanisms. These findings highlight the potential of Rutin as an effective adjuvant therapy to minimize antibiotic-associated organ toxicity.

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Introduction

The global rise in bacterial infections has heightened reliance on broad-spectrum antibiotics. Among these, macrolides such as Azithromycin are particularly useful due to their favorable pharmacokinetic and immunomodulatory properties [1, 2]. Owing to its long half-life, extensive tissue penetration, and ease of dosing, Azithromycin has become the drug of first choice in various respiratory, gastrointestinal, cutaneous, and sexually transmitted infections [3, 4]. In addition, its central role in public health is evidenced by its inclusion in mass-administration campaigns for neglected tropical diseases (NTDs) such as trachoma and yaws [5]. This sets it apart from other antibiotics, such as doxycycline, and highlights its relevance to global health efforts.

The growing reliance on Azithromycin for both treatment and prevention has prompted scrutiny of its safety profile, particularly concerning hepatic and renal toxicity. The liver, the main site of metabolism via cytochrome P450 enzymes, and, to a lesser extent, organs such as the kidneys, which have a limited role in biliary-mediated excretion under normal circumstances, are particularly relevant organs [7]. However, long-term use or overdose may cause drug-induced liver injury (DILI) and acute kidney injury (AKI), which is mainly attributed to oxidative stress, mitochondrial dysfunction, inflammation, and cellular apoptosis [8, 9].

Oxidative stress is certainly a central mechanism in azithromycin-induced hepatorenal toxicity, that is, the pathological condition in which ROS generation exceeds the biological system's ability to eliminate and/or repair oxidative damage. The main event is mitochondrial damage, in which Azithromycin accumulates and impairs the electron transport chain, causing substantial electron leakage and an influx of superoxide radicals and other ROS [8, 10]. This ROS burst scavenges natural antioxidant defence molecules that would otherwise selectively and safely neutralize the detrimental effects of free radicals and minimize oxidative harm, resulting in widespread deleterious oxidation of cellular macromolecules, including lipid peroxidation in membranes, protein dysfunction, and DNA injury [11]. In addition, increased oxidative stress induces proinflammatory signaling cascades, such as the nuclear factor-kappa B (NF-κB) pathway. It aggravates injury by producing cytokines that drive apoptotic and necrotic cell death in hepatic and renal tissues [8, 9].

Furthermore, azithromycin treatment results in antibiotic-induced gut dysbiosis that may contribute to systemic inflammation and organ damage via the gut–liver–kidney axis [12]. Dysbiosis leads to increased intestinal mucosal permeability, increased translocation of microbial toxins, and increased proinflammatory cytokine production, which may contribute to worsening hepatic and renal stress [13]. This complex pathophysiology begs for the identification of therapeutic strategies that protect microbial homeostasis and organ tissue structure [14].

Natural antioxidants, in the form of polyphenolic compounds found in plants, have shown promising potential to minimize drug-induced organ toxicity [15]. Rutin, a bioactive flavonoid glycoside found in fruits, vegetables, and teas, has been reported to possess multiple pharmacological activities, including antioxidant, anti-inflammatory, and cytoprotective effects [16]. The protective effects of Rutin, including the regulation of crucial cellular signaling pathways such as nuclear factor erythroid 2-related factor 2 (Nrf2) and NF-κB, the suppression of proinflammatory mediator release, and the neutralization of reactive oxygen species, make cells resistant to oxidative stress [17, 18]. The hepatoprotective and nephroprotective effects of Rutin against different toxic insults have been demonstrated in preclinical studies. Various studies have shown the effectiveness of Rutin in protecting against acetaminophen-, carbon tetrachloride-, and ethanol-induced hepatotoxicity, and against cisplatin-, gentamicin-, and other nephrotoxic agent-induced nephrotoxicity [12, 18]. In the gut-liver-kidney axis, the compound's actions on the triangle organs suggest possible systemic therapeutic effects, making it an ideal candidate for alleviating antibiotic-induced organ toxicity [19].

Based on this concept, the present study aimed to assess histopathological changes in the liver and kidneys of azithromycin-treated rats and the ameliorative role of Rutin in these rats. The present study aimed to clarify how Azithromycin induces hepatorenal toxicity and to assess whether natural antioxidants can diminish antibiotic-induced organ injury, focusing specifically on histopathological assessment and total pharmacological analysis. In this study, the proposed protective role of natural antioxidants against antibiotic-induced organ toxicity was investigated.

Materials and Methods

Experimental Animals

This trial was carried out in the Animal House of the College of Education / University of AL-Qadisiyah. Thirty-six healthy adult male albino rats (12-14 weeks

old, weighing 180–200 g) were kept under standard laboratory conditions on a 12:12h light/dark regime and normal room temperature (22±2°C), at a relative humidity of 50-60%. During the experimental period, all animals had free access to a regular pellet diet and clean drinking water.

Experimental Design

Animals were randomly assigned to one of five groups:

1. Control Group (G): Twelve rats were equally divided into two subgroups (n=6 each) and administered 1 ml/day of ordinary water orally by gavage for 7 days (Control-7d) and 14 days (Control-14d).

2. T1 (Azithromycin 7 days): Azithromycin (30 mg/kg body weight) was administered orally to six rats for 7 days.

3. Azithromycin 14-day cycle group (T2): Six rats were administered a similar dose of Azithromycin for 14 days.

4. Azithromycin + Rutin (7 days) group (T3): Six rats were administered azithromycin (30 mg/kg) for 7 days, followed by rutin (50 mg/kg) for the next 7 days.

5. Rats were treated with Azithromycin and Rutin for 14 days (T4), and six SD rats were orally administered azithromycin and rutin (50 mg/kg) for 14 days.

All compounds were dissolved in distilled water and freshly prepared for dosing on the day of treatment, before oral gavage, at a volume of 1 ml.

Chemicals

Azithromycin was purchased from a local pharmaceutical supplier. Rutin was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animal Sacrifice and Tissue Collection

Twenty-four hours after the final treatment, the animals were anesthetized with intraperitoneal ketamine and xylazine. Liver and kidney tissues were immediately excised, rinsed in saline, and fixed in 10% neutral buffered formalin (prepared from 37-40% formaldehyde stock) for histological analysis.

Histological Processing

Fixed liver and kidney samples were processed for routine light microscopy. The tissues were dehydrated using a graded series of ethanol (70, 80, 90, and 100%; Merck KGaA, Darmstadt, Germany), cleared in xylene (Merck KGaA, Darmstadt, Germany), and embedded in paraffin wax (Merck KGaA, Darmstadt, Germany). Serial sections of 5 µm thickness were cut using a Rotary Microtome (RM 2125 RTS, Leica Biosystems, Germany). Sections were mounted on glass slides and stained with Hematoxylin and Eosin (H&E) following the standard protocol described by Bancroft et al. [20]. Hematoxylin and Eosin stain was also procured from Merck KGaA (Darmstadt, Germany).

Microscopy

The prepared slides were examined under a light microscope. Images were captured using a digital camera attached to the microscope at 100x and 400x magnifications to document histopathological findings in the liver and kidney tissues.

Results

Liver Histology (Figure 1): Control, T1, and T2 Groups

Histological examination of liver sections from the control group (Figure 1A) revealed a normal hepatic architecture with polygonal hepatocytes arranged in cords around the central vein and clear sinusoidal capillaries. In the T1 group (Figure 1B), which received Azithromycin for 7 days, mild alterations such as central vein dilation and initial signs of fatty degeneration were observed. More severe histological damage was observed in the T2 group (Figure 1C–D), which was treated with Azithromycin for 14 days. These include disrupted hepatic lobular organization, hepatocyte necrosis, Pyknosis, sinusoidal dilatation, and inflammatory infiltrations.

Liver Histology Post-Rutin Cotreatment (Figure 2): T3 and T4 Groups

Histology showed dose-dependent restoration of the liver architecture. The T3 group showed a more uniform distribution of hepatocytes, significantly less necrosis, and better-preserved central veins than the T2 group. In the T4 group (Figure 2D), rats co-treated with Rutin showed almost complete restoration of hepatic histology, as evidenced by minimal inflammatory foci and preserved sinusoidal structures.

Kidney Histology (Figure 3): Control, T2, and T3 Groups

The glomerular and tubular structures of the kidneys in the control group were normal (Figure 3A). In the T2 group (Figure 3C), in which Azithromycin

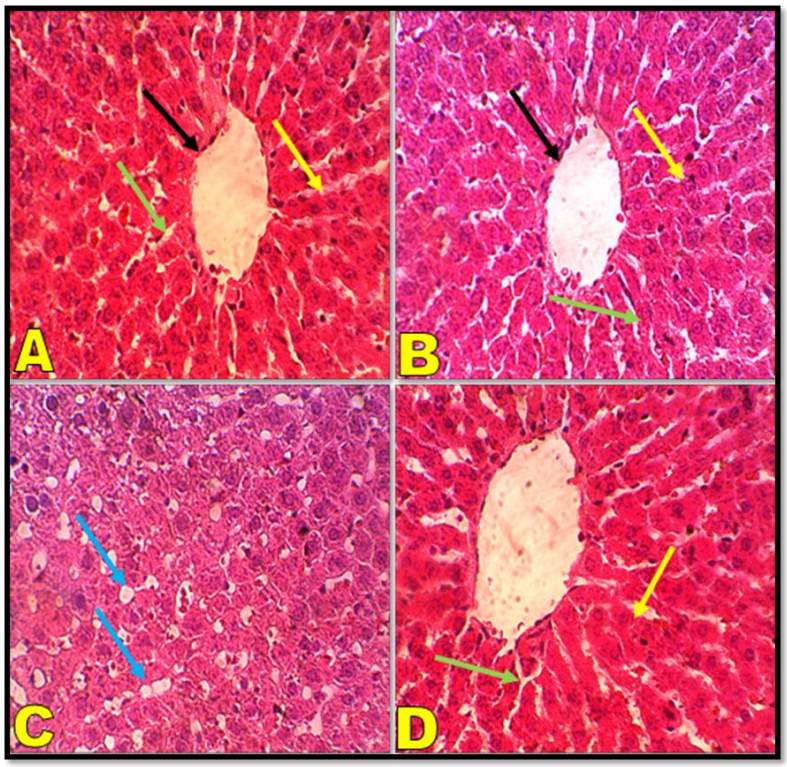

Figure 1. It shows the liver tissue of rats. Picture (A) shows the control group, demonstrating a normal arrangement of the hepatic cells (yellow arrow) around the central vein (black arrow) and sinusoidal capillaries (green arrow). Images (B)and (C) of the T1 group show a normal cellular arrangement pattern (yellow arrow), dilation of the central vein (black arrow), clear blood capillaries (green arrow), and fatty degeneration (blue arrow). (D) The T2 group shows loss of the normal hexagonal arrangement of hepatocytes (yellow arrow), a dilated central vein (black arrow), and blood capillaries (green arrow).

was simulated for 14 days, the kidney tissue showed glomerular atrophy, tubular necrosis, Bowman's capsule dilatation, and inflammatory cell infiltration. In contrast, the T3 group (Figure 3B), which received Azithromycin followed by Rutin for 7 days, demonstrated preservation of glomerular structure and less pronounced tubular damage.

Kidney Histology (Figure 4): T2 vs T4 Comparison

Figure 4A–C (T2 group) shows severe renal lesions, including glomerular shrinkage, interstitial hemorrhage, and necrotic tubules. Conversely, Figure 4D (T4 group) shows restored renal histoarchitecture, with normal glomeruli, intact proximal and distal tubules, and no hemorrhage or inflammatory cells.

Discussion

Histopathological examination revealed that Azithromycin induced severe architectural damage to liver cells, and Rutin clearly attenuated this effect. The lesions, including degenerating hepatocytes, congested sinusoids, and inflammatory cell infiltration, were consistent with previously reported azithromycin-induced hepatotoxicity in animal experiments [10]. The clinical presentation of azithromycin-induced hepatotoxicity is reflected in histopathological findings, which are mainly hepatocellular, associated with higher ALT levels, and generally begin 1 to 3 weeks after treatment onset [21].

The hepatotoxicity of Azithromycin, like that of other macrolides, is known to occur in a dose- and exposure-time-dependent manner, as supported by earlier studies [22, 23]. Mechanistic experiments indicated that cytochrome P450-catalysed metabolism of Azithromycin leads to the generation of reactive azithromycin intermediates, including species directly toxic to mitochondria. Moreover, the presence of immune cell infiltration at T2 suggests another possible secondary mechanism of immunological liver damage. This immunomodulatory aspect is important, as macrolides, including Azithromycin, are also immune-modulating antibiotics. As detailed by Altenburg et al. (2010) and Zarogoulidis et al. (2011), this same property may make patients more likely to experience exaggerated cytokine release with prolonged use, thereby promoting liver inflammation and injury [24, 25]. Taken together, these findings underscore the clinical significance of monitoring hepatic function during extended azithromycin therapy, especially in

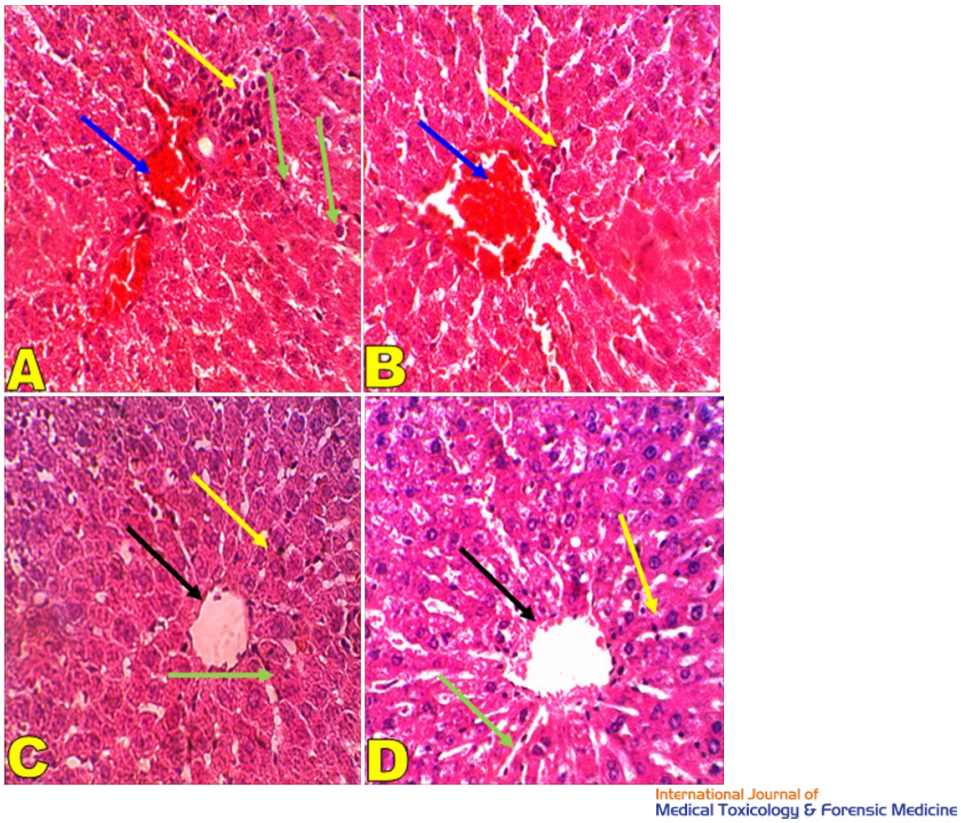

Figure 2. Picture (A and B) is the T2 group, which shows lymphoid infiltration (yellow arrow), Congestion in the central vein (blue arrow), and Pyknosis (green arrow). Picture (C) is the T3 group demonstrating normal arrangement of the hepatic cells (Yellow arrow) around the central vein (black arrow) sinusoidal capillaries (green arrow), Picture (D), the T4 group shows Normal cellular arrangement pattern (Yellow arrow) minor dilation central vein(black arrow) and minor dilation in the blood capillaries (green arrow).

vulnerable patients or those with preexisting liver conditions.

The significant improvement in hepatic architecture, approaching normalcy in T4 group, grossly indicates the protective effect of Rutin when co-administered with Azithromycin. Rutin protects against Azithromycin-induced hepatic damage by reducing inflammation, thereby preserving hepatocyte alignment with minimal sinusoidal damage. This is a biologically plausible observation given Rutin's antioxidant role. Choi et al. (2021) reported that Rutin directly scavenges ROS [26]. In contrast, Satari et al. (2021) and Lee et al. (2024) found that it upregulated endogenous antioxidant enzymes, including GPx and CAT, which play a key role in protection against lipid peroxidation and mitochondrial dysfunction [27, 28]. The hepatoprotective effects of Rutin against hepatic lesions may result from its potent antioxidant activity. Rutin upregulates the activity of major antioxidant enzymes, such as SOD, CAT, and GPx, and simultaneously activates the Nrf2/HO-1 pathway [18]. AZM-induced hepatotoxicity is caused by ROS production driven by oxidative stress, which disrupts normal cellular functions and promotes hepatocyte injury [29]. Rutin's capacity to protect organs from oxidative damage, through increased antioxidant enzyme activities, increased GSH concentration, and reduced malondialdehyde (MDA) levels, explains the significant amelioration of hepatic structure observed in the treated groups [18, 29].

Additionally, the anti-inflammatory effects of Rutin, especially its suppression of the NF-κB pathway, likely reduce proinflammatory cytokine release, thereby minimizing hepatocellular death and vascular injury [30]. Rutin also exhibits membrane-stabilizing effects, preserving hepatocyte integrity under oxidative assault conditions, as shown in acetaminophen-induced liver injury models [9]. Moreover, the results observed in T4 indicate a dose-duration-dependent (inverse) Rutin efficacy, with longer Rutin exposure being more effective, facilitating the restoration of redox homeostasis and tissue repair. These findings are consistent with previous investigations suggesting that flavonoids may be therapeutic agents for drug-induced organ injury and support the idea of adopting bioactive plant-based compounds as additional therapies to reduce the toxic side effects of drugs.

Tissue damage in the T2 group showed glomerular atrophy, tubular epithelial necrosis, interstitial hemorrhage, and inflammation, all classic signs of AKI. These are consistent with clinical descriptions of rare yet severe nephrotoxic allergic reactions to

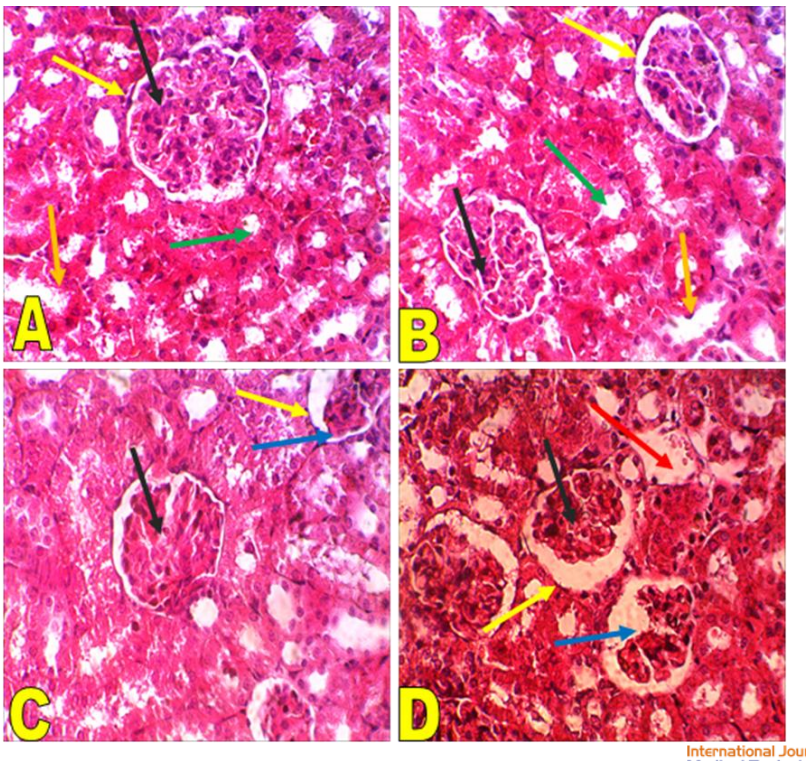

Figure 3. It show kidney tissue in rats: picture (A) is the control group (c), demonstrated normal kidney tissue, glomerulus (black arrow), Bowman's capsule (yellow arrow), Proximal convoluted Tubule (green arrow) and Distal convoluted tubule (brown arrow), picture (B) is T3 Group, demonstrated normal kidney tissue, glomerulus (black arrow), simple dilation in Bowman's capsule (yellow arrow), Proximal convoluted Tubule (green arrow) and Distal convoluted tubule (brown arrow), picture (C) is T2 Group, demonstrated simple tissue changes, glomerulus (black arrow), simple dilation in Bowman's capsule (yellow arrow) and atrophy in glomerulus (blue arrow), picture (D) is T4 Group, demonstrated tissue changes included dilation in Bowman's capsule (yellow arrow) and atrophy in glomerulus (blue arrow) and necrosis in Proximal convoluted Tubule (red arrow) (H and E, 40×).

Azithromycin, particularly associated with high doses or coexisting renal dysfunction [31]. The resulting injury in the T2 group may include tubular degeneration, glomerular atrophy, and vascular Congestion caused by oxygen radical production, followed by oxidative stress and apoptosis of proximal tubule cells. This is reinforced by increased proinflammatory cytokines and reduced mitochondrial membrane potential, consistent with Ranasinghe et al. (2023) [32]. T3 (rutin cotreatment group) was interestingly found to provide significant protection against these pathological changes. The reason may be Rutin's protective properties against oxidative stress, microvascular collapse, and inflammatory activation pathways. Rutin also suppresses tumor cell resistance to apoptosis by protecting mitochondrial structure. This was consistent with that of Diwan et al., who reported a protective effect of rutin treatment against impairment of glomerular histological structure and function in CKD rats [33]. Furthermore, Rutin reversed endothelial dysfunction and ameliorated renal tubular damage, demonstrating systemic therapeutic potential and antioxidative activity. More favorable renal histological features in T3 should encourage co-therapy regimens in early therapy to minimize drug-induced nephrotoxicity.

The significant difference between marked structural defects in group T2 and nearly normal nephros in group T4 once again indicates the protective role of Rutin as a potential therapeutic agent for the kidney. There was minimal hemorrhage and minimal tubular dilation in the preserved glomeruli, with complete histological recovery in T4 kidneys. This protective action is believed to occur through many pathways, including antioxidative effects against oxidative DNA and membrane damage, anti-inflammatory effects by reducing the accessibility of immune cells, and increased renal perfusion and reduced ischemic stress. Further, this hypothesis that Rutin protects renal epithelium from apoptosis was also supported by Pandey et al. (2021), suggesting that it can be used in the presence of established trauma [34]. It has been demonstrated that administration of Rutin significantly decreased proteinuria, BUN, and creatinine levels in animals with CRF. The protective effect is related to its ability to prevent Rutin-induced inhibition of the TGF-β1/Smad pathway, which plays a role in kidney fibrosis and nephropathy development.

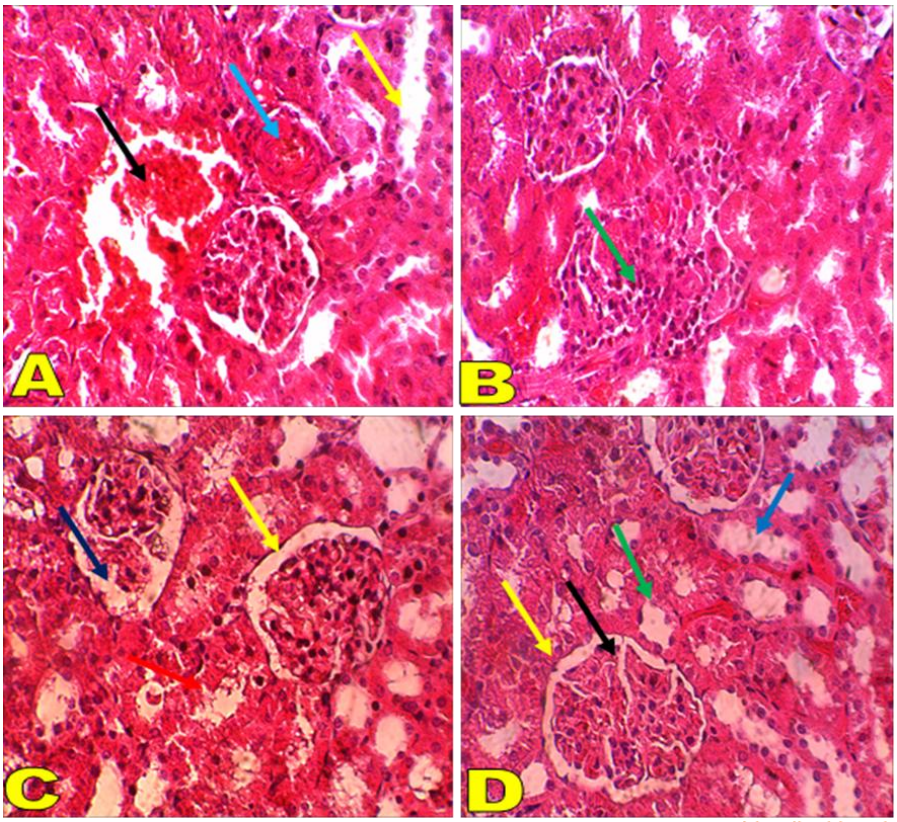

Figure 4. It show kidney tissue in rats: picture (A), (B) and (C) is T2 Group demonstrated tissue changes included dilation in Bowman's capsule and Distal convoluted tubule (yellow arrow), destruction in glomerulus (Purple arrow) and necrosis in Proximal convoluted Tubule (red arrow), bleeding (black arrow), Congestion in the vein(blue arrow) and Inflammatory cell infiltration (green arrow), picture (D) the T4 group, demonstrated normal kidney tissue, glomerulus (black arrow), Bowman's capsule(yellow arrow), Proximal convoluted Tubule (green arrow) and Distal convoluted tubule(blue arrow), (H and E, 40×).

Furthermore, as an antioxidant, Rutin helps restore the function of critical renal antioxidant enzymes (CAT, SOD, GSH-Px, GSR, and GST) that are known to be depleted during nephrotoxic responses [35]. Moreover, the restoration of renal structure in T4 may reflect Rutin's role in modulating the gut-kidney axis, as antibiotic-induced dysbiosis has been linked to systemic inflammation and renal decline [36, 37]. By preserving gut microbial balance and limiting systemic endotoxemia, Rutin may also confer indirect renal protection, an effect supported by the findings of Wang et al. [38].

Azithromycin was found to cause morphological changes in both hepatic and renal tissues, as demonstrated by histopathology. Azithromycin treatment induces marked necrosis, fatty degeneration, vacuolated cytoplasm, bile duct proliferation, and inflammatory cell infiltration in the liver [39]. Renal histopathology demonstrates equally severe changes, including tubular epithelial cell degeneration, dilated urinary spaces, glomerular atrophy, inflammatory cell infiltration, and pyknotic nuclei [40]. These morphological changes correspond to the functional impairments observed in biochemical parameters and reflect the progression from cellular damage to tissue-level architectural disruption [41].

Co-administration of Rutin was found to exhibit significant histoprotective effects, as evidenced by the retained normal architecture in both organs. Rutin-treated groups showed preserved liver histoarchitecture, minimal necrosis, a mild inflammatory infiltrate, and intact sinusoidal structures [42]. Renal walls reveal regenerating tubular epithelium, restored glomerular architecture, and reduced inflammatory alterations. The preserved normal histological appearance definitely correlated with the above-cited functional parameters, suggesting that Rutin demonstrated a protective effect at both the cellular and tissue levels [43]. This cytoprotection is most likely due to Rutin's ability to maintain cellular membrane integrity and reduce inflammation and apoptosis [41, 44].

Conclusion

This study revealed that the excessive use of Azithromycin leads to severe hepatorenal tissue damage in albino rats, characterized by inflammation, cellular degeneration, and vascular Congestion. Rutin cotreatment significantly attenuated the destructive effects and preserved the tissue architecture with a

near-normal appearance. The observed protective effect of Rutin is largely attributable to its potent antioxidant and anti-inflammatory activities, which help mitigate oxidative stress, suppress the activation of inflammatory signalling pathways, and stabilize cellular integrity. These results indicate that Rutin is an efficacious chemoprotective adjuvant modality for the prevention of organ-specific toxicity associated with prolonged administration of Azithromycin, without compromising its antibacterial activity. More clinical trials are needed to elucidate the explicit molecular mechanisms, systemic dosing regimens, translational potential, and safety of rutin supplementation.

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Ethical Approval

All the experimental protocols involving animals, including their care, were conducted in accordance with international standards on animal welfare and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Al-Qadisiyah (Reference Number: 14623/12/6/2023).

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Conflicts of Interest

The authors report there are no competing interests to declare.

References

[1] Mahmud H, Landskroner E, Amza A, Aragie S, Godwin WW, de Hostos Barth A, et al. Stopping azithromycin mass drug administration for trachoma: A systematic review. PLoS Negl Trop Dis. 2021;15(7):e0009491. [DOI: 10.1371/journal.pntd.0009491]

[2] Dongmo OLM, Epoh NJ, Tadjoua HT, Yousuf S, Telefo PB, Tapondjou LA, et al. Acute and sub-acute toxicity of the aqueous extract from the stem bark of Tetrapleura tetrapteura Taub. (Fabaceae) in mice and rats. J Ethnopharmacol. 2019;236:42–9. [DOI: 10.1016/j.jep.2019.02.026]

[3] Chen L, Qin F, Ma Y, Li F. Quantitative determination of Azithromycin in human plasma by ultra performance liquid chromatography-electrospray ionization mass spectrometry and its application in a pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci. 2007;855(2):255–61. [DOI: 10.1016/j.jchromb.2007.05.016]

[4] Sharma K, Mullangi R. A concise review of HPLC, LC-MS and LC-MS/MS methods for determination of Azithromycin in various biological matrices. Biomed Chromatogr. 2013;27(10):1243–58. [DOI: 10.1002/bmc.2898]

[5] Zhang Y, Bala V, Chhonker YS, Aldhafiri WN, John LN, Bjerum CM, et al. A simple, high-throughput and validated LC-MS/MS method for the determination of Azithromycin in human plasma and its application to a clinical pharmacokinetic study. Biomed Chromatogr. 2022;36(10):e5443. [DOI: 10.1002/bmc.5443]

[6] Westphal JF. Macrolide - induced clinically relevant drug interactions with cytochrome P-450A (CYP) 3A4: an update focused on clarithromycin, Azithromycin and dirithromycin. Br J Clin Pharmacol. 2000;50(4):285–95. [DOI: 10.1046/j.1365-2125.2000.00261.x]

[7] Woodhead JL, Yang K, Oldach D, MacLauchlin C, Fernandes P, Watkins PB, et al. Analyzing the Mechanisms Behind Macrolide Antibiotic-Induced Liver Injury Using Quantitative Systems Toxicology Modeling. Pharm Res. 2019;36(3):48. [DOI: 10.1007/s11095-019-2582-y]

[8] Villanueva-Paz M, Morán L, López-Alcántara N, Freixo C, Andrade RJ, Lucena MI, et al. Oxidative Stress in Drug-Induced Liver Injury (DILI): From Mechanisms to Biomarkers for Use in Clinical Practice. Antioxidants (Basel, Switzerland). 2021;10(3). [DOI: 10.3390/antiox10030390]

[9] Shinkai M, Henke MO, Rubin BK. Macrolide antibiotics as immunomodulatory medications: proposed mechanisms of action. Pharmacol Ther. 2008;117(3):393–405. [DOI: 10.1016/j.pharmthera.2007.11.001]

[10] Martinez MA, Vuppalanchi R, Fontana RJ, Stolz A, Kleiner DE, Hayashi PH, et al. Clinical and histologic features of azithromycin-induced liver injury. Clin Gastroenterol Hepatol. 2015;13(2):369-376.e3. [DOI: 10.1016/j.cgh.2014.07.054]

[11] Wu H, Bao X, Gutierrez AH, Nevzorova YA,

Cubero FJ. Role of oxidative stress and endoplasmic reticulum stress in drug-induced liver injury. Explor Dig Dis. 2023;2(3):83–99. [DOI: 10.37349/edd.2023.00020]

[12] Liu P, Zhang Y, Zhang Z, Huang X, Su X, Yang S, et al. Antibiotic-Induced Dysbiosis of the Gut Microbiota Impairs Gene Expression in Gut-Liver Axis of Mice. Genes (Basel). 2023;14(7). [DOI:10.3390/genes14071423]

[13] He M, Shi J, Liu A, Xu YJ, Liu Y. Antibiotic-induced gut microbiota dysbiosis altered host metabolism. Mol Omi. 2023;19(4):330–9. [DOI: 10.1039/d2mo00284a]

[14] Dahiya D, Nigam PS. Antibiotic-Therapy-Induced Gut Dysbiosis Affecting Gut Microbiota-Brain Axis and Cognition: Restoration by Intake of Probiotics and Synbiotics. Int J Mol Sci. 2023;24(4). [DOI: 10.3390/ijms24043074]

[15] Gao W, Guo L, Yang Y, Wang Y, Xia S, Gong H, et al. Dissecting the Crosstalk Between Nrf2 and NF-κB Response Pathways in Drug-Induced Toxicity. Front Cell Dev Biol. 2022;Volume 9. [Link]

[16] Chunmei Z, Shuai W. Molecular mechanisms of neuroprotective effect of Rutin. Front Pharmacol. 2025;Volume 16. [Link]

[17] Emeka PM, Badger-Emeka LI, Thirugnanasambantham K, Alatawi AS. Rutin-Activated Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) Attenuates Corneal and Heart Damage in Mice. Pharmaceuticals (Basel). 2024;17(11). [DOI: 10.3390/ph17111523]

[18] Rahmani S, Naraki K, Roohbakhsh A, Hayes AW, Karimi G. The protective effects of Rutin on the liver, kidneys, and heart by counteracting organ toxicity caused by synthetic and natural compounds. Food Sci Nutr. 2023;11(1):39–56. [DOI: 10.1002/fsn3.3041]

[19] Mahmoud AM, Ahmed RR, Soliman HA, Salah M. Ruta graveolens and its active constituent rutin protect against diethylnitrosamine-induced nephrotoxicity through modulation of oxidative stress. J Appl Pharm Sci. 2015;5(10):16–21. [DOI: 10.7324/JAPS.2015.50104]

[20] Bancroft JD, Gamble M. Theory and practice of histological techniques. Elsevier health sciences; 2008. [Link]

[21] Usadadia S V, Patel JM, Vihol PD, Urkude AP. Protective effect of quercetin on Azithromycin induced hepatotoxicity and nephrotoxicityin Wistar rats. Int J Curr Microbiol App Sci. 2020;9(9):2926–34. [DOI: 10.20546/ijcmas.2020.909.360]

[22] Martínez-Trejo A, Ruiz-Ruiz JM, Gonzalez-Avila LU, Saldaña-Padilla A, Hernández-Cortez C, Loyola-Cruz MA, et al. Evasion of antimicrobial activity in acinetobacter baumannii by target site modifications: an effective resistance mechanism. Int J Mol Sci. 2022;23(12):6582. [DOI: 10.3390/ijms23126582]

[23] Abushaheen MA, Muzaheed, Fatani AJ, Alosaimi M, Mansy W, George M, et al. Antimicrobial resistance, mechanisms and its clinical significance. Dis Mon. 2020;66(6):100971. [DOI: 10.1016/j.disamonth.2020.100971]

[24] Altenburg J, de Graaff CS, van der Werf TS, Boersma WG. Immunomodulatory effects of macrolide antibiotics - part 1: biological mechanisms. Respiration. 2011;81(1):67–74. [DOI: 10.1159/000320319]

[25] Zarogoulidis P, Papanas N, Kioumis I, Chatzaki E, Maltezos E, Zarogoulidis K. Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to clinical practice in respiratory diseases. Eur J Clin Pharmacol. 2012;68(5):479–503. [DOI: 10.1007/s00228-011-1161-x]

[26] Choi SS, Park HR, Lee KA. A Comparative Study of Rutin and Rutin Glycoside: Antioxidant Activity, Anti-Inflammatory Effect, Effect on Platelet Aggregation and Blood Coagulation. Antioxidants (Basel, Switzerland). 2021;10(11). [DOI: 10.3390/antiox10111696]

[27] Satari A, Ghasemi S, Habtemariam S, Asgharian S, Lorigooini Z. Rutin: A Flavonoid as an Effective Sensitizer for Anticancer Therapy; Insights into Multifaceted Mechanisms and Applicability for Combination Therapy. Evid Based Complement Alternat Med. 2021;2021:9913179. [DOI: 10.1155/2021/9913179]

[28] Lee OYA, Wong ANN, Ho CY, Tse KW, Chan AZ, Leung GPH, et al. Potentials of Natural Antioxidants in Reducing Inflammation and Oxidative Stress in Chronic Kidney Disease. Antioxidants (Basel, Switzerland). 2024;13(6). [DOI: 10.3390/antiox13060751]

[29] Liu Q, Pan R, Ding L, Zhang F, Hu L, Ding B, et al. Rutin exhibits hepatoprotective effects in a mouse model of non-alcoholic fatty liver disease by reducing hepatic lipid levels and mitigating lipid-induced oxidative injuries. Int Immunopharmacol. 2017;49:132–41. [DOI:

10.1016/j.intimp.2017.05.026]

[30] Farha AK, Gan RY, Li HB, Wu DT, Atanasov AG, Gul K, et al. The anticancer potential of the dietary polyphenol rutin: Current status, challenges, and perspectives. Crit Rev Food Sci Nutr. 2022;62(3):832–59. [DOI: 10.1080/10408398.2020.1829541]

[31] Molaei E, Molaei A, Abedi F, Hayes AW, Karimi G. Nephroprotective activity of natural products against chemical toxicants: The role of Nrf2/ARE signaling pathway. Food Sci Nutr. 2021;9(6):3362–84. [DOI: 10.1002/fsn3.2320]

[32] Ranasinghe R, Mathai M, Zulli A. Cytoprotective remedies for ameliorating nephrotoxicity induced by renal oxidative stress. Life Sci. 2023;318:121466. [DOI: 10.1016/j.lfs.2023.121466]

[33] Diwan V, Brown L, Gobe GC. The flavonoid rutin improves kidney and heart structure and function in an adenine-induced rat model of chronic kidney disease. J Funct Foods. 2017;33:85–93. [Link]

[34] Pandey P, Khan F, Qari HA, Oves M. Rutin (Bioflavonoid) as Cell Signaling Pathway Modulator: Prospects in Treatment and Chemoprevention. Pharmaceuticals (Basel). 2021;14(11). [DOI: 10.3390/ph14111069]

[35] Han Y, Lu JS, Xu Y, Zhang L, Hong BF. Rutin ameliorates renal fibrosis and proteinuria in 5/6-nephrectomized rats by anti-oxidation and inhibiting activation of TGFβ1-smad signaling. Int J Clin Exp Pathol. 2015;8(5):4725–34. [Link]

[36] Rysz J, Franczyk B, Ławiński J, Olszewski R, Ciałkowska-Rysz A, Gluba-Brzózka A. The Impact of CKD on Uremic Toxins and Gut Microbiota. Toxins (Basel). 2021;13(4). [DOI: 10.3390/toxins13040252]

[37] Shah T, Baloch Z, Shah Z, Cui X, Xia X. The Intestinal Microbiota: Impacts of Antibiotics Therapy, Colonization Resistance, and Diseases. Int J Mol Sci. 2021;22(12). [DOI: 10.3390/ijms22126597]

[38] Wang G, Gong H, Zou Y, Zhang H, Mao X. Bifidobacterium animalis Subsp. lactis PB200 Improves Intestinal Barrier Function and Flora Disturbance in Mice with Antibiotic-Induced Intestinal Injury. Nutrients [Internet]. 2025 ;17(10). [DOI: 10.3390/nu17101610]

[39] Hanafy SM, Zakaria SS. The Protective Role of Folic Acid in Biochemical and Histopathological Changes Induced by Azithromycin in the Livers of Pregnant Albino Rats. Medicina (Kaunas). 2025 ;61(3). [DOI: 10.3390/medicina61030415]

[40] Sakurai T, Kamio K, Sasaki K, Nishimoto T, Yamaguchi J ichi, Sasaki M, et al. Imaging Mass Microscopy of Kidneys from Azithromycin-Treated Rats with Phospholipidosis. Am J Pathol [Internet]. 2018;188(9):1993–2003. [Link]

[41] Ali MAM, Matouk AI, Hamza AA, Amin A, Heeba GH. Gallic and glycyrrhetinic acids prevent azithromycin-induced liver damage in rats by mitigating oxidative stress and inflammation. Sci Rep. 2025;15(1):9566. [DOI: 10.1038/s41598-025-93120-3]

[42] Mohsen AM, Wagdi MA, Salama A. Rutin loaded bilosomes for enhancing the oral activity and nephroprotective effects of Rutin in potassium dichromate induced acute nephrotoxicity in rats. Sci Rep. 2024 ;14(1):23799. [DOI: 10.1038/s41598-024-73567-6]

[43] Prasad R, Prasad SB. Histoprotective effect of Rutin against cisplatin-induced toxicities in tumor-bearing mice: Rutin lessens cisplatin-induced toxicities. Hum Exp Toxicol. 2020 ;40(2):245–58. [DOI: 10.1177/0960327120947793]

[44] Kwiatkowska E, Domański L, Dziedziejko V, Kajdy A, Stefańska K, Kwiatkowski S. The Mechanism of Drug Nephrotoxicity and the Methods for Preventing Kidney Damage. Int J Mol Sci. 2021;22(11). [DOI:10.3390/ijms22116109]