

## Research Article

# Renoprotective Effect of Pitavastatin against TAA-Induced Renal Injury: Involvement of the miR-93/PTEN/AKT/mTOR Pathway

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This research investigated if pitavastatin (Pita) might protect rats' kidneys against thioacetamide (TAA). By altering the PTEN/AKT/mTOR pathway, pitavastatin may boost kidney antioxidant capacity and minimize oxidative damage. Statins have several benefits, including antioxidant and anti-inflammatory characteristics. The principal hypothesis of this study was that Pita can regulate the miR-93/PTEN/AKT/mTOR pathways, which is thought to be responsible for its renoprotective effects. The experiment divided male rats into four groups. Group 1 included untreated rats as the control. Group 2 included rats which received TAA (100 mg/kg intraperitoneally thrice a week for two weeks) to destroy their kidneys. Groups 3 and 4 included rats which received Pita orally at 0.4 and 0.8 mg/kg for 14 days after TAA injections. Renal injury increased BUN, creatinine, and MDA levels and decreased glutathione (GSH) levels. Pitavastatin prevented these alterations. TAA decreased PTEN and increased miR-93, Akt, p-Akt, mTOR, and Stat3 in the kidneys. Pitavastatin also regulated the associated culprit pathway, miR-93/PTEN/Akt/mTOR. In addition, TAA induced adverse effects on the kidney tissue, which were significantly ameliorated by pitavastatin treatment. The findings suggest that pitavastatin can attenuate renal injury, likely by regulating the miR-93/PTEN/Akt/mTOR pathway. This modulation of the pathway appears to contribute to the protective effects of pitavastatin against TAA-induced renal injury, adding to the growing evidence of the pleiotropic benefits of statins in renal health.

## 1. Introduction

Acute kidney injury (AKI) is a clinical illness characterized by abruptly impaired renal function, retention of metabolites, and abnormalities of the water, electrolyte, and acid-base balance [1]. AKI is a severe condition that causes over 2 million fatalities globally each year and is linked to higher morbidity and mortality rates [2]. Prior research has shown that patients with AKI frequently experience incomplete recovery of their renal function. In addition, AKI is

a substantial risk factor for the development of both chronic kidney disease (CKD) and end-stage renal disease (ESRD) [3]. Inflammation and oxidative stress are intricately interconnected, leading to a detrimental cycle. Oxidative stress initiates the process of inflammation, which, in response, further triggers oxidative stress by generating reactive oxygen species (ROS). These harmful occurrences collectively contribute to tissue damage by inducing necrosis, apoptosis, and fibrosis [4]. AKI lacks specific treatments beyond standard supportive care and renal

replacement therapy, which significantly contributes to the unfavorable short-term outlook experienced by AKI patients [4, 5]. Despite substantial research on AKI, the molecular pathways underlying renal damage have not yet been thoroughly explored. In order to treat or stop the progression of AKI to CKD, new medications or mechanisms must be developed. Despite extensive research on AKI, there remains a lack of comprehensive understanding regarding the molecular processes underlying renal injury. Consequently, it is essential to explore new medications or mechanisms to effectively manage or prevent the progression of AKI to CKD [6].

MicroRNAs (miRs) are short RNA molecules, typically consisting of 18–25 nucleotides, which control protein translation by binding to complementary regions of mRNA. This binding typically occurs in the 3' untranslated region of the mRNA [7]. In studies involving preclinical AKI models, numerous miR variations have demonstrated promising therapeutic capabilities [8]. For instance, in cases of experimental acute kidney injury (AKI) caused by ischemia-reperfusion (I/R), administering miR-17-5p mimic via intravenous injection has been shown to hinder histologic ischemic damage. It also leads to a decrease in blood urea nitrogen and serum creatinine levels.

In addition, when miR-21 is overexpressed in mice, it offers protection against ischemic kidney injury by reducing apoptosis [9–11]. In addition, Liao and colleagues [12] provided evidence that miR-140-5p enhanced the recovery from cisplatin-induced AKI in mice. This effect was achieved by reducing oxidative stress through the activation of the Nrf2/ARE pathway. Moreover, recent research has unveiled the protective properties of miRNAs in different renal cell types, as they effectively inhibit apoptosis and inflammatory responses [13, 14].

Phosphatase and tensin homolog (PTEN), located on chromosome 10, acts as a tumor suppressor by exhibiting the bispecific phosphatase activity. This activity leads to the dephosphorylation of PI3P, converting it into PI2P. Consequently, the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway is deactivated [15]. PI3K becomes activated, leading to the phosphorylation of PIP2 and its conversion into PIP3. Consequently, Akt is activated by PIP3 [16]. Furthermore, there is a notable rise in the levels of PTEN, leading to the suppression of the Akt/mTOR signaling pathway [17]. Akt stimulates mTOR, leading to the initiation of downstream protein translation through the phosphorylation of 4EBP1 and P70S6K [18]. All renal tissues express PTEN. However, renal tubular cells are where it is most abundant [19].

Statins are a traditional medication utilized to address hyperlipidemia in obese and diabetic patients. Their mode of action involves the inhibition of the hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase enzyme, which not only leads to a direct reduction in endogenous cholesterol production but also exhibits additional beneficial effects known as pleiotropic effects, as demonstrated in both laboratory experiments (*in vitro*) and studies involving living organisms (*in vivo*) [20]. In addition to reducing oxidative stress, inflammation, and apoptosis, in

streptozotocin-induced type 1 diabetes models in rats, statins may improve pancreatic and renal function [21]. In addition, the beneficial impacts of statins on kidney dysfunction were observed in rats with diet-induced obesity and patients diagnosed with type 2 diabetes (T2DM) [22–26]. The kidney-protective benefits of atorvastatin, simvastatin, and rosuvastatin have been proven [27]. Statins demonstrate advantageous impacts in various animal model kidney diseases [28]. Despite numerous research papers focusing on statins' impact on AKI, this study had a distinct objective of examining pitavastatin (Pita)'s potential in mitigating thioacetamide- (TAA-) induced renal injury in rats. The main hypothesis of this investigation was that Pita's renoprotective properties are attributed to its regulation of miR-93/PTEN/AKT/mTOR pathways.

## 2. Materials and Methods

**2.1. Animals.** Twenty-four adult male Wistar rats weighing 150–200 g and five months old were acquired from the “Animal House Colony at the National Research Centre (NRC, Cairo, Egypt).” Rats were housed at ambient temperature (25°C) and on a 12-hour light/dark cycle. All operations and tests with permission number “1041112022” have been authorized by the National Research Center's Medical Research Ethics Committee. The report of the study follows the ARRIVE principles in accordance with ARRIVE guidelines.

**2.2. Chemicals.** From Sigma-Aldrich Co. (St. Louis, MO, USA), thioacetamide (TAA) was purchased. From Western pharmaceutical industries, in Egypt, pitavastatin was acquired. Every other chemical that was used in the experiments was of the highest purity and analytical grade. Freshly suspended Pita was orally administered at a dose of 0.4 mg/kg and 0.8 mg/kg in a 1% Tween 80 solution [29].

**2.3. Experimental Design.** The rats were divided into four groups (6 rats/group). Rats in group 1 (negative control group) were given an intraperitoneal (ip) injection of saline three times a week for two consecutive weeks. Rats in group 2 (TAA group) received an intraperitoneal (ip) injection of TAA (100 mg/kg) three times a week for two consecutive weeks to cause renal injury [30]. Rats in groups 3 and 4 were given Pita orally (0.4 and 0.8 mg/kg) [31] every day for two weeks following TAA injections.

**2.4. Preparation of Serum Samples.** After completing the experiment, the rats were deprived of food overnight. Their blood was then drawn, and the serum was extracted by spinning the samples for five minutes at 3000 rpm and 4°C. The obtained serum was then stored at –80°C for later use in measuring blood urea nitrogen (BUN) and creatinine levels.

**2.5. Collecting Renal Tissues.** A cold saline solution was used to rinse the kidneys. They were then split into three portions. To enable mRNA extraction and the evaluation of miRNA 93

and AKT gene expression, the first portion was promptly frozen in liquid nitrogen and kept at a temperature of  $-80^{\circ}\text{C}$ . After homogenizing the second portion with 0.5% potassium chloride (1 mL for every 100 mg of tissue), it was centrifuged for 10 min at  $4^{\circ}\text{C}$  and 3000 rpm. The resultant supernatant was separated and stored at  $80^{\circ}\text{C}$  to measure renal glutathione (GSH) and malondialdehyde (MDA) using colorimetric kits, as well as AKT, *p*-AKT, mTOR, *p*-mTOR, and PTEN using ELISA. At the same time, the second portion was utilized to estimate the renal content of miRNA 93 and the AKT RNA content. Finally, the last part was fixed in 10% buffered formalin for subsequent histopathological and immune-histochemical investigations.

**2.6. Renal Function Tests.** To assess the renal function, we measured serum BUN and creatinine levels using commercially available kits. BUN levels were determined colorimetrically with the BioVision kit (Catalog# K375-100, Milpitas Boulevard, Milpitas, USA). The principle of this is a kit comprised of the hydrolysis of urea by urease to produce ammonia and  $\text{CO}_2$ . The ammonia that is produced then combines with hypochlorite and a phenolic chromogen to generate a complex that is green in color. The color intensity that develops is closely correlated with the urea content of the sample and vice versa. Besides, creatinine levels were measured colorimetrically using the BioVision kit (Catalog# E4370-100, Milpitas Boulevard, Milpitas, USA). The principle of this kit comprised the modified kinetic Jaffe methodology for the determination of creatinine involving a protein-free filtrate and a reaction with picric acid in an alkaline solution, a surfactant, and other ingredients to minimize protein and carbohydrate interferences [32]. The assays were performed following the manufacturer's instructions.

**2.7. Renal Oxidative Stress Markers.** For the evaluation of renal oxidative stress, we measured MDA levels and the GSH content. The MDA level was assessed colorimetrically with the BioVision kit (Catalog# K739-100, Milpitas Boulevard, Milpitas, USA) that is based on the reaction of MDA in the sample with thiobarbituric acid (TBA) to generate the MDA-TBA adduct which can be easily quantified colorimetrically (OD 532 nm). The GSH content was determined colorimetrically using the BioVision kit (Catalog# K464-100, Milpitas Boulevard, Milpitas, USA) based on an enzymatic cycling method in the presence of GSH and a chromophore. The reduction of the chromophore produces a stable product, which can be followed kinetically at 450 nm. The procedures were conducted in accordance with the manufacturer's instructions to ensure accuracy and reliability.

**2.8. Detection of mRNA Expression of miRNA 93 and Akt in Renal Tissues via Real-Time qRT-PCR.** Thermo Fisher Scientific, Waltham, Massachusetts, USA, supplied the SuperScript IV One-Step RT-PCR kit for reverse transcription of extracted RNA and PCR. The following thermal profile was created using a StepOne instrument (Applied

Biosystem, USA): 10 minutes at  $45^{\circ}\text{C}$  for reverse transcription, 2 minutes at  $98^{\circ}\text{C}$  for RT inactivation, and initial denaturation by 40 cycles of 10 s at  $98^{\circ}\text{C}$ , 10 s at  $55^{\circ}\text{C}$ , and 30 s at  $72^{\circ}\text{C}$  for the amplification step. Data were expressed as cycle threshold (Ct) for the target genes and housekeeping gene following the RT-PCR run. Using the mean critical threshold (CT) expression levels of the housekeeping gene GAPDH, the target genes' expression was normalized for variation using the  $\Delta\Delta\text{Ct}$  method. Each target gene's relative quantitation (RQ) is determined using the  $2^{-\Delta\Delta\text{Ct}}$  method of calculation. The AKT gene's primer sequence was 5'-CGG ATACCATGAACGACGTAG-3' and 5'-GCAGGCAGC GGATGATAAAG-3'; the GenBank accession number for this sequence is NM\_033230.2 which was normalized versus the GAPDH as a housekeeping gene; forward 5'-TGGCCT TCCGTGTTCCCTAC-3' and reverse 5'-GAGTTGCTGTTG AAGTCGCA-3' (GenBank accession number is XM\_036165840.1). The miRNA 93 gene's primer sequence is as follows: forward 5'-CAAAGTGCTGTTCGTGCAGGT AG-3'; reverse 5'-AACGCTTACGAATTTGCGT-3'; normalization was performed against housekeeping gene U6 forward 5'-CTCGCTTCGGCAGCACACA-3', and reverse 5'-AACGCTTACGAATTTGCGT-3' (GenBank accession number is K00784.1).

**2.9. ELISA Assay.** The protein concentrations of *p*-AKT were measured using commercially available ELISA kits (Catalog# PEL-AKT-S473-T, Ray Biotech, Norcross, USA), mTOR or *p*-mTOR (Catalog# SL1350Ra and SL5329R SunLong Biotech Co., Zhejiang, China), and AKT or PTEN (Catalog# MBS3807575 and MBS452729, MyBioSource, San Diego, USA) in renal tissue homogenate. Each ELISA kit's manufacturer's instructions were followed.

**2.10. Histopathological Examination.** The renal tissues were fixed for 24 h in 10% buffered formalin. After that, it was dried in various alcohol concentrations, cleaned in xylene, and finally embedded in paraffin wax. The paraffin slices (4 mm) were stained with hematoxylin and eosin (H&E) stain. The cells were inspected under a light microscope by a blinded pathologist to prevent prejudice.

**2.11. Immune-Histochemical Examination of STAT3 Expression.** The other paraffin section from each group was used for immunohistochemical detection of the expression of Stat3 in various experimental groups using avidin-biotin-peroxidase according to the method described in [33]. Kidney slices were treated with monoclonal antibodies for Stat3 (Abcam, Cambridge, MA, USA) at a dilution of 1:200 and (Vectastain ABC peroxidase kit, Vector Laboratories, Newark, USA) for the aim of identifying antigen-antibody complexes. Each marker's expression was visualized using chromagen 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co.). The positive brown region of each marker's expression was assessed using seven high-power microscopic fields and image analysis software (ImageJ, 1.46a, NIH, Bethesda, USA).

**2.12. Statistical Analysis.** The outcomes are represented as the “means  $\pm$  S.E.”. Data were processed by “one-way ANOVA followed by the Tukey–Kramer post hoc test.” “GraphPad Prism software (version 9, USA)” was used to conduct the statistical analysis and create the graphs shown. The significance level was set to “ $p < 0.05$ ” for all statistical tests [34].

### 3. Results

**3.1. Effect of Pitavastatin on Renal Function Tests.** The serum levels of creatinine and BUN significantly increased after the injection of TAA. Pita administration successfully reduced serum creatinine and BUN levels in both treatment groups, as indicated in Figure 1.

**3.2. Effect of Pitavastatin on the Oxidative Stress Status.** In the TAA group, compared to the control group, there was a significantly higher level of renal MDA ( $p < 0.05$ ) and a significantly lower level of renal GSH ( $p < 0.05$ ). As indicated in Figure 2, Pita treatment successfully reduced the renal MDA content in both treated groups ( $p < 0.05$ ) and enhanced the GSH content when compared to the TAA group ( $p < 0.05$ ).

**3.3. Effect of Pitavastatin on the PTEN/AKT/mTOR Pathway.** The level of PTEN and the downstream variables AKT, *p*-AKT, mTOR, and *p*-mTOR were examined in order to clarify further the mechanism underlying the protective effect of Pita against renal injury because these factors are crucial in the development of renal injury. PTEN levels were significantly decreased, and AKT, *p*-AKT, mTOR, and *p*-mTOR levels were significantly increased by TAA-induced renal injury compared to the control group ( $p < 0.05$ ). In contrast, PTEN levels were significantly decreased, and AKT, *p*-AKT, mTOR, and *p*-mTOR levels were significantly increased by the Pita treatment groups ( $p < 0.05$ , Figures 3(a)–3(e)).

**3.4. Effect of Pitavastatin on Renal miR-93 and AKT Gene Expression.** In the TAA model group ( $p < 0.05$ ), as compared to the control group, real-time qRT-PCR analysis revealed a significantly increased expression of miR-93 and AKT. Comparing the gene expression of miR-93 and AKT in both Pita-treated groups to TAA-renal damaged rats, Figure 4 demonstrates a significant decrease in both genes' expression ( $p < 0.05$ ).

**3.5. Histopathological Findings.** Pita markedly prevented the adverse effects of TAA on the kidney tissue. Normal histological structures of cortical and medullary elements, as well as the renal pelvis, were clearly noticed under light microscopic examination of the kidney tissues of control rats (Figure 5(a)). Whilst TAA administration had an adverse effect on kidney tissue, particularly in the cortex, which showed variation in the tinctorial intensity of the renal tubules with marked dilatation and congestion of the

interstitial blood vessels with interstitial edema and few pockets of hemorrhages (Figure 5(b)). The renal tubules showed epithelial lining swelling, degeneration, some pyknotic nuclei, necrosis, and sloughing of a few cells. Other tubules were cystically dilated with an attenuated epithelium (Figure 5(c)). Some cellular debris was observed in the lumen of a few tubules. Occasional granular casts were present in medullary collecting ducts and tubules (Figure 5(d)). Most of the glomeruli showed membrane thickening of the glomerular tuft with few cellular infiltrates and few others appeared with atrophied tufts. Daily treatment with Pita in rats for two weeks prior to being administrated TAA showed marked protection of their kidney tissues against the harmful effect of TAA, particularly in the high-dose administrated rats. Rats administrated Pita at a low dose (Figures 5(e) and 5(f)) showed mild degenerative changes of the renal tubular epithelium with scarred glomeruli appearing with atrophied tuft. While at the high dose of Pita (Figures 5(g) and 5(h)), mild degenerative changes were noticed in some tubular epithelium with the appearance of scattered foci of regenerated tubules.

Pita reduced TAA's kidney damage. Under light microscopy, the kidney tissues of control rats showed the normal histological structure of cortical, medullary, and renal pelvic components (Figure 5(a)). TAA treatment damaged the kidney tissue, notably in the cortex, which demonstrated variance in renal tubule tinctorial intensity, significant dilatation and congestion of interstitial blood vessels, interstitial edema, and rare hemorrhages (Figure 5(b)). The renal tubules revealed epithelial lining enlargement, degeneration, some pyknotic nuclei, necrosis, and sloughing of a few cells. Other cystically dilated tubules have attenuated epithelium (Figure 5(c)). Few tubules had cellular debris. Medullary collecting ducts and tubules sometimes have granular deposits (Figure 5(d)). Most glomeruli had membrane thickening and minimal cellular infiltrates, whereas a few had atrophied tufts. Pita given daily to rats for two weeks before TAA administration protected their kidney tissues, especially in high-dose animals. Low-dose Pita-treated rats (Figures 5(e) and 5(f)) demonstrated modest renal tubular epithelial degeneration with scarred glomeruli and atrophied tuft. At high Pita doses (Figures 5(g) and 5(h)), some tubular epithelium showed modest degeneration and scattered foci of regenerated tubules.

**3.6. Immunohistochemistry Findings.** A remarkable increased expression of Stat3 was noticed in the kidney tissues of the rats' group administrated TAA (Figure 6(b)) compared to the control group (Figure 6(a)). Meanwhile, Pita (0.4 and 0.8 mg/kg) treatment following TAA administration displayed a dose-related reduced Stat3 expression compared to the TAA group (Figures 6(c) and 6(d)). The quantitative analysis of the positive brown color of Stat3, displayed as a staining score, demonstrated that the TAA rats significantly ( $p < 0.05$ ) overexpressed Stat3 in comparison to the other treated groups.

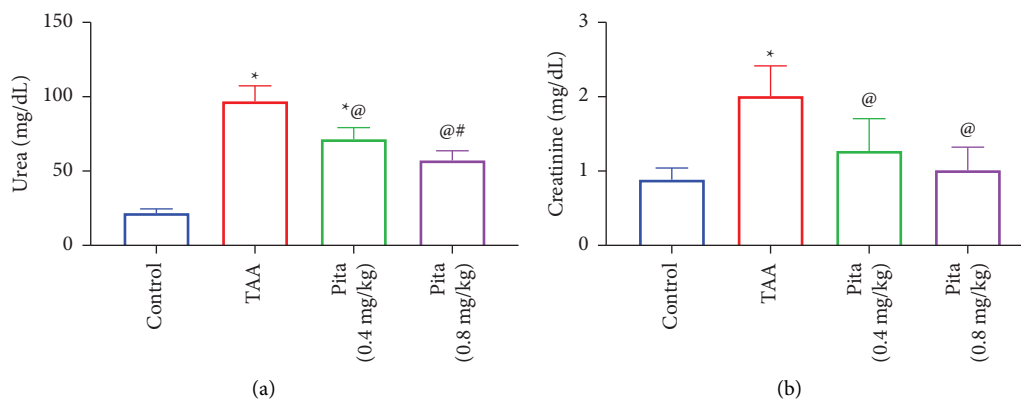


FIGURE 1: Effect of pitavastatin on serum renal function: (a) urea and (b) creatinine. A bar shows the mean and standard error of six rats: \* compared to the normal control group, @ to the TAA group, and # to pita (0.4 mg/kg) at  $p < 0.05$ . Pita: pitavastatin and TAA: thioacetamide.

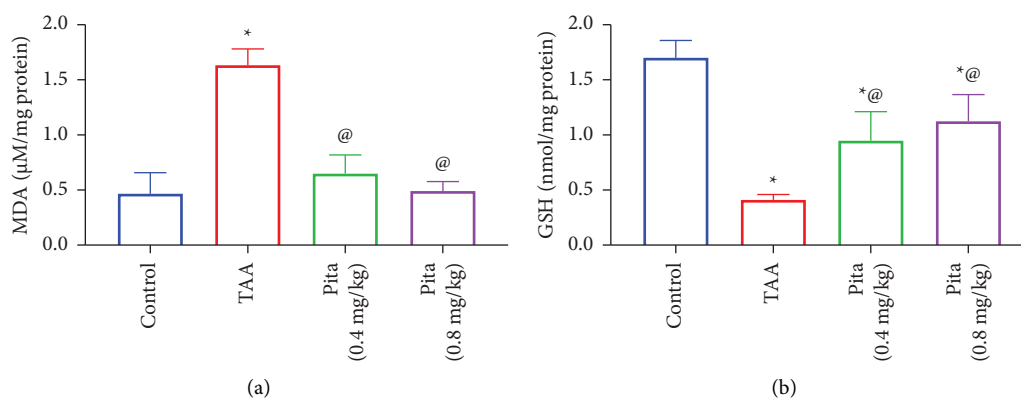


FIGURE 2: Effect of pitavastatin on renal (a) MDA and (b) GSH. A bar shows the mean and standard error of six rats: compared \* to the normal control group and @ to the TAA group at  $p < 0.05$ . Pita: pitavastatin and TAA: thioacetamide.

TAA-treated rats had significantly higher Stat3 expression in their renal tissues (Figure 6(b)) than controls (Figure 6(a)). Pita (0.4 and 0.8 mg/kg) therapy after TAA lowered Stat3 expression compared to the TAA group (Figures 6(c) and 6(d)). TAA rats overexpressed Stat3 based on the Stat3 staining score.

#### 4. Discussion

Statins work by blocking cholesterol synthesis, leading to a reduction in blood lipids. The recent focus on the pleiotropic effects of statins arises from numerous clinical and preclinical investigations that highlight the additional benefits of statins beyond cholesterol control. These studies reveal advantages that are not directly related to cholesterol levels [35]. Pita stands as an innovative statin with remarkable pharmacokinetic and pharmacodynamic qualities. Unlike other statins, it undergoes minimal metabolism by CYP3A4 (cytochrome P450), reducing the likelihood of drug interactions with substances metabolized by this enzyme. Additional research has revealed several advantageous attributes of Pita, including safeguarding endothelial function and suppressing vascular inflammation and oxidative stress [36]. Thioacetamide (TAA), an organosulfur compound, is

commonly used as a fungicide [37]. TAA is converted *in vivo* to the free radical derivatives TAA sulfoxide and TAA-S, S-dioxide. This causes enhanced lipid peroxidation, which leads to the generation of reactive oxygen species (ROS), which causes damage to the liver [38–41]. The metabolites produced can affect amine lipids and proteins, increasing systemic oxidative stress, releasing cytokines, and altering kidney function since they are dispersed laterally throughout various organs, including the liver, kidney, adrenals, bone marrow, and other tissues [42–44].

While previous studies have shown the nephroprotective effects of statins, including pitavastatin [45–51], the specific involvement of the miR-93/PTEN/Akt/mTOR pathway in mediating these effects has not been elucidated *in vivo* in the context of xenobiotic-induced kidney injury. Our study is the first to demonstrate that pitavastatin attenuates TAA-induced kidney damage in rats by regulating this essential signaling cascade. We used the TAA model because it is a well-established approach for studying mechanisms of kidney injury due to oxidative stress and xenobiotic toxicity.

Our findings significantly advance understanding of the mechanisms underlying statins' pleiotropic benefits in the kidney. We show for the first time the integral role of miR-93 and PTEN regulation in activating downstream Akt/mTOR

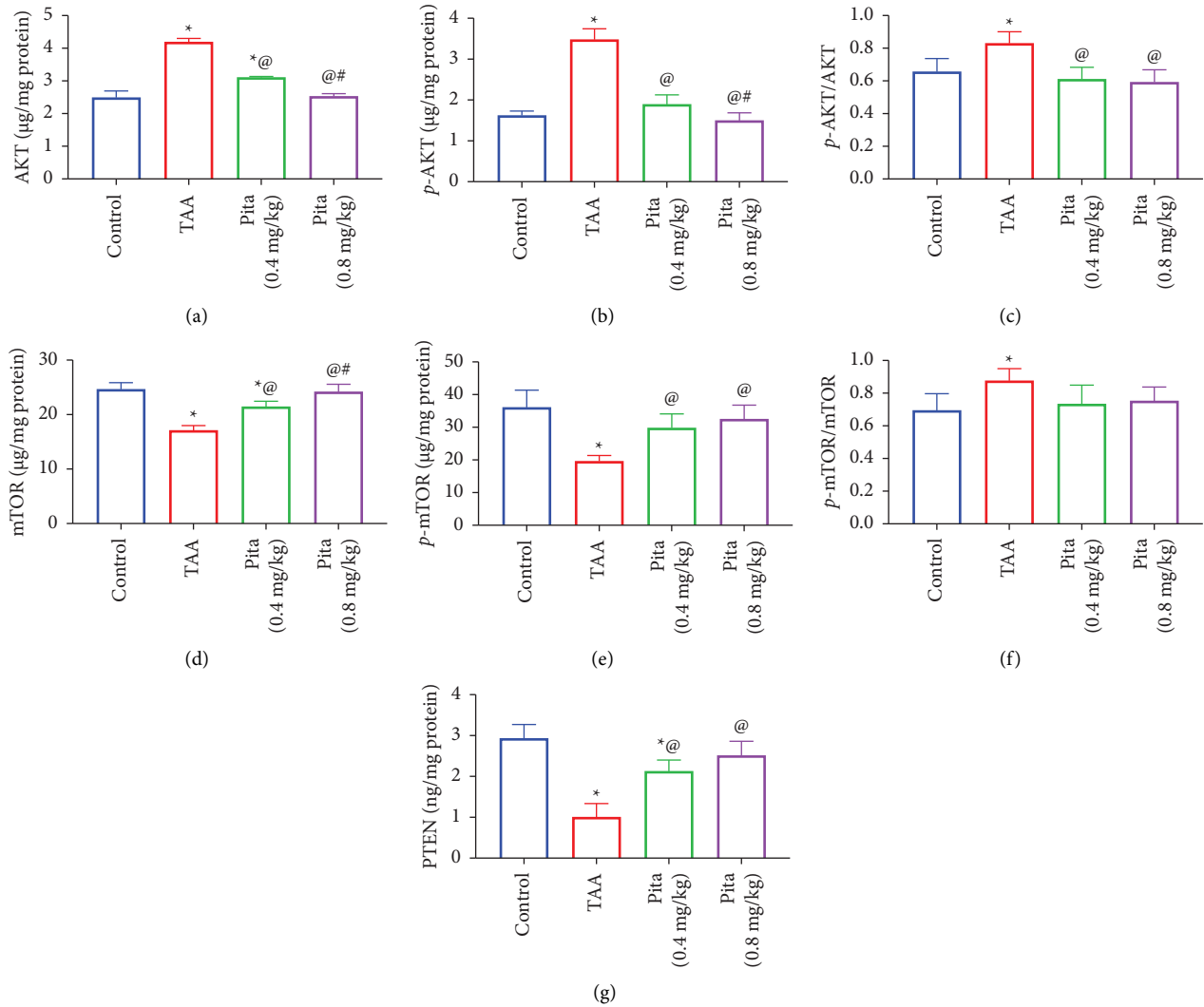


FIGURE 3: Effect of pitavastatin on (a) AKT, (b) *p*-AKT, (c) *p*-AKT/AKT, (d) mTOR, (e) *p*-mTOR, (f) *p*-mTOR/m-TOR, and (g) PTEN. A bar shows the mean and standard error of six rats: \* compared to the normal control group, @ to the TAA group, and # to pita (0.4 mg/kg) at  $p < 0.05$ . Pita: pitavastatin and TAA: thioacetamide.

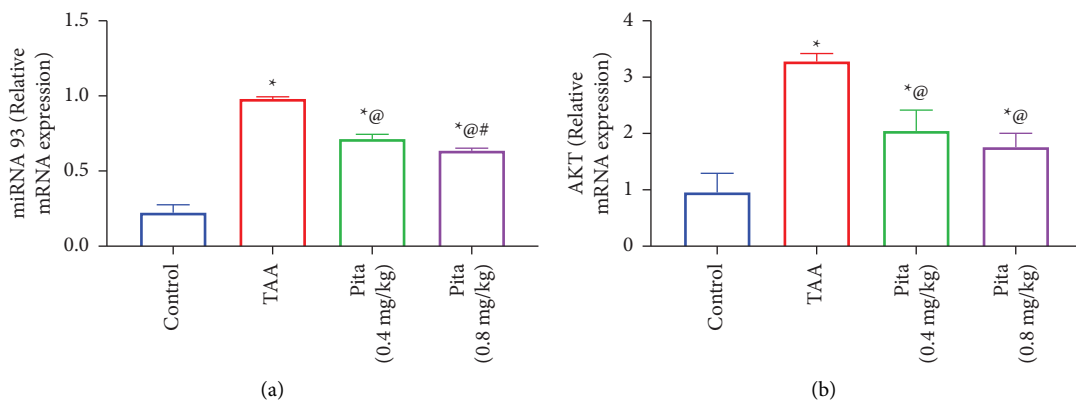


FIGURE 4: Effect of pitavastatin on renal gene expression of (a) miR-93 and (b) AKT. A bar shows the mean and standard error of six rats: \* compared to the normal control group, @ to the TAA group, and # to pita (0.4 mg/kg) at  $p < 0.05$ . Pita: pitavastatin and TAA: thioacetamide.



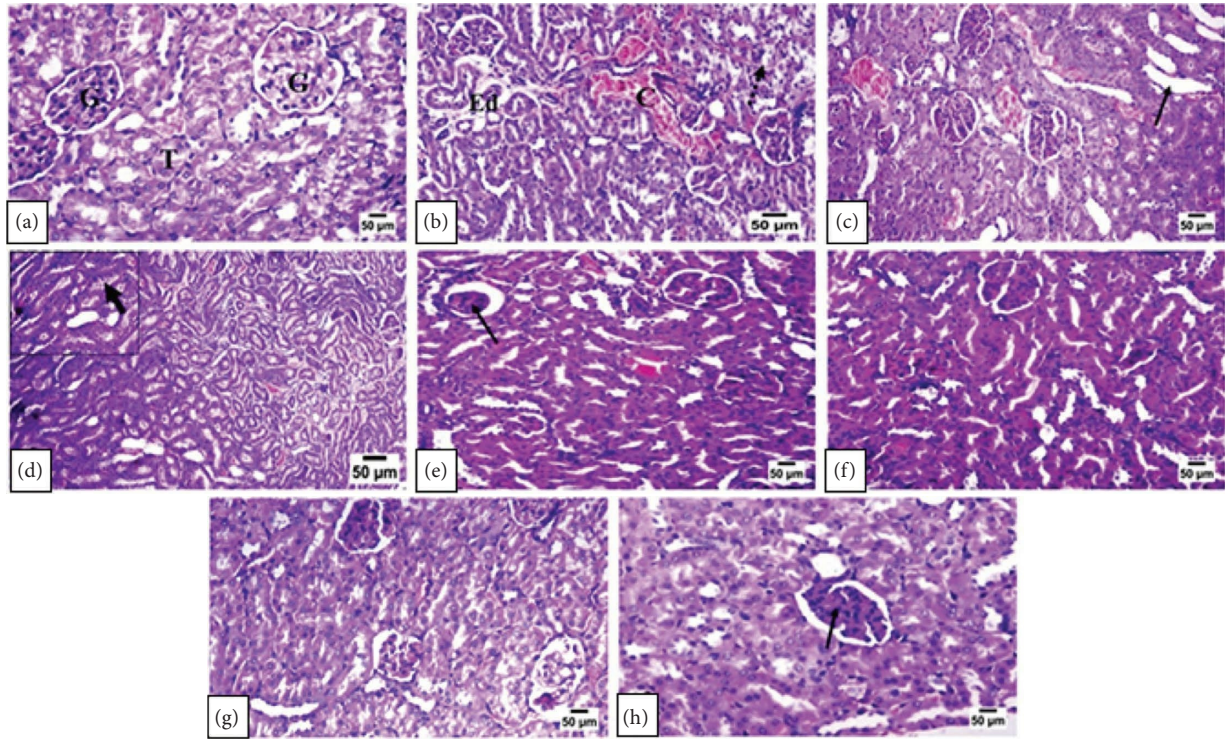


FIGURE 5: Effect of *p* on histopathology findings. Normal control (a), TAA group (b–d), low dose of pitavastatin-treated groups (e–f), and high dose of the pitavastatin-treated group (g–h).

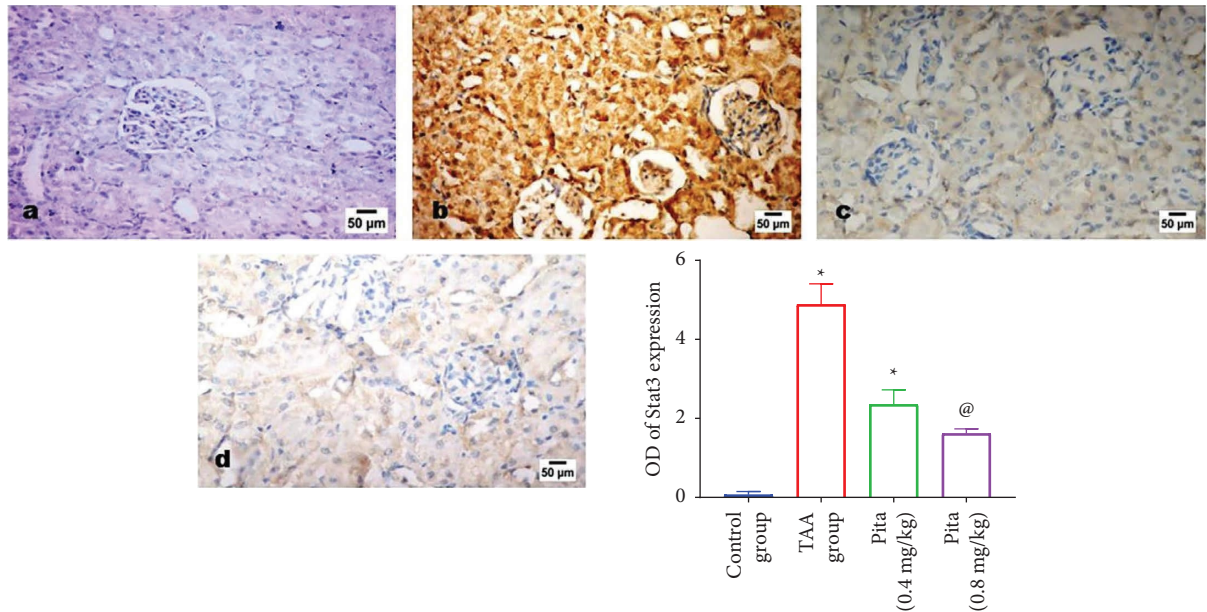


FIGURE 6: Effect of pitavastatin on Stat3 expression in kidney tissues. (a) Normal group, (b) TAA group, (c) low dose of Pita, and (d) high dose of Pita. A bar shows the mean and standard error of six rats: compared \*to the normal control group and <sup>@</sup>to the TAA group at  $p < 0.05$ . Pita: pitavastatin and TAA: thioacetamide.

signaling to mitigate renal injury. These novel insights expand the evidence for the multifaceted protective properties of statins and support their potential therapeutic use to preserve kidney function in contexts of toxicant exposure or oxidative damage. Our study also suggests that the miR-93/

PTEN/Akt/mTOR axis could be a promising target for future nephroprotective strategies.

In our research, we observed notable disruption in kidney function among rats treated with TAA, as indicated by increased levels of BUN and creatinine in the

bloodstream. Prior studies had already indicated the potential impact of TAA on measures of the kidney function [52]. Pita normalized the levels of BUN and creatinine in a dose-dependent manner. Our findings are in accordance with [53]. The histopathological examination revealed considerable tissue damage and loss of structure in the kidneys of the group treated with TAA. However, the group treated with Pita showed a noteworthy decrease in tissue damage, along with improved preservation of cellular nuclei and organelles.

In alignment with prior research, it was observed that alongside abnormal kidney function and histological changes, there was an elevation in MDA levels and a decrease in the antioxidant content (specifically GSH). These results correspond with earlier studies [54, 55]. TAA can cause a significant amount of ROS to be produced, which can hinder the antioxidant defense system. GSH and other thiols, which are components of the intracellular antioxidant system, may not be adequate to repair this damage [37]. Statins have been reported to block the generation of reactive oxygen and nitrogen species, remove harmful free radicals, and boost the synthesis of vascular endothelial nitric oxide [56]. Accordingly, Pita was found to reestablish the oxidant-antioxidant equilibrium in the renal tissue.

Importantly, the effects of miR-93/AKT/mTOR signaling pathways and the link to Pita in the pathophysiology of renal tissue injury have never been elucidated *in vivo* through the context of xenobiotic toxicity.

MicroRNAs (miRNAs) play a significant role in various biological processes, including kidney injury and repair. One specific miRNA that has been studied in the context of kidney injury is miR-93. In addition, PTEN (phosphatase and tensin homolog) is a critical protein involved in regulating cell growth and survival [57]. Studies have shown that miR-93 can target PTEN, leading to its downregulation. This downregulation of PTEN, in turn, can contribute to kidney injury by promoting cell proliferation, survival, and inflammation. During kidney injury, there can be an upregulation of miR-93, leading to decreased PTEN levels and subsequent alterations in cell mir-93/AKT/mTOR signaling pathways. These changes may impact the overall injury and repair processes in the kidney [58]. It is worth noting that the study of miRNAs in kidney injury is an active area of research, and ongoing investigations continue to shed light on their specific roles and potential therapeutic implications. Our findings are consistent with the data presented, showing that miR-93 is linked to the activation of the AKT/mTOR pathway, resulting in kidney injury. On the contrary, the administration of Pita treatment in the current study successfully halted the miR-93 level, leading to the upregulation of the PTEN level. This, in turn, suppressed the AKT/mTOR pathway in the kidney, leading to the improvement and resolution of kidney injury.

A crucial transcription factor known as Stat3 controls cytokine-dependent inflammation and immunity [59, 60]. Cytokine receptors stimulate the Janus kinase (JAK)-Stats signaling cascade and phosphorylate Stat3. As a result, Stat3 either pairs up with other Stat proteins to form stable homodimers or heterodimers. These activated Stats then

react to cytokines, creating a feed-forward inflammation loop within the affected microenvironment [61]. Studies using experimental models of kidney diseases have examined the role of Stat3 in terms of pathophysiology and the protective effects of Stat3 inhibition [62]. Stat3 is the target of miR-93 [63]. As shown by the decreased Stat3 expression generated by TAA in the renal tissue, Pita was able to lessen Stat3 activation in this case, protecting against renal injury. The observed results are consistent with the investigation of Al-Shabrawey et al. [64], who demonstrated that simvastatin could protect against the early signs of diabetic retinopathy by preventing NADPH oxidase-mediated activation of Stat3.

Further research is needed utilizing genetic manipulation of miR-93 to conclusively demonstrate its direct interactions with target genes like PTEN and precise impacts on Akt/mTOR signaling in this context. While beyond the scope of the current study, such molecular examination through gain and loss of function experiments represents an important future direction to complement our findings.

## 5. Conclusions

In this study, we demonstrate for the first time that pitavastatin attenuates xenobiotic-induced kidney injury in rats by regulating the miR-93/PTEN/Akt/mTOR signaling pathway. Specifically, we show that thioacetamide causes marked impairment in renal function and histopathological damage associated with oxidative stress, inflammation, and apoptosis. Pitavastatin treatment mitigated these harmful effects and preserved normal kidney structure and function. Mechanistically, our central finding is that the renoprotective impact of pitavastatin involves the suppression of miR-93 expression and subsequent activation of PTEN. This shifts the balance of Akt/mTOR signaling to ameliorate molecular processes driving kidney toxicity. Taken together, these novel results substantially advance understanding of the pleiotropic benefits of statins in the kidney by defining a specific molecular cascade—the miR-93/PTEN/Akt/mTOR axis—underlying their protective capacities.

Overall, this research highlights the therapeutic potential of pitavastatin in contexts of toxicant-mediated oxidative injury and inflammation in the kidney. Our work suggests that the modulation of miR-93 and associated signaling could be a promising pharmacological approach to attenuate the progression of acute kidney disease. These findings merit further investigation of the clinical applicability of statins to preserve renal function in patient populations at high risk of kidney damage due to xenobiotic exposures or conditions causing oxidative stress.

## Data Availability

The data used to support the findings of this study are included within the article.

## Disclosure

The funders had no role in the study design, data collection and analysis, the decision to publish, or preparation of the manuscript.



## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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## References

- [1] R. Bellomo, J. A. Kellum, and C. Ronco, "Acute kidney injury," *The Lancet*, vol. 380, no. 9843, pp. 756–766, 2012.
- [2] Y. Fang, J. Teng, and X. Ding, "Acute kidney injury in China," *Hemodialysis International*, vol. 19, no. 1, pp. 2–10, 2015.
- [3] G. Zhang, Q. Wang, W. Wang et al., "Tempol protects against acute renal injury by regulating PI3K/Akt/mTOR and GSK3 $\beta$  signaling cascades and afferent arteriolar activity," *Kidney and Blood Pressure Research*, vol. 43, no. 3, pp. 904–913, 2018.
- [4] L. He, Q. Wei, J. Liu et al., "AKI on CKD: heightened injury, suppressed repair, and the underlying mechanisms," *Kidney International*, vol. 92, no. 5, pp. 1071–1083, 2017.
- [5] R. L. Mehta, J. A. Kellum, S. V. Shah et al., "Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury," *Critical Care*, vol. 11, no. 2, p. R31, 2007.
- [6] F. Tong and X. Zhou, "The Nrf2/HO-1 pathway mediates the antagonist effect of L-arginine on renal ischemia/reperfusion injury in rats," *Kidney and Blood Pressure Research*, vol. 42, no. 3, pp. 519–529, 2017.
- [7] J. T. Mendell and E. N. Olson, "MicroRNAs in stress signaling and human disease," *Cell*, vol. 148, no. 6, pp. 1172–1187, 2012.
- [8] T. Brandenburger and J. M. Lorenzen, "Diagnostic and therapeutic potential of microRNAs in acute kidney injury," *Frontiers in Pharmacology*, vol. 11, p. 657, 2020.
- [9] H. Hu, W. Jiang, X. Xi, C. Zou, and Z. Ye, "MicroRNA-21 attenuates renal ischemia reperfusion injury via targeting caspase signaling in mice," *American Journal of Nephrology*, vol. 40, no. 3, pp. 215–223, 2014.
- [10] J. Hao, Q. Wei, S. Mei et al., "Induction of microRNA-17-5p by p53 protects against renal ischemia-reperfusion injury by targeting death receptor 6," *Kidney International*, vol. 91, no. 1, pp. 106–118, 2017.
- [11] J. V. Bonventre and L. Yang, "Cellular pathophysiology of ischemic acute kidney injury," *Journal of Clinical Investigation*, vol. 121, no. 11, pp. 4210–4221, 2011.
- [12] W. Liao, Z. Fu, Y. Zou et al., "MicroRNA-140-5p attenuated oxidative stress in Cisplatin induced acute kidney injury by activating Nrf2/ARE pathway through a Keap1-independent mechanism," *Experimental Cell Research*, vol. 360, no. 2, pp. 292–302, 2017.
- [13] L. L. Lv, Y. Feng, M. Wu et al., "Exosomal miRNA-19b-3p of tubular epithelial cells promotes M1 macrophage activation in kidney injury," *Cell Death and Differentiation*, vol. 27, no. 1, pp. 210–226, 2020.
- [14] L. Jiang, X. Q. Liu, Q. Ma et al., "hsa-miR-500a-3P alleviates kidney injury by targeting MLKL-mediated necroptosis in renal epithelial cells," *The FASEB Journal*, vol. 33, no. 3, pp. 3523–3535, 2019.
- [15] L. Kotelevets, B. Trifault, E. Chastre, and M. Scott, "Post-translational regulation and conformational plasticity of PTEN," *Cold Spring Harbor Perspectives in Medicine*, vol. 10, no. 7, Article ID a036095, 2020.
- [16] A. S. Alzahrani, "PI3K/Akt/mTOR inhibitors in cancer: at the bench and bedside," *Seminars in Cancer Biology*, vol. 59, pp. 125–132, 2019.
- [17] H. Zhu, R. Xie, X. Liu et al., "MicroRNA-494 improves functional recovery and inhibits apoptosis by modulating PTEN/AKT/mTOR pathway in rats after spinal cord injury," *Biomedicine and Pharmacotherapy*, vol. 92, pp. 879–887, 2017.
- [18] K. Li, J. Liu, L. Song, W. Lv, X. Tian, and Z. Li, "Effect of Electroacupuncture Treatment at Dazhui (GV14) and Mingmen (GV4) Modulates the PI3K/AKT/mTOR Signaling Pathway in Rats after Spinal Cord Injury," *Neural Plasticity*, vol. 2020, Article ID 5474608, 13 pages, 2020.
- [19] R. Samarakoon, S. Helo, A. D. Dobberfuhr et al., "Loss of tumour suppressor PTEN expression in renal injury initiates SMAD3- and p53-dependent fibrotic responses," *The Journal of Pathology*, vol. 236, no. 4, pp. 421–432, 2015.
- [20] A. Vlad, M. Vlad, L. Petrica et al., "Therapy with atorvastatin versus rosuvastatin reduces urinary podocytes, podocyte-associated molecules, and proximal tubule dysfunction biomarkers in patients with type 2 diabetes mellitus: a pilot study," *Renal Failure*, vol. 39, no. 1, pp. 112–119, 2017.
- [21] L. Thongnak, A. Pongchaidecha, K. Jaikumkao, V. Chatsudthipong, N. Chattipakorn, and A. Lungkaphin, "The additive effects of atorvastatin and insulin on renal function and renal organic anion transporter 3 function in diabetic rats," *Scientific Reports*, vol. 7, no. 1, Article ID 13532, 2017.
- [22] N. Pengrattanachot, R. Cherngwell, K. Jaikumkao et al., "Atorvastatin attenuates obese-induced kidney injury and impaired renal organic anion transporter 3 function through inhibition of oxidative stress and inflammation," *Biochimica et Biophysica Acta, Molecular Basis of Disease*, vol. 1866, no. 6, Article ID 165741, 2020.
- [23] A. M. A. Elseidy, S. A. Bashandy, F. A. A. Ibrahim et al., "Zinc oxide nanoparticles characterization and therapeutic evaluation on high fat/sucrose diet induced-obesity," *Egyptian Journal of Chemistry*, vol. 65, pp. 497–511, 2022.
- [24] S. A. E. Bashandy, A. M. A. El-Seidy, F. A. A. Ibrahim, M. S. Abdelrahman, S. A. Abdelmottaleb Moussa, and M. A. ElBaset, "Zinc nanoparticles ameliorated obesity-induced cardiovascular disease: role of metabolic syndrome and iron overload," *Scientific Reports*, vol. 13, no. 1, Article ID 16010, 2023.
- [25] R. F. Ahmed, M. Nasr, M. Abd Elbaset et al., "Combating hematopoietic and hepatocellular abnormalities resulting from administration of cisplatin: role of liver targeted glycyrrhetic acid nanoliposomes loaded with amino acids," *Pharmaceutical Development and Technology*, vol. 27, no. 9, pp. 925–941, 2022.
- [26] S. A. M. Abdelmottaleb, F. A. A. Ibrahim, M. A. Elbaset et al., "Goldenberry (*Physalis peruviana*) alleviates hepatic oxidative stress and metabolic syndrome in obese rats," *Journal of Applied Pharmaceutical Science*, vol. 12, 2022.
- [27] H. Ma, Y. Liu, H. Xie et al., "The renoprotective effects of simvastatin and atorvastatin in patients with acute coronary syndrome undergoing percutaneous coronary intervention: an observational study," *Medicine*, vol. 96, no. 32, p. e7351, 2017.
- [28] L. Yan, L. Jiaqiong, G. Yue et al., "Atorvastatin protects against contrast-induced acute kidney injury via upregulation of

- endogenous hydrogen sulfide," *Renal Failure*, vol. 42, no. 1, pp. 270–281, 2020.
- [29] F. Qadir, S. M. Alam, A. Q. Siddiqi, and A. Kamran, "Pitavastatin is a potent anti-inflammatory agent in the rat paw model of acute inflammation," *Pakistan Journal of Pharmaceutical Sciences*, vol. 27, no. 6 Spec No, pp. 2169–2175, 2014.
- [30] R. F. Abdel-Rahman, H. M. Fayed, G. F. Asaad et al., "The involvement of TGF- $\beta$ 1/FAK/ $\alpha$ -SMA pathway in the anti-fibrotic impact of rice bran oil on thioacetamide-induced liver fibrosis in rats," *PLoS One*, vol. 16, no. 12, Article ID e0260130, 2021.
- [31] H. Cetin, S. Olgar, F. Oktem et al., "Novel evidence suggesting an anti-oxidant property for erythropoietin on vancomycin-induced nephrotoxicity in a rat model," *Clinical and Experimental Pharmacology and Physiology*, vol. 34, no. 11, pp. 1181–1185, 2007.
- [32] J. R. Delanghe and M. M. Speeckaert, "Creatinine determination according to Jaffe-what does it stand for?" *Clinical Kidney Journal*, vol. 4, no. 2, pp. 83–86, 2011.
- [33] W. Zhang, X. Chen, S. Shi et al., "Expression and activation of STAT3 in chronic proliferative immune complex glomerulonephritis and the effect of fosinopril," *Nephrology Dialysis Transplantation*, vol. 20, no. 5, pp. 892–901, 2005.
- [34] M. A. ElBaset, R. S. Salem, F. Ayman et al., "Effect of empagliflozin on thioacetamide-induced liver injury in rats: role of AMPK/SIRT-1/HIF-1 $\alpha$  pathway in halting liver fibrosis," *Antioxidants*, vol. 11, no. 11, p. 2152, 2022.
- [35] P. M. Ridker, "Rosuvastatin in the primary prevention of cardiovascular disease among patients with low levels of low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: rationale and design of the JUPITER trial," *Circulation*, vol. 108, no. 19, pp. 2292–2297, 2003.
- [36] M. Margaritis, K. M. Channon, and C. Antoniades, "Statins as regulators of redox state in the vascular endothelium: beyond lipid lowering," *Antioxidants and Redox Signaling*, vol. 20, no. 8, pp. 1198–1215, 2014.
- [37] A. Ramadan, N. Afifi, N. Z. Yassin, R. F. Abdel-Rahman, S. S. Abd El-Rahman, and H. M. Fayed, "Mesalazine, an osteopontin inhibitor: the potential prophylactic and remedial roles in induced liver fibrosis in rats," *Chemico-Biological Interactions*, vol. 289, pp. 109–118, 2018.
- [38] S. S. Abd El-Rahman and H. M. Fayed, "Targeting AngII/AT1R signaling pathway by perindopril inhibits ongoing liver fibrosis in rat," *Journal of Tissue Engineering and Regenerative Medicine*, vol. 13, no. 12, pp. 2131–2141, 2019.
- [39] M. A. Elbaset, B. M. S. A. Mohamed, P. E. Moustafa et al., "Erythropoietin suppresses the hepatic fibrosis caused by thioacetamide: role of the PI3K/Akt and TLR4 signaling pathways," in *Oxidative Medicine and Cellular Longevity*, J. L. Franco, Ed., vol. 2023, Article ID 5514248, 14 pages, 2023.
- [40] M. A. Elbaset, B. M. S. A. Mohamed, S. A. Gad et al., "Erythropoietin mitigated thioacetamide-induced renal injury via JAK2/STAT5 and AMPK pathway," *Scientific Reports*, vol. 13, no. 1, Article ID 14929, 2023.
- [41] O. S. Mohammed, H. G. Attia, B. Mohamed, M. A. Elbaset, and H. M. Fayed, "Current investigations for liver fibrosis treatment: between repurposing the FDA-approved drugs and the other emerging approaches," *Journal of Pharmacy and Pharmaceutical Sciences*, vol. 26, Article ID 11808, 2023.
- [42] S. Ogura and T. Shimosawa, "Oxidative stress and organ damages," *Current Hypertension Reports*, vol. 16, no. 8, pp. 452–455, 2014.
- [43] I. M. Ayoub, M. A. El-Baset, M. M. Elghonemy et al., "Chemical profile of *Cyperus laevigatus* and its protective effects against thioacetamide-induced hepatorenal toxicity in rats," *Molecules*, vol. 27, no. 19, p. 6470, 2022.
- [44] H. A. Ogaly, R. F. Abdel-Rahman, M. A. E. Mohamed, A. F. Oa, M. S. Khattab, and R. M. Abd-Elsalam, "Thymol ameliorated neurotoxicity and cognitive deterioration in a thioacetamide-induced hepatic encephalopathy rat model; involvement of the BDNF/CREB signaling pathway," *Food and Function*, vol. 13, no. 11, pp. 6180–6194, 2022.
- [45] M. Shibasaki, J. G. Wang, J. L. Figueiredo et al., "Pitavastatin reduces inflammation in atherosclerotic plaques in apolipoprotein E-deficient mice with late stage renal disease," *PLoS One*, vol. 10, no. 9, Article ID e0138047, 2015.
- [46] X. M. Liang, H. Otani, Q. Zhou et al., "Renal protective effects of pitavastatin on spontaneously hypercholesterolaemic Imai Rats," *Nephrology Dialysis Transplantation*, vol. 22, no. 8, pp. 2156–2164, 2007.
- [47] S. Kaushik, A. Tomar, S. Puthanmadhom Narayanan, T. C. Nag, D. S. Arya, and J. Bhatia, "Pitavastatin attenuates cisplatin-induced renal injury by targeting MAPK and apoptotic pathways," *Journal of Pharmacy and Pharmacology*, vol. 71, no. 7, pp. 1072–1081, 2019.
- [48] W. C. Kang, M. Kim, S. M. Park, B. K. Kim, B. K. Lee, and H. M. Kwon, "Preventive effect of pretreatment with pitavastatin on contrast-induced nephropathy in patients with renal dysfunction undergoing coronary procedure: PRINCIPLE-II randomized clinical trial," *Journal of Clinical Medicine*, vol. 9, no. 11, p. 3689, 2020.
- [49] H. Takahashi, Y. Takeishi, T. Arimoto et al., "Pitavastatin inhibits cardiac hypertrophy in a rat model of progressive renal injury," *Journal of Cardiovascular Pharmacology*, vol. 46, no. 4, pp. 487–493, 2005.
- [50] H. Kakuda, K. Kanasaki, D. Koya, and N. Takekoshi, "The administration of pitavastatin augments creatinine clearance associated with reduction in oxidative stress parameters: acute and early effects," *Clinical and Experimental Nephrology*, vol. 17, no. 2, pp. 240–247, 2013.
- [51] M. Abdelbaset, M. M. Safar, S. S. Mahmoud, S. A. Negm, and A. M. Agha, "Red yeast rice and coenzyme Q10 as safe alternatives to surmount atorvastatin-induced myopathy in hyperlipidemic rats," *Canadian Journal of Physiology and Pharmacology*, vol. 92, no. 6, pp. 481–489, 2014.
- [52] A. M. Al-Attar, A. A. Alrobai, and D. A. Almalki, "Protective effect of olive and juniper leaves extracts on nephrotoxicity induced by thioacetamide in male mice," *Saudi Journal of Biological Sciences*, vol. 24, no. 1, pp. 15–22, 2017.
- [53] T. Nakamura, E. Sato, N. Fujiwara et al., "Co-administration of ezetimibe enhances proteinuria-lowering effects of pitavastatin in chronic kidney disease patients partly via a cholesterol-independent manner," *Pharmacological Research*, vol. 61, no. 1, pp. 58–61, 2010.
- [54] W. A. Keshk and S. M. Zahran, "Mechanistic role of cAMP and hepatocyte growth factor signaling in thioacetamide-induced nephrotoxicity: unraveling the role of platelet rich plasma," *Biomedicine and Pharmacotherapy*, vol. 109, pp. 1078–1084, 2019.
- [55] N. B. Türkmen, H. Yüce, A. Taşlıdere, Y. Şahin, and O. Çiftçi, "The ameliorate effects of nerolidol on thioacetamide-induced oxidative damage in heart and kidney tissue," *Turkish Journal of Pharmaceutical Sciences*, vol. 19, no. 1, pp. 1–8, 2022.
- [56] J. Beltowski, "Statins and modulation of oxidative stress," *Toxicology Mechanisms and Methods*, vol. 15, no. 2, pp. 61–92, 2005.
- [57] Y. Zhan, M. Zhu, S. Liu et al., "MicroRNA-93 inhibits the apoptosis and inflammatory response of tubular epithelial

- cells via the PTEN/AKT/mTOR pathway in acute kidney injury,” *Molecular Medicine Reports*, vol. 24, no. 3, p. 666, 2021.
- [58] B. M. Hussen, S. R. Abdullah, M. F. Rasul et al., “MiRNA-93: a novel signature in human disorders and drug resistance,” *Cell Communication and Signaling*, vol. 21, no. 1, p. 79, 2023.
- [59] H. Yu, D. Pardoll, and R. Jove, “STATs in cancer inflammation and immunity: a leading role for STAT3,” *Nature Reviews Cancer*, vol. 9, no. 11, pp. 798–809, 2009.
- [60] N. F. Abdelkader, M. A. Elbaset, P. E. Moustafa, and S. M. Ibrahim, “Empagliflozin mitigates type 2 diabetes-associated peripheral neuropathy: a glucose-independent effect through AMPK signaling,” *Archives of Pharmacal Research*, vol. 45, no. 7, pp. 475–493, 2022.
- [61] P. Przanowski, M. Dabrowski, A. Ellert-Miklaszewska et al., “The signal transducers Stat1 and Stat3 and their novel target Jmjd3 drive the expression of inflammatory genes in microglia,” *Journal of Molecular Medicine (Berlin)*, vol. 92, no. 3, pp. 239–254, 2014.
- [62] J. Y. Park, K. D. Yoo, E. Bae et al., “Blockade of STAT3 signaling alleviates the progression of acute kidney injury to chronic kidney disease through antiapoptosis,” *American Journal of Physiology- Renal Physiology*, vol. 322, no. 5, pp. F553–F572, 2022.
- [63] Y. Wang, S. Chen, J. Wang et al., “MicroRNA-93/STAT3 signalling pathway mediates retinal microglial activation and protects retinal ganglion cells in an acute ocular hypertension model,” *Cell Death and Disease*, vol. 12, no. 1, p. 41, 2021.
- [64] M. Al-Shabrawey, M. Bartoli, A. B. El-Remessy et al., “Role of NADPH oxidase and Stat3 in statin-mediated protection against diabetic retinopathy,” *Investigative Ophthalmology and Visual Science*, vol. 49, no. 7, pp. 3231–3238, 2008.