



Article

# A Novel Protective Strategy Against Metformin-Induced Renal Injury Involving Adenosine Triphosphate and Thiamine Pyrophosphate

Huseyin Kocaturk <sup>1,\*</sup>, Fevzi Bedir <sup>1</sup>, Bulent Yavuzer <sup>2</sup>, Esra Tuba Sezgin <sup>3</sup>, Renad Mammadov <sup>2</sup>, Bahadir Suleyman <sup>2</sup>, Cengiz Sarigul <sup>4</sup>, Ferda Keskin Cimen <sup>5</sup>, Mehmet Sefa Altay <sup>1</sup> and Halis Suleyman <sup>2</sup>

<sup>1</sup> Department of Urology, Erzurum City Hospital, University of Health Sciences, Erzurum 25240, Turkey; fevzi.bedir@sbu.edu.tr (F.B.); memsefaaltay@gmail.com (M.S.A.)

<sup>2</sup> Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkey; bulent.yavuzer@erzincan.edu.tr (B.Y.); rammadov@erzincan.edu.tr (R.M.); bsuleyman@erzincan.edu.tr (B.S.); hsuleyman@erzincan.edu.tr (H.S.)

<sup>3</sup> Anesthesia Program, Vocational School of Health Services, Erzincan Binali Yildirim University, Erzincan 24036, Turkey; esra.demir@erzincan.edu.tr

<sup>4</sup> Department of Medical Biochemistry, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkey; cengiz.sarigul@erzincan.edu.tr

<sup>5</sup> Department of Pathology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkey; ferda.keskin@erzincan.edu.tr

\* Correspondence: huseyin.kocaturk@sbu.edu.tr

## Abstract

Metformin is widely used in type 2 diabetes, but its effects on oxidative and inflammatory pathways remain controversial. Beyond glycemic control, it may promote lactic acidosis by impairing mitochondrial metabolism and pyruvate flux. The potential renoprotective roles of adenosine triphosphate (ATP) and thiamine pyrophosphate (TPP) remain poorly defined. This study aimed to evaluate whether ATP and TPP mitigate metformin-induced renal injury through biochemical and histopathological assessments. Wistar rats were randomly divided into six groups: control, ATP, TPP, metformin, ATP + metformin, and TPP + metformin. Metformin (50 mg/kg, oral), ATP (4 mg/kg, intraperitoneal), or TPP (20 mg/kg, intraperitoneal) was administered daily for 10 days. Oxidative stress markers, inflammatory cytokines, renal histopathology, and serum creatinine, BUN, lactate, and LDH levels were evaluated. Metformin induced significant oxidative stress, inflammation, metabolic disturbance, and renal injury. ATP provided partial protection, whereas TPP markedly restored redox balance, reduced inflammation, and preserved renal histology. TPP confers superior protection against metformin-induced renal injury compared with ATP by modulating oxidative, inflammatory, and metabolic pathways, highlighting its therapeutic potential in preventing metformin-related nephrotoxicity.

**Keywords:** metformin; ATP; TPP; oxidative stress; inflammation; renal injury



Academic Editors: Matteo Perra,  
Maria Letizia Manca, Amparo Nacher,  
Ines Castangia and Mohamad Allaw

Received: 28 January 2026

Revised: 8 February 2026

Accepted: 12 February 2026

Published: 14 February 2026

**Copyright:** © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

## 1. Introduction

Metformin, a dimethylbiguanide-derived pharmaceutical agent, was first synthesized in 1929, and its potent hypoglycemic effect was subsequently demonstrated [1]. Its pharmacological development was inspired by its natural origin, *Galega officinalis*, a plant that has been used for centuries in traditional folk medicine and is commonly referred to as French lilac [2]. As a member of the biguanide class, metformin is widely accepted as a first-line

therapeutic agent in the management of type 2 diabetes mellitus (T2DM) [3]. Additionally, in 2022, it received approval for use in Europe for the treatment of T2DM during pregnancy and the periconceptional period, as well as for the management of polycystic ovary syndrome [4]. In diabetes, chronic hyperglycemia is characterized by increased production of reactive oxygen species (ROS), depletion of antioxidant defenses, and the presence of low-grade systemic inflammation [5]. In this context, the literature provides numerous lines of evidence indicating that metformin exerts antioxidant and anti-inflammatory actions that extend beyond its well-established role in glycemic control [6]. At the clinical level, studies in patients with T2DM have reported reductions in lipid and protein oxidation and in markers of nitrosative stress, accompanied by increases in superoxide dismutase (SOD) and catalase (CAT) activities and decreases in pro-inflammatory cytokine levels [7]. From a renal perspective, data in the literature also indicate that the combined use of insulin and metformin confers a renoprotective effect by alleviating oxidative stress [8]. On the other hand, there is also evidence in the literature indicating that the effects of metformin may vary depending on tissue type, dose, duration of exposure, oxygenation status, and the direction of mitochondrial electron flow, potentially resulting in pro-oxidant and/or pro-inflammatory out-comes. In certain experimental models, metformin has been reported to enhance ROS production, suppress antioxidant regulatory pathways, and amplify early-phase pro-inflammatory responses [9–12]. At the mechanistic level, metformin's inhibition of mitochondrial complex I can, under certain conditions, increase ROS production via electron leakage; moreover, the direction of the oxidative response may vary depending on the context of electron flow [5,7–9]. Thus, the existing evidence presents a markedly heterogeneous and at times contradictory picture of metformin's effects on oxidative stress and inflammatory pathways. It is proposed that metformin's fundamental glycemic effect in T2DM arises primarily through the inhibition of hepatic gluconeogenesis [13]. In addition, during treatment, a decrease in lactate elimination, an increase in lactate production, and the resulting development of lactic acidosis have been reported [14]. This condition is referred to in the literature as metformin-associated lactic acidosis (MALA) [15]. Corchia et al. emphasized that MALA can be a life-threatening condition, often necessitates renal replacement therapy, and may require discontinuation of metformin in patients with renal disease [16]. It is also stated that hemodialysis represents the most effective therapeutic approach in the management of MALA [17]. Metformin reduces ATP production by inhibiting complex I of the mitochondrial respiratory chain in these tissues, and the accompanying suppression of gluconeogenesis increases lactate production [18]. An increase in lactate production is often accompanied by a rise in pyruvate levels [19]. The conversion of pyruvate to acetyl-CoA prior to its entry into the Krebs cycle is catalyzed by the PDH complex, a process for which TPP serves as an obligatory cofactor [20]. Moreover, in clinical settings, the acidosis observed in the coexistence of MALA and acute renal injury may further deepen mitochondrial dysfunction and thereby intensify this oxidative-inflammatory cycle [21]. This biochemical and pathophysiological framework supports the notion that providing TPP, a cofactor at the PDH junction, may redirect pyruvate flux toward oxidative phosphorylation and thereby reduce the lactate/pyruvate burden. Moreover, it suggests that strengthening cellular energy balance through ATP supplementation could indirectly mitigate the severity of oxidative stress and inflammation. Indeed, TPP deficiency/lability has been identified as a factor that increases the risk of lactic acidosis. Moreover, decreases in ATP levels are known to facilitate the injury cascade, particularly within tubular cells [22,23]. Evidence derived from the existing literature underscores the critical role of reduced intracellular ATP levels in the pathogenesis of metformin-associated renal injury. Moreover, a review of the available literature indicates that no studies have been identified that investigate the potential protective effects of ATP

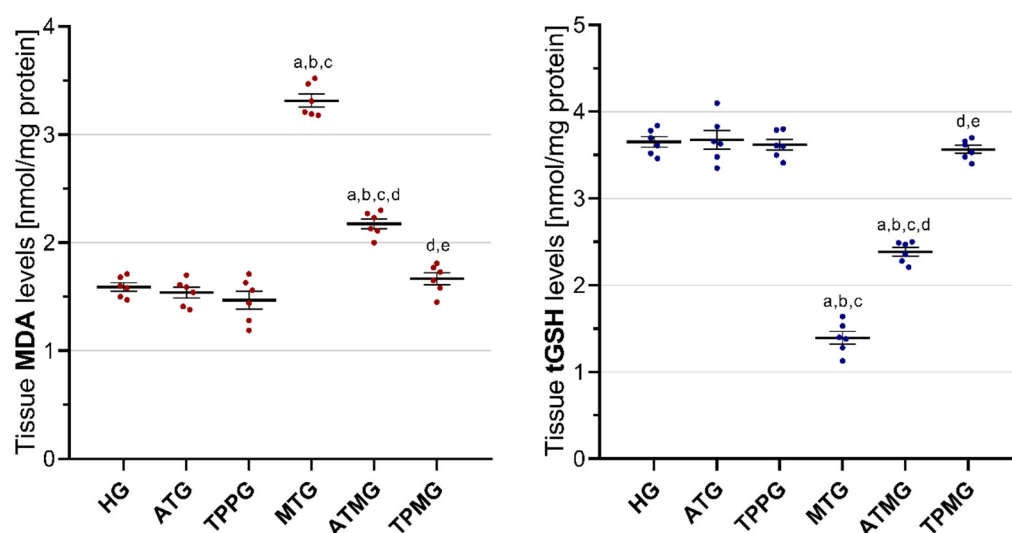
and TPP against metformin-induced renal damage. Accordingly, the present study aims to evaluate the potential renoprotective effects of ATP and TPP against metformin-induced renal injury in rats through comprehensive biochemical and histopathological assessments.

## 2. Results

### 2.1. Biochemical Findings

#### 2.1.1. Analysis of MDA and tGSH Levels in Kidney Tissue

As shown in Figure 1, the malondialdehyde (MDA) levels in renal tissue of rats receiving ATP alone (ATG;  $1.54 \pm 0.05$ ) or TPP alone (TPPG;  $1.47 \pm 0.08$ ) were comparable to those of the healthy control group (HG;  $1.59 \pm 0.04$ ), and the differences were not statistically significant (ATP alone (ATG) vs. HG,  $p = 0.987$ ; TPP alone (TPPG) vs. HG,  $p = 0.667$ ). In contrast, MDA levels in the metformin-only group (metformin alone (MTG);  $3.31 \pm 0.06$ ) were markedly higher compared with the healthy, ATP, and TPP groups (MTG vs. HG,  $p < 0.001$ ; MTG vs. ATG,  $p < 0.001$ ; MTG vs. TPPG,  $p < 0.001$ ). Co-administration of ATP (ATP and metformin (ATMG);  $2.17 \pm 0.05$ ) or TPP (TPP and metformin (TPMG);  $1.67 \pm 0.05$ ) significantly suppressed the metformin-induced increase in MDA levels (ATMG vs. MTG,  $p < 0.001$ ; TPMG vs. MTG,  $p < 0.001$ ). Notably, TPP exerted a more pronounced suppressive effect on MDA elevation than ATP (TPMG vs. ATMG,  $p < 0.001$ ).

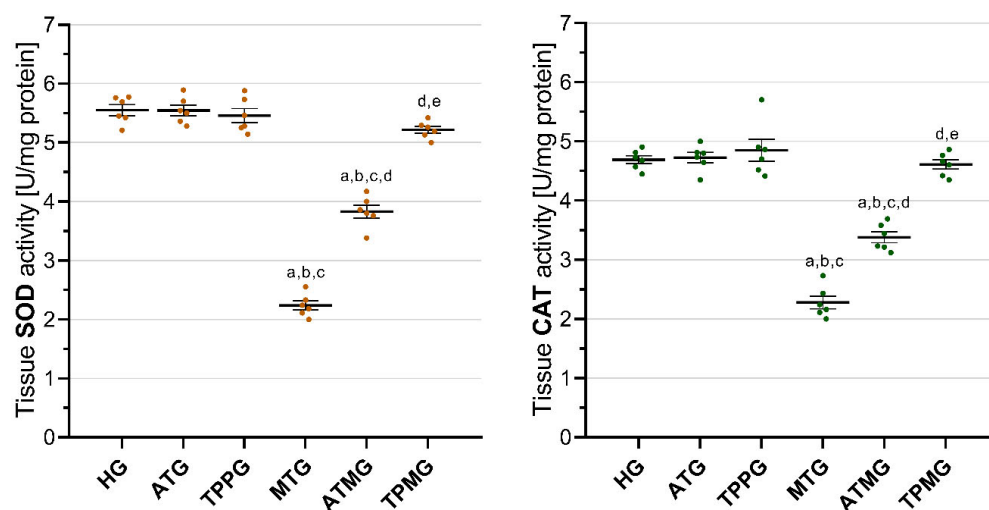


**Figure 1.** Renal MDA and tGSH levels in experimental groups. Data are expressed as mean  $\pm$  SEM ( $n = 6$ ). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. a indicates vs. HG  $p < 0.001$ , b indicates vs. ATG  $p < 0.001$ , c indicates vs. TPPG  $p < 0.001$ , d indicates vs. MTG  $p < 0.001$ , e indicates vs. ATMG  $p < 0.001$ . All values are expressed as mean  $\pm$  SEM (standard error of the mean). Abbreviations: HG, healthy group; ATG, ATP-only group; TPPG, TPP-only group; MTG, metformin-only group; ATMG, ATP + metformin group; TPMG, TPP + metformin; MDA, malondialdehyde; tGSH, total glutathione.

Total glutathione (tGSH) levels in renal tissue were comparable among the HG ( $3.65 \pm 0.06$ ), ATG ( $3.68 \pm 0.11$ ), and TPPG ( $3.62 \pm 0.06$ ) groups, with no statistically significant differences (ATG vs. HG,  $p = 1.000$ ; TPPG vs. HG,  $p = 0.999$ ). In contrast, tGSH levels were significantly reduced in the MTG ( $1.39 \pm 0.07$ ) compared with the HG, ATG, and TPPG groups (all  $p < 0.001$ ). Co-administration of ATP (ATMG;  $2.39 \pm 0.05$ ) or TPP (TPMG;  $3.57 \pm 0.05$ ) significantly attenuated the metformin-induced depletion of tGSH (both  $p < 0.001$  vs. MTG). Notably, TPP provided significantly greater protection against tGSH reduction than ATP (TPMG vs. ATMG,  $p < 0.001$ ) (Figure 1).

### 2.1.2. Analysis of SOD and CAT Activities in Kidney Tissue

Figure 2 shows that renal SOD activity was comparable among the HG ( $5.55 \pm 0.09$ ), ATG ( $5.55 \pm 0.09$ ), and TPPG ( $5.46 \pm 0.12$ ) groups, with no statistically significant differences (ATG vs. HG,  $p = 1.000$ ; TPPG vs. HG,  $p = 0.980$ ). In contrast, SOD activity was markedly reduced in the MTG ( $2.24 \pm 0.08$ ) compared with the HG, ATG, and TPPG groups (all  $p < 0.001$ ). Co-administration of ATP (ATMG;  $3.83 \pm 0.11$ ) or TPP (TPMG;  $5.22 \pm 0.06$ ) significantly attenuated the metformin-induced reduction in SOD activity (both  $p < 0.001$  vs. MTG), with TPP providing significantly greater protection than ATP (TPMG vs. ATMG,  $p < 0.001$ ).

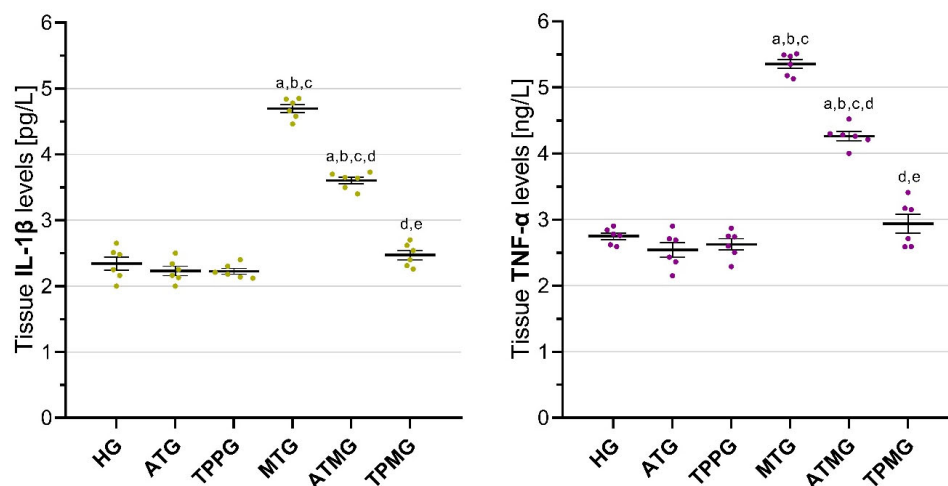


**Figure 2.** Renal SOD and CAT activities in experimental groups. Data are expressed as mean  $\pm$  SEM ( $n = 6$ ). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. a indicates vs. HG  $p < 0.001$ , b indicates vs. ATG  $p < 0.001$ , c indicates vs. TPPG  $p < 0.001$ , d indicates vs. MTG  $p < 0.001$ , e indicates vs. ATMG  $p < 0.001$ . All values are expressed as mean  $\pm$  SEM (standard error of the mean). Abbreviations: HG, healthy group; ATG, ATP-only group; TPPG, TPP-only group; MTG, metformin-only group; ATMG, ATP + metformin group; TPMG, TPP + metformin; SOD, superoxide dismutase; CAT, catalase.

Similarly, renal CAT activity did not differ significantly among the HG ( $4.69 \pm 0.07$ ), ATG ( $4.72 \pm 0.09$ ), and TPPG ( $4.85 \pm 0.19$ ) groups (ATG vs. HG,  $p = 1.000$ ; TPPG vs. HG,  $p = 0.911$ ), whereas it was significantly decreased in the MTG ( $2.28 \pm 0.11$ ) compared with the HG, ATG, and TPPG groups (all  $p < 0.001$ ). Co-administration of ATP (ATMG;  $3.38 \pm 0.09$ ) or TPP (TPMG;  $4.61 \pm 0.08$ ) significantly mitigated the metformin-induced suppression of CAT activity (both  $p < 0.001$  vs. MTG), with TPP exerting a more pronounced protective effect than ATP (TPMG vs. ATMG,  $p < 0.001$ ) (Figure 2).

### 2.1.3. Analysis of IL-1 $\beta$ and TNF- $\alpha$ Levels in Kidney Tissue

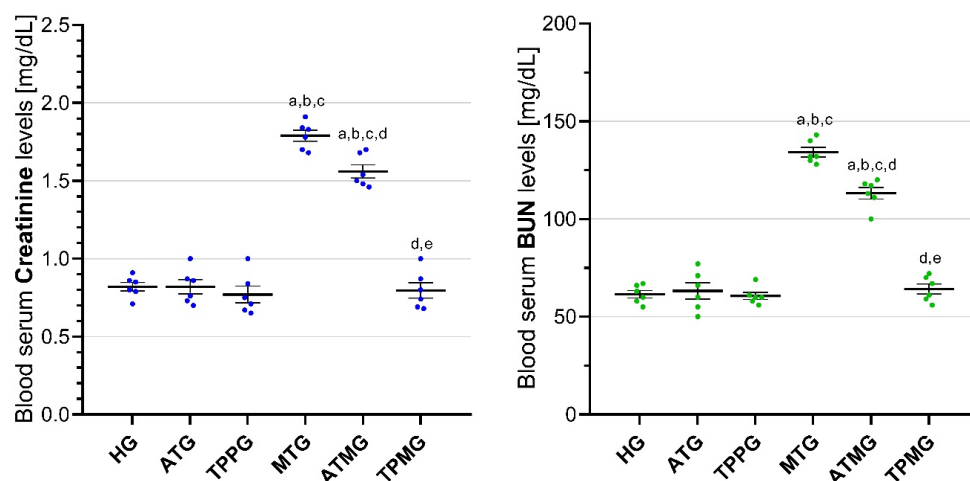
Figure 3 shows that renal IL-1 $\beta$  and TNF- $\alpha$  levels were comparable among the HG (IL-1 $\beta$ ,  $2.34 \pm 0.10$ ; TNF- $\alpha$ ,  $2.75 \pm 0.05$ ), ATG (IL-1 $\beta$ ,  $2.23 \pm 0.07$ ; TNF- $\alpha$ ,  $2.54 \pm 0.11$ ), and TPPG (IL-1 $\beta$ ,  $2.23 \pm 0.04$ ; TNF- $\alpha$ ,  $2.63 \pm 0.09$ ) groups, with no statistically significant differences. In contrast, both cytokines were significantly elevated in the MTG (IL-1 $\beta$ ,  $4.70 \pm 0.06$ ; TNF- $\alpha$ ,  $5.36 \pm 0.07$ ) compared with the HG, ATG, and TPPG groups (all  $p < 0.001$ ). Co-administration of ATP (ATMG; IL-1 $\beta$ ,  $3.60 \pm 0.05$ ; TNF- $\alpha$ ,  $4.26 \pm 0.07$ ) or TPP (TPMG; IL-1 $\beta$ ,  $2.47 \pm 0.07$ ; TNF- $\alpha$ ,  $2.94 \pm 0.14$ ) significantly attenuated the metformin-induced increases in IL-1 $\beta$  and TNF- $\alpha$  (both  $p < 0.001$  vs. MTG). Notably, TPP exerted a significantly stronger inhibitory effect than ATP on both cytokines (TPMG vs. ATMG,  $p < 0.001$ ) (Figure 3).



**Figure 3.** Renal IL-1 $\beta$  and TNF- $\alpha$  levels in experimental groups. Data are expressed as mean  $\pm$  SEM ( $n = 6$ ). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. a indicates vs. HG  $p < 0.001$ , b indicates vs. ATG  $p < 0.001$ , c indicates vs. TPPG  $p < 0.001$ , d indicates vs. MTG  $p < 0.001$ , e indicates vs. ATMG  $p < 0.001$ . All values are expressed as mean  $\pm$  SEM (standard error of the mean). Abbreviations: HG, healthy group; ATG, ATP-only group; TPPG, TPP-only group; MTG, metformin-only group; ATMG, ATP + metformin group; TPMG, TPP + metformin; IL-1 $\beta$ , interleukin-1 beta; TNF- $\alpha$ , tumor necrosis factor-alpha.

#### 2.1.4. Analysis of Serum Creatinine and BUN Levels as Renal Function Indicators

As shown in Figure 4, serum creatinine and BUN levels were similar among the HG ( $0.82 \pm 0.03$  and  $61.50 \pm 1.91$ ), ATG ( $0.82 \pm 0.05$  and  $63.17 \pm 4.13$ ), and TPPG ( $0.77 \pm 0.05$  and  $60.67 \pm 1.82$ ) groups, with no statistically significant differences. In contrast, both parameters were significantly elevated in the MTG ( $1.79 \pm 0.04$  and  $134.17 \pm 2.43$ ) compared with the HG, ATG, and TPPG groups (all  $p < 0.001$ ). Co-administration of ATP (ATMG;  $1.56 \pm 0.04$  and  $113.17 \pm 2.96$ ) or TPP (TPMG;  $0.80 \pm 0.05$  and  $64.17 \pm 2.63$ ) significantly attenuated the metformin-induced increases in serum creatinine and BUN (ATMG vs. MTG:  $p = 0.009$  and  $p < 0.001$ ; TPMG vs. MTG: both  $p < 0.001$ ).

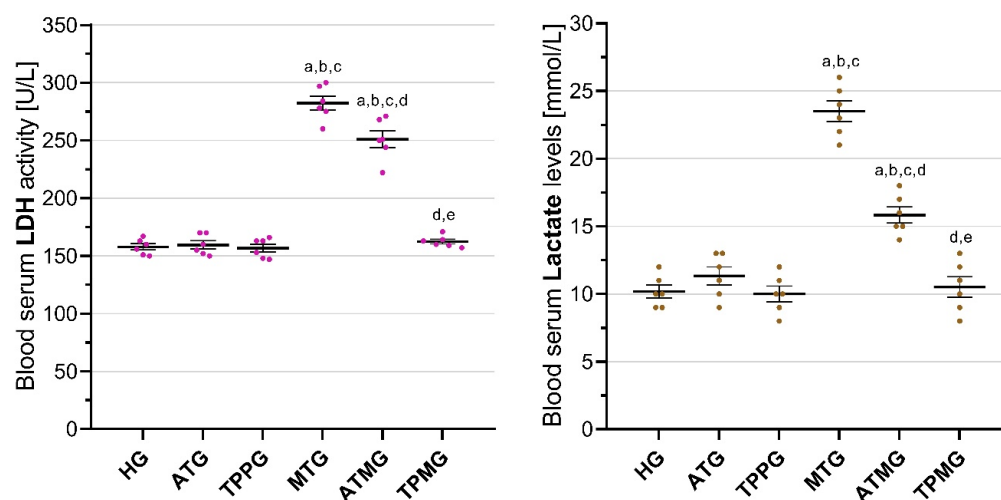


**Figure 4.** Serum creatinine and BUN levels in experimental groups. Data are expressed as mean  $\pm$  SEM ( $n = 6$ ). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. a indicates vs. HG  $p < 0.001$ , b indicates vs. ATG  $p < 0.001$ , c indicates vs. TPPG  $p < 0.001$ , d indicates vs. MTG  $p < 0.05$ , e indicates vs. ATMG  $p < 0.001$ . All values are expressed as mean  $\pm$  SEM (standard error of the mean). Abbreviations: HG, healthy group; ATG, ATP-only group; TPPG, TPP-only group; MTG, metformin-only group; ATMG, ATP + metformin group; TPMG, TPP + metformin; BUN, blood urea nitrogen.

Notably, TPP exerted a significantly stronger protective effect than ATP, restoring both parameters to near-control levels (TPMG vs. ATMG,  $p < 0.001$ ) (Figure 4).

### 2.1.5. Serum LDH Levels Analysis Results

As shown in Figure 5, serum LDH levels were comparable among the HG ( $157.83 \pm 2.75$ ), ATG ( $159.50 \pm 3.59$ ), and TPPG ( $156.67 \pm 3.41$ ) groups, with no statistically significant differences. In contrast, LDH levels were significantly elevated in the MTG ( $282.33 \pm 6.06$ ) compared with the HG, ATG, and TPPG groups (all  $p < 0.001$ ). Co-administration of ATP (ATMG;  $251.00 \pm 7.26$ ) or TPP (TPMG;  $162.33 \pm 2.03$ ) significantly attenuated the metformin-induced increase in LDH (both  $p < 0.001$  vs. MTG). Notably, TPP exerted a significantly stronger inhibitory effect than ATP, reducing LDH levels to near-control values (TPMG vs. ATMG,  $p < 0.001$ ; HG vs. TPMG,  $p = 0.981$ ) (Figure 5).



**Figure 5.** Serum LDH activity and blood lactate levels in experimental groups. Data are expressed as mean  $\pm$  SEM ( $n = 6$ ). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. a indicates vs. HG  $p < 0.001$ , b indicates vs. ATG  $p < 0.001$ , c indicates vs. TPPG  $p < 0.001$ , d indicates vs. MTG  $p < 0.001$ , e indicates vs. ATMG  $p < 0.001$ . All values are expressed as mean  $\pm$  SEM (standard error of the mean). Abbreviations: HG, healthy group; ATG, ATP-only group; TPPG, TPP-only group; MTG, metformin-only group; ATMG, ATP + metformin group; TPMG, TPP + metformin; LDH, lactate dehydrogenase.

### 2.1.6. Blood Lactate Levels Analysis Results

As shown in Figure 5, blood lactate levels were comparable among the HG ( $10.17 \pm 0.48$ ), ATG ( $11.33 \pm 0.67$ ), and TPPG ( $10.00 \pm 0.58$ ) groups, with no statistically significant differences. In contrast, lactate levels were significantly elevated in the MTG ( $23.50 \pm 0.76$ ) compared with the HG, ATG, and TPPG groups (all  $p < 0.001$ ). Co-administration of ATP (ATMG;  $15.83 \pm 0.60$ ) or TPP (TPMG;  $10.50 \pm 0.76$ ) significantly attenuated the metformin-induced increase in lactate levels (both  $p < 0.001$  vs. MTG). Notably, TPP exerted a significantly greater protective effect than ATP, restoring lactate levels to near-control values (TPMG vs. ATMG,  $p < 0.001$ ; HG vs. TPMG,  $p = 0.999$ ) (Figure 5).

## 2.2. Histopathological Findings

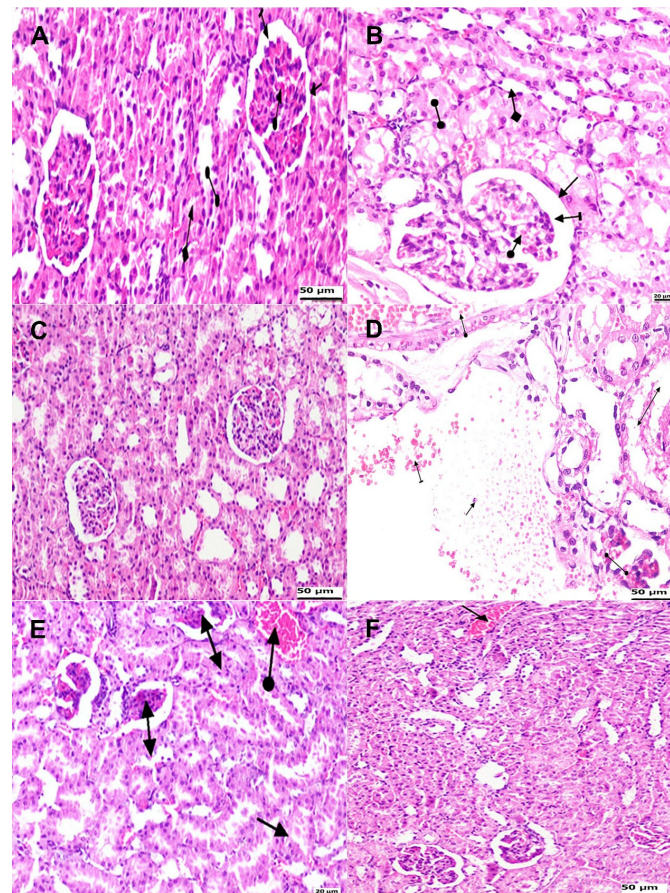
A detailed semi-quantitative evaluation of renal histopathological alterations, together with intergroup statistical comparisons, is presented in Table 1. As shown in Figure 6A, renal tissue from the HG exhibited normal microscopic architecture, characterized by intact glomerular capsule and capsular space, well-defined glomerular capillaries, and preserved morphology of proximal and distal tubules. Similarly, renal tissues from the ATG and TPPG groups displayed a normal histological appearance comparable to that of the HG, with no

detectable pathological alterations (Figure 6B,C). In contrast, renal tissues from the MTG demonstrated marked histopathological damage, with severe (grade 3) injury characterized by prominent glomerular damage, tubular degeneration, hemorrhage, interstitial edema accompanied by polymorphonuclear leukocyte infiltration, and markedly dilated and congested blood vessels (Figure 6D). In the ATMG group, these pathological changes were partially attenuated, consistent with moderate (grade 2) renal injury, including glomerular and tubular damage and vascular congestion (Figure 6E). Notably, renal tissue from the TPMG exhibited substantial histological improvement, showing an almost normal microscopic architecture, with only mildly dilated and congested blood vessels corresponding to grade 1 injury (Figure 6F).

**Table 1.** Semi-quantitative scoring of histopathological lesions in rat renal tissue.

| Groups | Glomerular Injury     | Tubular Damage        | Hemorrhage            | Interstitial Edema with PMNL Infiltration | Dilated/Congested Blood Vessels |
|--------|-----------------------|-----------------------|-----------------------|-------------------------------------------|---------------------------------|
| HG     | 0 (0–0) <sup>a</sup>  | 0 (0–0) <sup>a</sup>  | 0 (0–0) <sup>a</sup>  | 0 (0–0) <sup>a</sup>                      | 0 (0–0) <sup>a</sup>            |
| ATG    | 0 (0–0) <sup>a</sup>  | 0 (0–0) <sup>a</sup>  | 0 (0–0) <sup>a</sup>  | 0 (0–0) <sup>a</sup>                      | 0 (0–0) <sup>a</sup>            |
| TPPG   | 0 (0–0) <sup>a</sup>  | 0 (0–0) <sup>a</sup>  | 0 (0–0) <sup>a</sup>  | 0 (0–0) <sup>a</sup>                      | 0 (0–0) <sup>a</sup>            |
| MTG    | 3 (2–3) <sup>c</sup>  | 3 (2–3) <sup>c</sup>  | 3 (2–3) <sup>c</sup>  | 3 (2–3) <sup>c</sup>                      | 3 (2–3) <sup>c</sup>            |
| ATMG   | 2 (2–3) <sup>b</sup>  | 2 (2–3) <sup>b</sup>  | 2 (2–3) <sup>b</sup>  | 2 (2–3) <sup>b</sup>                      | 2 (2–3) <sup>b</sup>            |
| TPMG   | 0 (0–1) <sup>ab</sup> | 0 (0–1) <sup>ab</sup> | 0 (0–1) <sup>ab</sup> | 0 (0–1) <sup>ab</sup>                     | 1 (0–2) <sup>ab</sup>           |

Data are presented as median (min–max). Overall group differences were analyzed using the Kruskal–Wallis test. When a statistically significant difference was detected, pairwise comparisons were performed using Dunn’s post hoc test with adjustment for multiple comparisons. Groups sharing the same superscript letter are not significantly different ( $p < 0.05$ ). All groups consisted of six animals ( $n = 6$ ).



**Figure 6.** Representative photomicrographs of the renal tissue. (A) HG group showing the normal microscopic architecture of the glomerular capsule (straight arrow), the capsular space (striped arrow),

the glomerular capillaries (round-tipped arrow), the distal tubule (double round-tipped arrow) and the proximal tubule (square-tipped arrow) (H&E,  $\times 100$ ). (B) ATG group demonstrating the normal microscopic architecture of the glomerular capsule (straight arrow), the capsular space (striped arrow), glomerular capillaries (round-tipped arrow), distal tubule (square-tipped arrow), and proximal tubule (double round-tipped arrow) (H&E,  $\times 200$ ). (C) Representative photomicrograph of the renal tissue from the TPPG group demonstrating normal microscopic architecture (H&E,  $\times 100$ ). (D) Representative photomicrograph of the renal tissue from the MTG group demonstrating severe glomerular injury (double round-tipped arrow), tubular damage (double-headed arrow), hemorrhage (striped arrow), edema with PMNL infiltration (straight arrow), and dilated-congested blood vessels (round-tipped arrow) (H&E,  $\times 100$ ). (E) Representative photomicrograph of the renal tissue from the ATMG group demonstrating partial glomerular injury (double-headed arrow), tubular damage (single-headed arrow), and dilated-congested blood vessels (round-tipped arrow), consistent with moderate histopathological damage (H&E,  $\times 100$ ). (F) Representative photomicrograph of the renal tissue from the TPMG group demonstrating an almost normal microscopic appearance, with the exception of dilated-congested blood vessels (straight arrow) (H&E,  $\times 100$ ).

### 3. Discussion

In this study, the effects of metformin-induced oxidative stress, inflammation, and lactic acidosis on renal tissue were investigated using biochemical and histopathological analyses, and the therapeutic potential of ATP and TPP against this toxicity was evaluated. Our findings demonstrated that metformin induces marked biochemical and histopathological alterations in renal tissue, and that treatment with ATP and, in particular, TPP significantly alleviates these changes. Metformin has been widely reported to exert renoprotective effects, particularly in experimental and clinical models of metabolic syndrome and diabetic nephropathy [24,25]. These protective effects have been attributed to mechanisms such as attenuation of oxidative stress, suppression of inflammatory signaling pathways, and improvement of mitochondrial function [24]. Nevertheless, an increasing body of evidence indicates that the renal effects of metformin may vary depending on factors including dose, duration of exposure, metabolic status, and the underlying pathological environment [26]. In this context, the renal injury observed in the present study should be interpreted not as a contradiction of metformin's established renoprotective profile, but rather within the framework of the specific experimental conditions employed.

Although the clinical use of metformin is well established, its cellular effects are primarily explained by the inhibition of mitochondrial complex I, activation of adenosine monophosphate-activated protein kinase (AMPK), and suppression of gluconeogenesis [1,2,5,11,13]. The effect on complex I may weaken oxidative phosphorylation and thereby disrupt redox balance and alter ROS dynamics, as well as oxidant/antioxidant parameters [5,11,13]. In this context, metformin may create a basis for increased oxidative stress by reducing ATP production [18]. Although the mechanism underlying metformin-induced nephrotoxicity has not been fully elucidated, oxidative stress stands out as a critical contributing factor [27,28]. Given the high energy demands of the kidney, it is understood that reduced ATP levels and increased oxidative stress may contribute to microvascular dysfunction [29]. The literature regarding the relationship between metformin and oxidative stress is heterogeneous. While some studies emphasize its antioxidant and anti-inflammatory effects [6–8], others have reported increased ROS generation, suppression of antioxidant regulatory pathways, and a strengthened early-phase pro-inflammatory response [9–12]. In our study, MDA, a terminal and cumulative product of lipid peroxidation, was found to be significantly elevated in the metformin-treated group compared with the healthy control group. This finding is consistent with the results reported by Deng et al., who observed increased MDA levels following metformin administration [10], although it contrasts with studies reporting reductions in MDA levels [30,31]. Studies demonstrat-

ing that levels of hexanoyl-lysine (HEL), an early-stage marker of lipid peroxidation, are increased in experimental models of renal injury and reflect enhanced lipid peroxidation under nephrotoxic conditions are available in the literature [32]. Although HEL was not directly assessed in the present study, the observed elevation in MDA levels supports the presence of ongoing and sustained lipid peroxidation in renal tissue following metformin exposure. GSH, one of the principal components of the endogenous antioxidant defense system, is a non-protein intracellular thiol [33], and its stores have been shown to decline in the kidney under conditions of drug-induced oxidative stress [34]. According to our experimental results, tGSH levels were markedly reduced in the metformin group compared with the healthy group, and, in parallel with this decrease, the activities of the ROS-scavenging enzymes SOD and CAT were likewise significantly reduced. Although these findings are at odds with studies reporting that metformin enhances the antioxidant system [6,30,31], they remain consistent with preclinical evidence demonstrating reductions in tGSH, SOD, and CAT [10]. Taken together, these findings indicate that metformin suppresses antioxidant defenses by increasing oxidative imbalance.

It is well established that oxidative stress elevates cytokine levels by triggering pro-inflammatory signaling cascades, most notably nuclear factor-kappa B (NF- $\kappa$ B), within affected tissues [35]. It has been reported that metformin suppresses NF- $\kappa$ B through AMPK activation across multiple tissues, thereby diminishing IL-1 $\beta$  and TNF- $\alpha$  expression [5,36,37], and reducing NF- $\kappa$ B nuclear translocation in vascular cells and hepatocytes [7]. However, this effect may vary depending on factors such as tissue type, dose, duration of administration, and the extent of oxidative stress [5,13]. Indeed, Yoon et al. demonstrated that metformin can enhance pro-inflammatory cytokine expression in certain connective tissues [12]. In our study as well, metformin administration led to a marked elevation in renal IL-1 $\beta$  and TNF- $\alpha$  levels. These findings contradict several reports that have described metformin as exerting anti-inflammatory effects [36,37].

As is well known, BUN and creatinine are widely used parameters for the evaluation of renal function [38]. These metabolites are waste products of protein metabolism, and elevations in their serum levels indicate a reduction in glomerular filtration rate and functional impairment attributable to the nephrotoxic agent [39]. Multiple case reports have noted increases in BUN and creatinine in association with metformin administration [40,41]. Conversely, several preclinical models have shown that metformin may also lower these parameters [42,43]. Our data show that metformin significantly elevates BUN and creatinine levels, a result that accords with the aforementioned case reports [40,41], and is interpreted as evidence of nephrotoxicity.

While metformin reduces glycemia at therapeutic doses by inhibiting hepatic gluconeogenesis, it may, under certain conditions, diminish lactate clearance and increase lactate production, thereby causing MALA [14,44]. Lactate formation in the body begins with the conversion of intracellular alanine and glucose to pyruvate via the LDH enzyme [45]. In vivo and in vitro evidence indicates that metformin inhibits complex I, thereby slowing NADH oxidation, increasing the NADH/NAD<sup>+</sup> ratio, and ultimately reducing oxidative phosphorylation and ATP synthesis [46–48]. This decline in cellular energy charge redirects pyruvate flux away from oxidative pathways toward LDH-mediated lactate formation, prompting the cell to accelerate lactate production in order to regenerate cytosolic NAD<sup>+</sup> [47,48]. Therefore, increases in circulating LDH and lactate are considered indicative of tissue injury or hypoxia, as well as heightened anaerobic glycolysis [49,50]. In light of our data, the marked elevations in LDH and lactate observed in the metformin group relative to healthy controls align with the redox-dependent mechanisms [46–48] that underlie MALA.

It has been reported that ATP influences renal pathophysiology not as a direct metabolic substrate, but rather as a signaling molecule acting through purinergic signaling

and the ATP-to-adenosine axis [51]. The kidney ranks among the most energy-dependent organs in the body, and the substantial metabolic demands of renal cells necessitate highly efficient, ATP-dependent energy generation [16,29]. ATP not only provides cellular energy but also functions as a critical source for ROS scavenging and the synthesis of endogenous antioxidants [52]. Additionally, activation of adenosine receptors following the rapid hydrolysis of ATP to adenosine may impart tissue-protective effects by suppressing the oxidative stress-inflammation cascade across multiple tissues, including the kidneys [53]. Within this framework, ATP supplementation may be anticipated to alleviate the oxidative, inflammatory, and nephrotoxic profile accompanying metformin administration. In our study, ATP not only suppressed the metformin-induced elevation in MDA but also markedly prevented the decreases in tGSH, SOD, and CAT levels. Moreover, ATP significantly suppressed the metformin-induced elevations in pro-inflammatory cytokines. This finding aligns with previous reports indicating that ATP exerts tissue-protective effects, particularly against drug-induced oxidative stress and inflammation [26,52,54]. Although ATP exerted more moderate effects on BUN, creatinine, and lactate, these effects were nonetheless consistent. ATP's ability to avert the metformin-induced increases in BUN and creatinine is consistent with reports documenting its renoprotective effects in models of drug-induced renal dysfunction [26,52]. Furthermore, ATP's suppression of the metformin-associated increase in lactate levels is mechanistically reinforced by evidence demonstrating that metformin diminishes ATP synthesis [46–48]. A previous study reported that elevations in blood lactic acid and serum LDH levels lead to reductions in tissue ATP and GSH content, whereas increases in ATP levels diminish these parameters [55]. These insights from the literature are concordant with our findings showing that ATP suppresses the metformin-induced increases in lactate and LDH levels.

Our study further examined the protective effect of TPP against metformin-induced renal injury. TPP more effectively than ATP prevented the metformin-induced increases in MDA, IL-1 $\beta$ , and TNF- $\alpha$ , as well as the reductions in antioxidant markers such as tGSH, SOD, and CAT in the renal tissue of the animals. This finding indicates that TPP confers substantial improvement in metformin-associated disruptions of the oxidant–antioxidant balance and in the accompanying inflammatory processes. As is well established, TPP serves as an essential cofactor for the PDH complex, thereby restoring oxidative carbon flux, stabilizing the NADH/NAD<sup>+</sup> ratio, and reactivating the GSH/GSSG cycle [56]. Dägel et al. reported that TPP protects renal tissue from amiodarone-induced oxidative injury through its antioxidant and anti-inflammatory actions [57]. Isik et al. reported that TPP ameliorates drug-induced hepatic injury through its antioxidant activity [23]. At this antioxidant-effective dose, TPP also significantly suppressed the metformin-induced elevations in renal IL-1 $\beta$  and TNF- $\alpha$  levels, exerting a more pronounced effect than ATP. The literature reports that TPP, at doses that suppress the aberrant elevation of pro-inflammatory cytokines, protects renal tissue from inflammatory injury [57].

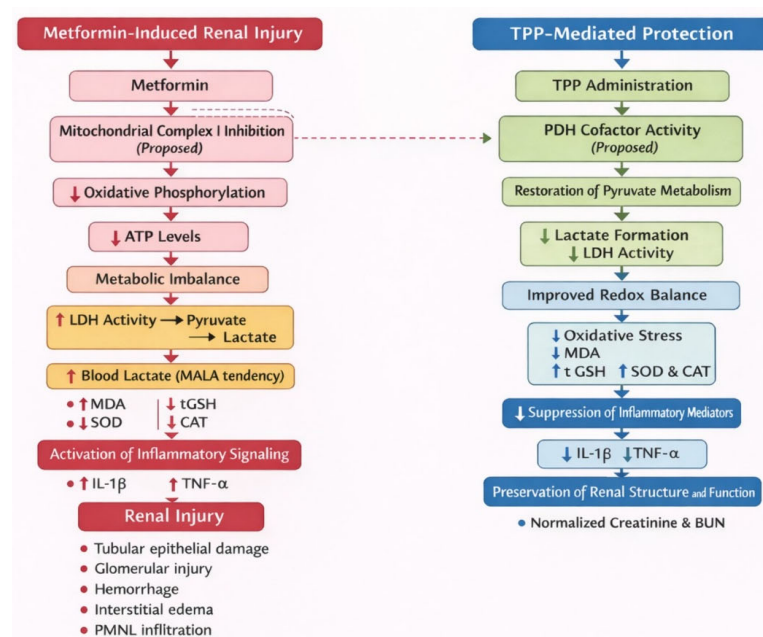
TPP not only significantly suppressed the metformin-induced elevations in oxidants and pro-inflammatory cytokines but also markedly prevented the increases in creatinine and BUN, exhibiting a more pronounced protective effect than ATP. As is well recognized, BUN and creatinine are widely employed parameters for evaluating renal function [38]. Mahdavi-fard et al. demonstrated that TPP enhances renal function in diabetic rats [58]. In this animal study, our findings on the effects of TPP on creatinine and BUN align with our previously obtained experimental results [57].

Our study also investigated the effects of TPP on serum LDH and lactate levels. The rationale for evaluating these two parameters together is that lactate is produced via the catalytic activity of the LDH enzyme [59,60]. In the setting of TPP deficiency, LDH shifts pyruvate toward lactate production, and the ensuing accumulation of lactic acid may

culminate in lactic acidosis [61]. In our study, LDH and lactate levels in the TPP group likewise supported its therapeutic efficacy, as both parameters were substantially restored toward normal following TPP administration. A study supporting our findings reported that TPP, the active metabolite of thiamine, prevents lactate accumulation, improves glucose metabolism, and enhances aerobic capacity [62]. Recent studies have highlighted that TPP inhibits the rise in LDH activity and significantly ameliorates lactic acidosis [23].

The histopathological findings obtained from all animal groups were consistent with the biochemical outcomes. In the metformin-treated group, pronounced glomerular and tubular injury, hemorrhage, and interstitial edema were evident, findings that are consistent with previous reports of metformin-induced renal damage [27,63]. However, these findings are at odds with reports suggesting that metformin may exert protective effects [64–66]. Although ATP administration yielded partial improvement in renal tissue, the TPP-treated group displayed an appearance that was nearly normal, except for the presence of dilated, congested vessels. Taken together, and consistent with previous studies, ATP [26] confers partial protection, whereas TPP [57] provides a more pronounced protective and ameliorative effect.

Although significant biochemical and histopathological improvements were observed following ATP and TPP administration, the underlying molecular mechanisms were not directly investigated in the present study. Therefore, the proposed associations between oxidative stress modulation and tissue protection should be interpreted with caution. The hypothetical model presented in Figure 7 provides a conceptual framework based on the observed findings and does not constitute direct mechanistic evidence.



**Figure 7.** Presents a hypothetical model illustrating the potential association between oxidative stress, histopathological alterations, and the observed protective effects of ATP and TPP based on biochemical and histological findings. This schematic representation is designed to provide a conceptual framework and therefore does not constitute direct mechanistic evidence. Upward arrow (↑) indicates increase, whereas downward arrow (↓) indicates decrease.

This study has several noteworthy limitations. Functional parameters of experimental animals, such as body weight, food and water intake, and hydration status of the subjects, were not quantitatively assessed, representing a limiting factor of the study. Although TPP demonstrated a protective effect against metformin-induced renal toxicity, it is plausible that metformin may have inhibited thiamine pyrophosphokinase, the enzyme responsible

for converting thiamine into its active metabolite TPP. To clarify this possibility, future investigations should include direct quantification of tissue and serum TPP levels, along with comprehensive assessments of thiamine pyrophosphokinase activity in animals receiving metformin. Such analyses would help determine whether the observed benefits of exogenously administered TPP stem from a metformin-related disruption of endogenous thiamine to TPP conversion. Exogenous ATP is inherently unstable and undergoes rapid enzymatic degradation, thereby limiting its bioavailability *in vivo*, and is rapidly processed through purinergic signaling pathways. In the present study, it was not directly investigated whether the antioxidant effects of ATP arise from direct actions or occur indirectly through modulation of endogenous antioxidant defense mechanisms. Similarly, although TPP is a well-established metabolic cofactor, its limited permeability across the plasma membrane suggests that its effects are unlikely to result from direct mitochondrial or intracellular uptake. Accordingly, the mechanistic interpretations related to both ATP and TPP should be considered hypothesis-generating, and further studies will be required to elucidate the precise mechanisms underlying these effects. In addition, the potential synergistic or additive effects of combined TPP and ATP administration were not evaluated in the present study and should be addressed in future investigations. Although renal injury was comprehensively evaluated using biochemical oxidative stress markers, inflammatory mediators, and histopathological assessment, immunohistochemical markers such as KIM-1 and NGAL, which are early indicators of kidney injury, as well as 4-HNE and HO-1, which play important roles in the development of acute kidney injury, could not be assessed due to limitations related to the predefined experimental design and the availability of tissue material. Histopathological evaluation was performed using a semi-quantitative, observer-dependent scoring system. We acknowledge that the lack of computer-assisted digital image analysis and objective quantitative measurements represents a limitation of the study. Furthermore, histopathological scores were not statistically correlated with biochemical or functional parameters. Another limitation of our study is that key pathways, including PDH activity, mitochondrial function, and AMPK signaling, were not directly assessed. Therefore, the proposed mechanistic interpretations are inferential, and further studies are required to validate the suggested mechanisms. Also, renal function was evaluated only by serum creatinine and urea levels, while more sensitive and specific functional parameters were not included in this study. Future studies should incorporate quantitative analyses as well as correlation-based assessments. The inclusion of only six animals per group and the absence of an *a priori* power analysis may limit the statistical power of the study. The lack of a positive control group also makes it difficult to place the efficacy of ATP and TPP on a solid comparative basis. In addition, intraperitoneal administration of the compounds does not directly reflect potential clinical use. Further comprehensive studies are required to translate the present findings into clinical applications.

## 4. Materials and Methods

### 4.1. Animals

A total of 36 male albino Wistar rats, 9–10 weeks of age and weighing between 277 and 281 g, were used as experimental subjects in the present study. All animals were sourced from the Experimental Animals Application and Research Center of Erzincan Binali Yildirim University (Erzincan, Turkey). The animals were randomly assigned to six experimental groups ( $n = 6$  in each), with baseline body weights balanced across groups. Before initiating the experimental procedures, the animals underwent a one-week acclimatization period and were housed in standard wire cages (20 cm height  $\times$  35 cm width  $\times$  55 cm length; floor area 1925 cm<sup>2</sup>), with six rats per enclosure. Environmental conditions were rigorously standardized: a 12 h light/12 h dark cycle, ambient temperature maintained

at  $22 \pm 2$  °C, and relative humidity between 30% and 70%. Animals had unrestricted access to standard laboratory chow (Bayramoglu Feed and Flour Industry Inc., Erzurum, Turkey) and tap water. All experimental procedures were performed within the laboratory facilities of the Experimental Animal Application and Research Center at Erzincan Binali Yildirim University.

The study protocol was prepared and implemented in strict accordance with Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes (Protocol ID: 2016-24-199). All procedures conformed to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines [67].

#### 4.2. Reagents and Chemicals

All reagents and chemicals used in this study were of analytical grade and purchased from certified suppliers. Thiopental sodium (Pental Sodyum<sup>®</sup>, 0.5 g vial, Catalog No.: 8699508270385) was obtained from Menarini Health and Pharmaceuticals Industry Trade Inc. (Istanbul, Turkey). Metformin hydrochloride (Diaformin<sup>®</sup>, 1000 mg tablet, Catalog No.: 8699543090092) was supplied by Ali Raif Pharmaceutical Industry Inc. (Istanbul, Turkey). ATP (ATP<sup>®</sup>, 10 mg/mL injectable solution, Catalog No.: 4820117741513) was purchased from Zdorovye Narodu Pharmaceutical LLC. (Kharkiv, Ukraine), and thiamine pyrophosphate (Cocarboxylase hydrochloride<sup>®</sup>, 50 mg/2 mL injectable solution, Catalog No.: 4820011070436) was sourced from BioPharma (Kyiv, Ukraine).

#### 4.3. Experimental Design and Randomization

The number of animals included in the study was determined in accordance with the principle of using the smallest possible sample size capable of yielding robust and reproducible findings, in line with the 4R guidelines (Reduction, Refinement, Replacement, and Responsibility) [68]. Exclusion criteria were defined for two distinct phases of the experiment. Pre-experimental criteria included abnormal body posture, reduced spontaneous activity, or injuries resulting from aggressive interactions between cage mates, leading to exclusion prior to randomization and initiation of the intervention. Peri- and post-experimental criteria comprised events such as unexpected death or complications related to anesthesia or drug administration prior to planned endpoints; errors during dosing procedures, including failed oral gavage or extravasation during injection; deviations from the planned treatment schedule or incomplete administration of study compounds; weight loss exceeding 15–20% of baseline, dehydration, or signs of systemic illness; severe distress, self-mutilation, or persistent vocalization indicating uncontrolled pain or suffering; failure to complete behavioral assessments due to non-compliance or motor deficits unrelated to experimental interventions; and loss of tissue integrity during collection or processing, rendering histological or biochemical analyses unreliable. These criteria were systematically applied throughout the intervention period and during subsequent data evaluation. None of the animals met the predefined exclusion criteria at any phase, and no subjects were excluded from the study. Group allocation was conducted using a random number table to ensure unbiased distribution. To further minimize potential confounding effects and systematic bias, each cage and individual animal was labeled with a numerical code, which was maintained consistently throughout the experimental period.

#### 4.4. Experimental Groups

Six experimental groups were established through random allocation of the animals. The groups were defined as follows: HG; ATG (ATP alone, 4 mg/kg, intraperitoneal); TPPG (TPP alone, 20 mg/kg, intraperitoneal); MTG (metformin alone, 50 mg/kg, oral); ATMG (ATP 4 mg/kg, intraperitoneal + metformin 50 mg/kg, oral); and TPMG (TPP 20 mg/kg, intraperitoneal + metformin 50 mg/kg, oral).

#### 4.5. Experimental Procedure

ATP (4 mg/kg, intraperitoneal) was administered to the ATG ( $n = 6$ ) and ATMG ( $n = 6$ ) groups, whereas TPP (20 mg/kg, intraperitoneal) was injected into the TPG ( $n = 6$ ) and TPMG ( $n = 6$ ) groups. The dosing regimens and routes of administration for ATP [69] and TPP [70] were informed by previously validated experimental models in which these agents consistently demonstrated potent antioxidant, anti-inflammatory, and tissue-protective efficacy. Animals in the HG ( $n = 6$ ) and MTG ( $n = 6$ ) groups received physiological saline (0.9% NaCl) as a vehicle. One hour after ATP, TPP, or saline administration, metformin (50 mg/kg, oral) was given to the ATMG, TPMG, and MTG groups by oral gavage. The 50 mg/kg metformin dose was selected based on prior experimental studies in rats [12]. This procedure was repeated once daily for 10 consecutive days. At the end of the treatment period, all animals were euthanized with a high dose of thiopental anesthesia (50 mg/kg, intraperitoneal), and their kidneys were collected. Kidney tissues were analyzed for MDA, tGSH, SOD, CAT, IL-1 $\beta$ , and TNF- $\alpha$  levels. Histopathological examination of the kidney tissues was also performed. Before euthanasia, blood samples obtained from the tail vein were analyzed for creatinine, BUN, LDH, and lactate levels. The experimental outcomes were then compared among groups.

#### 4.6. Biochemical Analyses

##### 4.6.1. Preparation of Samples

Kidney tissue was carefully excised from each animal and briefly washed with ice-cold 0.9% sodium chloride solution to remove residual blood and tissue debris. Approximately 200 mg of tissue was weighed, minced into small pieces, rapidly snap-frozen in liquid nitrogen, and pulverized to a fine powder using a pre-cooled mortar and pestle. The powdered tissue was homogenized in phosphate-buffered saline (PBS, pH 7.4) at a ratio of 1:10 ( $w/v$ ). Homogenates were vortexed for 10 s and centrifuged at  $10,000 \times g$  for 20 min at 4 °C. The resulting supernatant was carefully collected and stored at  $-80$  °C until biochemical analyses were performed. All biochemical analyses were carried out using commercially available assay kits in accordance with the manufacturers' instructions. To ensure consistency and allow reliable intergroup comparisons, biochemical parameters were normalized to total protein content determined by a validated colorimetric assay and expressed as nmol/mg protein for MDA and tGSH and U/mg protein for SOD and CAT. Variations in absolute biochemical values may reflect assay-specific sensitivity and tissue homogenization conditions rather than exaggerated biological effects; therefore, relative differences between experimental groups were emphasized in the statistical analysis.

##### 4.6.2. Quantification of MDA, tGSH, SOD, CAT, and Total Protein Levels in Kidney Tissue

The levels of MDA and tGSH, along with SOD activity, in kidney tissue were quantified using rat-specific ELISA kits (catalog numbers: 10009055 for MDA, 703002 for tGSH, and 706002 for SOD; Cayman Chemical Co., Ann Arbor, MI, USA) in accordance with the manufacturer's instructions. Catalase enzymatic activity was assessed according to Goth's method [71]. Total protein concentration was determined by the Bradford method [72].

##### 4.6.3. Quantification of IL-1 $\beta$ and TNF- $\alpha$ Levels in Kidney Tissue

IL-1 $\beta$  (pg/L; Catalog No.: 201-11-0120) and TNF- $\alpha$  (ng/L; Catalog No.: 201-11-0765) levels were determined using rat-specific ELISA kits (SunRed Biotechnology Co.<sup>®</sup>, Shanghai, China) according to the manufacturer's validated protocols.

#### 4.6.4. Quantification of Serum Creatinine Levels as a Marker of Renal Function

Serum creatinine concentrations were quantified spectrophotometrically using a kinetic colorimetric assay based on the Jaffé reaction, employing commercially available diagnostic test kits on a Beckman Coulter AU5800 automated analyzer (Beckman Coulter Inc.<sup>®</sup>, Brea, CA, USA, 2023). In this method, creatinine reacts with picric acid under alkaline conditions to form a yellow–orange chromogenic complex. The absorbance of the resulting complex was determined spectrophotometrically at 505 nm, with color intensity directly proportional to the creatinine concentration. To minimize analytical interference attributable to bilirubin, a rate-blanking technique was employed. In addition, a correction factor of  $-26 \mu\text{mol/L}$  ( $-0.3 \text{ mg/dL}$ ) was applied to compensate for nonspecific reactions caused by pseudo-creatinine chromogens, including proteins and ketone bodies.

#### 4.6.5. Quantification of Serum BUN Levels as a Marker of Renal Function

Quantitative analysis of serum urea was performed using an enzymatic spectrophotometric assay on a Beckman Coulter AU5800 automated analyzer (Beckman Coulter Inc.<sup>®</sup>, Brea, CA, USA, 2023), in accordance with the manufacturer's instructions. BUN levels were calculated by applying a conversion factor of 0.48 ( $\text{BUN} = \text{Urea} \times 0.48$ ).

#### 4.6.6. Determination of Serum LDH Activity

Quantitative determination of serum LDH activity was performed using a spectrophotometric method on a Beckman Coulter AU5800 automated analyzer (Beckman Coulter Inc.<sup>®</sup>, Brea, CA, USA, 2023).

#### 4.6.7. Determination of Blood Lactate Levels

Tail vein blood samples were collected from rats using pre-heparinized lithium heparin-coated syringes to prevent coagulation and minimize glycolytic activity. Lactate concentrations were measured using an ABL800 FLEX blood gas analyzer (Radiometer<sup>®</sup>, Copenhagen, Denmark), in accordance with the manufacturer's instructions.

#### 4.7. Histopathological Procedures

Kidney tissue specimens were immediately fixed in 10% neutral buffered formalin to preserve cellular and structural integrity. After fixation, tissues were placed in cassettes and rinsed under running tap water for 24 h to remove residual fixative. Dehydration was performed using a graded ethanol series (70%, 80%, 90%, and 100%), followed by clearing in xylol and embedding in paraffin. Paraffin blocks were sectioned at a thickness of 4–5  $\mu\text{m}$  using a rotary microtome, and the sections were mounted on glass slides. For histological evaluation, serial paraffin sections obtained from the kidneys of animals in each experimental group ( $n = 6$ ) were stained with hematoxylin and eosin (H&E) to assess overall tissue architecture. Histopathological evaluation was performed by an experienced pathologist blinded to group allocation. To ensure unbiased assessment, microscopic fields were selected using a systematic sampling approach. At least five non-overlapping fields per animal were evaluated at predetermined magnifications ( $\times 100$  and  $\times 200$ ), and representative photomicrographs were captured using an Olympus BX53 microscope equipped with a DP2-SAL imaging system (version 3.3.1.198; Olympus<sup>®</sup> Inc., Tokyo, Japan). Histopathological alterations were defined by the presence of glomerular injury, tubular damage, hemorrhage, interstitial edema, polymorphonuclear leukocyte (PMNL) infiltration, and dilated, congested blood vessels. Tissue damage was assessed using a semi-quantitative scoring system ranging from 0 to 3, where 0 indicated no detectable injury, and 3 indicated severe injury. Scores obtained from each microscopic field were averaged to generate a

representative histopathological score for each animal. Digital image quantification was not performed due to the semi-quantitative design of the histopathological scoring system.

#### 4.8. Statistical Analyses

All statistical analyses were performed using IBM SPSS® Statistics for Windows (Version 27.0; IBM Corp., Armonk, NY, USA). Graphical representations were generated using GraphPad Prism® (Version 8.0.1; GraphPad Software, San Diego, CA, USA). Biochemical data are presented as mean  $\pm$  standard error of the mean (SEM). Normality of biochemical data was assessed using the Shapiro–Wilk test, and homogeneity of variances was evaluated using Levene’s test (Tables S1–S3). As the assumptions of normality and homogeneity were met, group comparisons for biochemical parameters were performed using one-way ANOVA, followed by Tukey’s post hoc test for multiple comparisons (Tables S4 and S5).

Histopathological scores are expressed as median values with minimum and maximum ranges. Because these data were ordinal in nature, statistical analysis was conducted using the Kruskal–Wallis test as a global non-parametric method. When statistically significant differences were detected, pairwise comparisons between groups were performed using Dunn’s post hoc test with adjustment for multiple comparisons. All statistical tests were two-tailed, and a  $p$  value  $< 0.05$  was considered statistically significant.

## 5. Conclusions

In this study, metformin was shown to induce biochemical alterations and histopathological injury in renal tissue, consistent with oxidative stress, pro-inflammatory responses, and metabolic imbalance. While ATP attenuated these changes to a lesser extent, TPP exerted a more pronounced protective effect against metformin-associated renal injury. Although the underlying mechanisms were not directly investigated, the greater efficacy of TPP may be related to its established role as a metabolic cofactor, as supported by the literature. Accordingly, this mechanistic interpretation should be considered hypothesis-generating. In the context of metformin-induced nephrotoxicity, our findings suggest that TPP may represent a more protective agent than ATP. From a translational perspective, these findings suggest that TPP may warrant further investigation as a potential tissue-protective agent in metformin-associated renal toxicity.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms27041825/s1>.

**Author Contributions:** H.K.: Conceptualization, Formal Analysis, Funding Acquisition, Project Administration, Resources, Supervision, Validation, Visualization, Writing—Original Draft Preparation, Writing—Review and Editing; F.B.: Conceptualization, Data Curation, Investigation, Resources, Writing—Original Draft Preparation, Writing—Review and Editing; B.Y.: Data Curation, Formal Analysis, Investigation, Resources, Software, Visualization, Writing—Original Draft Preparation, Writing—Review and Editing; E.T.S.: Data Curation, Resources; Writing—Original Draft Preparation, Writing—Review and Editing; R.M.: Data Curation, Project Administration, Investigation, Resources, Validation, Writing—Original Draft Preparation, Writing—Review and Editing; B.S.: Data Curation, Investigation, Resources, Validation, Writing—Original Draft Preparation, Writing—Review and Editing; C.S.: Data Curation, Formal Analysis, Investigation, Resources, Validation, Writing—Original Draft Preparation, Writing—Review and Editing; F.K.C.: Data Curation, Formal Analysis, Investigation, Resources, Validation, Writing—Original Draft Preparation, Writing—Review and Editing; M.S.A.: Conceptualization, Data Curation, Investigation, Resources, Writing—Original Draft Preparation, Writing—Review and Editing; H.S.: Conceptualization, Formal Analysis, Methodology, Project Administration, Supervision, Validation, Writing—Original Draft Preparation, Writing—Review and Editing. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The animal study protocol was approved by the Local Animal Ethics Committee of Erzincan Binali Yıldırım University (Approval No.: 40; Session: August 2025; Date: 21 August 2025).

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

|               |                                                  |
|---------------|--------------------------------------------------|
| T2DM          | Type 2 diabetes mellitus                         |
| TPP           | Thiamine pyrophosphate                           |
| ATP           | Adenosine Triphosphate                           |
| MALA          | Metformin-associated lactic acidosis             |
| MDA           | Malondialdehyde                                  |
| tGSH          | Total Glutathione                                |
| SOD           | Superoxide Dismutase                             |
| CAT           | Catalase                                         |
| TNF- $\alpha$ | Tumor necrosis factor-alpha                      |
| IL-1 $\beta$  | Interleukin-1 beta                               |
| BUN           | Blood urea nitrogen                              |
| LDH           | Lactate dehydrogenase                            |
| ROS           | Reactive Oxygen Species                          |
| ELISA         | Enzyme-linked Immunosorbent Assay                |
| HEL           | Hexanoyl-lysine                                  |
| AMPK          | Adenosine monophosphate-activated protein kinase |

## References

1. Bell, P.M.; Hadden, D.R. Metformin. *Endocrinol. Metab. Clin. N. Am.* **1997**, *26*, 523–537. [[CrossRef](#)] [[PubMed](#)]
2. Bailey, C.J. Metformin: Historical overview. *Diabetologia* **2017**, *60*, 1566–1576. [[CrossRef](#)]
3. Tokhirovna, E. The role of metformin (GLIFORMIN) in the treatment of patients with type 2 diabetes mellitus. *Eur. J. Mod. Med. Pract.* **2024**, *4*, 171–177.
4. Group, M. *Glucophage® Is the First Oral Diabetes Treatment Approved in Europe for Use During Pregnancy*; Merck: Darmstadt, Germany, 2022.
5. Foretz, M.; Guigas, B.; Viollet, B. Metformin: Update on mechanisms of action and repurposing potential. *Nat. Rev. Endocrinol.* **2023**, *19*, 460–476. [[CrossRef](#)] [[PubMed](#)]
6. Buczynska, A.; Sidorkiewicz, I.; Kretowski, A.J.; Adamska, A. Examining the clinical relevance of metformin as an antioxidant intervention. *Front. Pharmacol.* **2024**, *15*, 1330797. [[CrossRef](#)] [[PubMed](#)]
7. Chakraborty, A.; Chowdhury, S.; Bhattacharyya, M. Effect of metformin on oxidative stress, nitrosative stress and inflammatory biomarkers in type 2 diabetes patients. *Diabetes Res. Clin. Pract.* **2011**, *93*, 56–62. [[CrossRef](#)]
8. Driver, C.; Hayangah, J.A.; Nyane, N.A.; Owira, P.M.O. Metformin with insulin relieves oxidative stress and confers renoprotection in type 1 diabetes in vivo. *J. Nephropathol.* **2017**, *7*, 171–181. [[CrossRef](#)]
9. Anedda, A.; Rial, E.; Gonzalez-Barroso, M.M. Metformin induces oxidative stress in white adipocytes and raises uncoupling protein 2 levels. *J. Endocrinol.* **2008**, *199*, 33–40. [[CrossRef](#)]
10. Deng, C.; Xiong, L.; Chen, Y.; Wu, K.; Wu, J. Metformin induces ferroptosis through the Nrf2/HO-1 signaling in lung cancer. *BMC Pulm. Med.* **2023**, *23*, 360. [[CrossRef](#)]
11. Bridges, H.R.; Jones, A.J.; Pollak, M.N.; Hirst, J. Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. *Biochem. J.* **2014**, *462*, 475–487. [[CrossRef](#)]
12. Yoon, J.P.; Park, S.J.; Kim, D.H.; Chung, S.W. Metformin increases the expression of proinflammatory cytokines and inhibits suprascapular fatty infiltration. *J. Orthop. Surg. Res.* **2023**, *18*, 674. [[CrossRef](#)] [[PubMed](#)]
13. LaMoia, T.E.; Shulman, G.I. Cellular and Molecular Mechanisms of Metformin Action. *Endocr. Rev.* **2021**, *42*, 77–96. [[CrossRef](#)] [[PubMed](#)]

14. Dyatlova, N.; Tobarran, N.V.; Kannan, L.; North, R.; Wills, B.K. Metformin-Associated Lactic Acidosis (MALA). In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2025.
15. Narade, S. Biguanides and lactic acidosis: Unraveling the complex relationship. *Pharmacol. Discov.* **2024**, *4*, 18. [\[CrossRef\]](#)
16. Corchia, A.; Wynckel, A.; Journet, J.; Moussi Frances, J.; Skandrani, N.; Lautrette, A.; Zafrani, L.; Lewandowski, E.; Reboul, P.; Vrigneaud, L.; et al. Metformin-related lactic acidosis with acute kidney injury: Results of a French observational multicenter study. *Clin. Toxicol.* **2020**, *58*, 375–382. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Mullins, M.E.; Kraut, J.A. The Role of the Nephrologist in Management of Poisoning and Intoxication: Core Curriculum 2022. *Am. J. Kidney Dis.* **2022**, *79*, 877–889. [\[CrossRef\]](#)
18. DeFronzo, R.; Fleming, G.A.; Chen, K.; Bicsak, T.A. Metformin-associated lactic acidosis: Current perspectives on causes and risk. *Metabolism* **2016**, *65*, 20–29. Correction in *Metabolism* **2016**, *65*, 1432–1433. [\[CrossRef\]](#)
19. Peake, K.N.; Tessier, S.; Longo, S.; Stahlnecker, D.M.; Idahosa, O.; Zanders, T.; Ido, F. Metformin toxicity in the intensive care unit: A case series and review of the literature. *Int. J. Crit. Illn. Inj. Sci.* **2024**, *14*, 51–58. [\[CrossRef\]](#)
20. Yuan, W.; Du, Y.; Yu, K.; Xu, S.; Liu, M.; Wang, S.; Yang, Y.; Zhang, Y.; Sun, J. The Production of Pyruvate in Biological Technology: A Critical Review. *Microorganisms* **2022**, *10*, 2454. [\[CrossRef\]](#)
21. Visconti, L.; Cernaro, V.; Ferrara, D.; Costantino, G.; Aloisi, C.; Amico, L.; Chirico, V.; Santoro, D.; Noto, A.; David, A.; et al. Metformin-related lactic acidosis: Is it a myth or an underestimated reality? *Ren. Fail.* **2016**, *38*, 1560–1565. [\[CrossRef\]](#)
22. Ott, M.; Werneke, U. Metformin-associated lactic acidosis may be treatable with thiamine. *Med. Hypotheses* **2024**, *189*, 111416. [\[CrossRef\]](#)
23. Isik, B.; Ates, I.; Yucel, N.; Suleyman, B.; Mendil, A.S.; Sezgin, E.T.; Suleyman, H. The Protective Effect of Thiamine and Thiamine Pyrophosphate Against Linezolid-Induced Oxidative Liver Damage and Lactic Acidosis in Rats. *Antioxidants* **2025**, *14*, 920. [\[CrossRef\]](#)
24. Zhang, W.L.; Zhang, L.J.; Liang, P.; Fang, H.L.; Wang, X.L.; Liu, Y.J.; Deng, H.F. Metformin protects against acute kidney injury induced by lipopolysaccharide via up-regulating the MCP1/SIRT1 pathway. *Biochem. Genet.* **2024**, *62*, 4591–4602. [\[CrossRef\]](#)
25. Cavaglieri, R.C.; Day, R.T.; Feliars, D.; Abboud, H.E. Metformin prevents renal interstitial fibrosis in experimental models. *Am. J. Physiol. Renal. Physiol.* **2015**, *309*, F728–F738.
26. Kajbaf, F.; Lalau, J.D. The renal effects of metformin: Current knowledge. *Diabetes Metab.* **2014**, *40*, 19–27.
27. Cai, Z.; Wu, X.; Song, Z.; Sun, S.; Su, Y.; Wang, T.; Cheng, X.; Yu, Y.; Yu, C.; Chen, E.; et al. Metformin potentiates nephrotoxicity by promoting NETosis in response to renal ferroptosis. *Cell Discov.* **2023**, *9*, 104. Correction in *Cell Discov.* **2024**, *10*, 4. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Momenzadeh, M.; Lakkakula, B.V.K.S. Metformin induced acute kidney injury; a systematic review. *J. Nephropharmacol.* **2020**, *10*, e13. [\[CrossRef\]](#)
29. Pavlakou, P.; Liakopoulos, V.; Eleftheriadis, T.; Mitsis, M.; Dounousi, E. Oxidative Stress and Acute Kidney Injury in Critical Illness: Pathophysiologic Mechanisms-Biomarkers-Interventions, and Future Perspectives. *Oxid. Med. Cell Longev.* **2017**, *2017*, 6193694. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Tombulturk, F.K. Reducing oxidative stress and enhancing antioxidant defenses for faster healing in diabetic wounds: The role of topical metformin. *Int. J. Med. Biochem.* **2025**, *8*, 89–98. [\[CrossRef\]](#)
31. Ahmed Mobasher, M.; Galal El-Tantawi, H.; Samy El-Said, K. Metformin Ameliorates Oxidative Stress Induced by Diabetes Mellitus and Hepatocellular Carcinoma in Rats. *Rep. Biochem. Mol. Biol.* **2020**, *9*, 115–128. [\[CrossRef\]](#)
32. Sugiyama, A.; Sun, J.; Nishinohara, M.; Fujita, Y.; Masuda, A.; Ochi, T.; Takeuchi, T. Expressions of lipid oxidation markers, N( $\epsilon$ )-hexanoyl lysine and acrolein in cisplatin-induced nephrotoxicity in rats. *J. Vet. Med. Sci.* **2011**, *73*, 821–826. [\[CrossRef\]](#)
33. Dong, X.Q.; Chu, L.K.; Cao, X.; Xiong, Q.W.; Mao, Y.M.; Chen, C.H.; Bi, Y.L.; Liu, J.; Yan, X.M. Glutathione metabolism rewiring protects renal tubule cells against cisplatin-induced apoptosis and ferroptosis. *Redox Rep.* **2023**, *28*, 2152607. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Hosohata, K. Role of Oxidative Stress in Drug-Induced Kidney Injury. *Int. J. Mol. Sci.* **2016**, *17*, 1826. [\[CrossRef\]](#)
35. Lingappan, K. NF-kappaB in Oxidative Stress. *Curr. Opin. Toxicol.* **2018**, *7*, 81–86. [\[CrossRef\]](#)
36. Isoda, K.; Young, J.L.; Zirlik, A.; MacFarlane, L.A.; Tsuboi, N.; Gerdes, N.; Schonbeck, U.; Libby, P. Metformin inhibits proinflammatory responses and nuclear factor-kappaB in human vascular wall cells. *Arterioscler. Thromb. Vasc. Biol.* **2006**, *26*, 611–617. [\[CrossRef\]](#)
37. Buczynska, A.; Malinowski, P.; Zbikowski, A.; Kretowski, A.J.; Zbucka-Kretowska, M. Metformin modulates oxidative stress via activation of AMPK/NF-kappaB signaling in Trisomy 21 fibroblasts: An in vitro study. *Front. Mol. Biosci.* **2025**, *12*, 1577044. [\[CrossRef\]](#)
38. Kalamkar, P.; Gadgoli, C.; Girase, G.; Dalvi, R.; Dadrekar, P.; Karulkar, N.; Deshmukh, S.; Kalamkar, V. Novel biomarkers for detection of nephrotoxicity. *J. Ren. Inj. Prev.* **2024**, *13*, e33294. [\[CrossRef\]](#)
39. Griffin, B.R.; Faubel, S.; Edelstein, C.L. Biomarkers of Drug-Induced Kidney Toxicity. *Ther. Drug Monit.* **2019**, *41*, 213–226. [\[CrossRef\]](#)

40. Fukuda, M.; Hirayu, N.; Nabeta, M.; Goto, M.; Takasu, O. Metformin-Associated Lactic Acidosis in Individuals Without Chronic Kidney Disease on Therapeutic Dose: A Case Report. *Cureus* **2023**, *15*, e48683. [CrossRef] [PubMed]
41. Hsu, W.H.; Hsiao, P.J.; Lin, P.C.; Chen, S.C.; Lee, M.Y.; Shin, S.J. Effect of metformin on kidney function in patients with type 2 diabetes mellitus and moderate chronic kidney disease. *Oncotarget* **2018**, *9*, 5416–5423. [CrossRef]
42. Zhang, S.; Xu, H.; Yu, X.; Wu, Y.; Sui, D. Metformin ameliorates diabetic nephropathy in a rat model of low-dose streptozotocin-induced diabetes. *Exp. Ther. Med.* **2017**, *14*, 383–390. [CrossRef]
43. Lin, C.X.; Li, Y.; Liang, S.; Tao, J.; Zhang, L.S.; Su, Y.F.; Huang, Y.X.; Zhao, Z.K.; Liu, S.Y.; Zheng, J.M. Metformin Attenuates Cyclosporine A-induced Renal Fibrosis in Rats. *Transplantation* **2019**, *103*, e285–e296. [CrossRef]
44. Paige, A.; Mirza, N.; Rayad, M.; Miller, R.A. Metformin-Induced Lactic Acidosis Complicated by Acute Liver Failure. *Chest* **2023**, *164*, A1875. [CrossRef]
45. See, K.C. Metformin-associated lactic acidosis: A mini review of pathophysiology, diagnosis and management in critically ill patients. *World J. Diabetes* **2024**, *15*, 1178–1186. [CrossRef]
46. Madiraju, A.K.; Erion, D.M.; Rahimi, Y.; Zhang, X.M.; Braddock, D.T.; Albright, R.A.; Prigaro, B.J.; Wood, J.L.; Bhanot, S.; MacDonald, M.J.; et al. Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature* **2014**, *510*, 542–546. [CrossRef] [PubMed]
47. Reczek, C.R.; Chakrabarty, R.P.; D'Alessandro, K.B.; Sebo, Z.L.; Grant, R.A.; Gao, P.; Budinger, G.R.; Chandel, N.S. Metformin targets mitochondrial complex I to lower blood glucose levels. *Sci. Adv.* **2024**, *10*, eads5466. [CrossRef] [PubMed]
48. Fontaine, E. Metformin-Induced Mitochondrial Complex I Inhibition: Facts, Uncertainties, and Consequences. *Front. Endocrinol.* **2018**, *9*, 753. [CrossRef]
49. Farhana, A.; Lappin, S.L. Biochemistry, Lactate Dehydrogenase. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2023. Available online: <https://www.ncbi.nlm.nih.gov/books/NBK557536/> (accessed on 3 November 2025).
50. Gupta, G.S. The Lactate and the Lactate Dehydrogenase in Inflammatory Diseases and Major Risk Factors in COVID-19 Patients. *Inflammation* **2022**, *45*, 2091–2123. [CrossRef]
51. Solini, A.; Uselli, V.; Fiorina, P. The dark side of extracellular ATP in kidney diseases. *J. Am. Soc. Nephrol.* **2015**, *26*, 1007–1016. [CrossRef]
52. Dagel, T.; Altuner, D.; Suleyman, B.; Mammadov, R.; Bulut, S.; Bal Tastan, T.; Gulaboglu, M.; Suleyman, H. Effects of adenosine triphosphate, Lacidipine, and Benidipine on 5-fluorouracil-induced kidney damage in rats. *Eur. Rev. Med. Pharmacol. Sci.* **2024**, *28*, 2538–2549. [CrossRef] [PubMed]
53. Venkatachalam, M.A.; Weinberg, J.M. The conundrum of protection from AKI by adenosine in rodent clamp ischemia models. *Kidney Int.* **2013**, *84*, 16–19. [CrossRef]
54. Somuncu, A.M.; Parlak Somuncu, B.; Ozbay, A.D.; Cicek, I.; Suleyman, B.; Mammadov, R.; Bulut, S.; Bal Tastan, T.; Coban, T.A.; Suleyman, H.; et al. Potential effects of adenosine triphosphate and melatonin on oxidative and inflammatory optic nerve damage in rats caused by 5-fluorouracil. *Int. J. Ophthalmol.* **2025**, *18*, 222–228. [CrossRef]
55. Zhang, Y.; Yan, H.; Lv, S.G.; Wang, L.; Liang, G.P.; Wan, Q.X.; Peng, X. Effects of glycyl-glutamine dipeptide supplementation on myocardial damage and cardiac function in rats after severe burn injury. *Int. J. Clin. Exp. Pathol.* **2013**, *6*, 821–830.
56. Secgin, S.; Zafar, M.; Khakimov, M.; Keles, H.; Kahraman, T. Thiamine pyrophosphate may protect indomethacin-induced small intestinal enteropathy in rats by inhibiting intestinal inflammation and oxidative stress. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2025**, *398*, 14441–14450. [CrossRef] [PubMed]
57. Dagel, T.; Mammadov, R.; Bulut, S.; Yeter, B.; Tastan, T.B.; Ekici, O.; Coban, T.A.; Suleyman, H. Effect of Thiamine Pyrophosphate on Amiodarone-Induced Oxidative Kidney Damage in Rats. *Int. J. Pharmacol.* **2023**, *19*, 521–530. [CrossRef]
58. Mahdavi, S.; Nakhjavani, M. Thiamine pyrophosphate improved vascular complications of diabetes in rats with type 2 diabetes by reducing glycation, oxidative stress, and inflammation markers. *Med. J. Islam. Repub. Iran* **2020**, *34*, 47. [CrossRef] [PubMed]
59. Connor, H.; Woods, H.F.; Ledingham, J.G. Comparison of the kinetics and utilisation of D(–)- and L(+)-sodium lactate in normal man. *Ann. Nutr. Metab.* **1983**, *27*, 481–487. [CrossRef] [PubMed]
60. Perriello, G.; Jorde, R.; Nurjhan, N.; Stumvoll, M.; Dailey, G.; Jenssen, T.; Bier, D.M.; Gerich, J.E. Estimation of glucose-alanine-lactate-glutamine cycles in postabsorptive humans: Role of skeletal muscle. *Am. J. Physiol.* **1995**, *269*, E443–E450. [CrossRef]
61. Attaluri, P.; Castillo, A.; Edriss, H.; Nugent, K. Thiamine Deficiency: An Important Consideration in Critically Ill Patients. *Am. J. Med. Sci.* **2018**, *356*, 382–390. [CrossRef]
62. Laus, F.; Faillace, V.; Tesesi, B.; Paggi, E.; Serri, E.; Marini, C.; Marvasi, L.; Vullo, C.; Spaterna, A. Effect of Thiamine Pyrophosphate (Bicarbossilasi®) Administration on the Exercising Horse Metabolism. *Isr. J. Vet. Med.* **2017**, *72*, 15–21.
63. Mohammed Ameen, A.; Al-Barwary, M. Assessment of Metformin's Impact on Liver, Kidneys and Spleen Tissues in Normoglycemic Female Albino Rats. *Egypt. J. Vet. Sci.* **2024**, *56*, 627–635. [CrossRef]
64. Ahmad, S.; Aftab, A.; Siraj, F.; Hameed, A.; Iqbal, Z.; Moeed, K. Effect of Metformin on the Kidney Histology of Rats in Gentamicin Induced Toxicity. *Pak. J. Med. Health Sci.* **2021**, *15*, 3524–3526. [CrossRef]

65. Wang, F.; Sun, H.; Zuo, B.; Shi, K.; Zhang, X.; Zhang, C.; Sun, D. Metformin attenuates renal tubulointerstitial fibrosis via upgrading autophagy in the early stage of diabetic nephropathy. *Sci. Rep.* **2021**, *11*, 16362. [[CrossRef](#)]
66. Song, A.; Zhang, C.; Meng, X. Mechanism and application of metformin in kidney diseases: An update. *Biomed. Pharmacother.* **2021**, *138*, 111454. [[CrossRef](#)] [[PubMed](#)]
67. Percie du Sert, N.; Hurst, V.; Ahluwalia, A.; Alam, S.; Avey, M.T.; Baker, M.; Browne, W.J.; Clark, A.; Cuthill, I.C.; Dirnagl, U.; et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol.* **2020**, *18*, e3000410. [[CrossRef](#)]
68. Kiani, A.K.; Pheby, D.; Henahan, G.; Brown, R.; Sieving, P.; Sykora, P.; Marks, R.; Falsini, B.; Capodicasa, N.; Miertus, S.; et al. Ethical considerations regarding animal experimentation. *J. Prev. Med. Hyg.* **2022**, *63*, E255–E266. [[CrossRef](#)]
69. Yeter, B.; Suleyman, Z.; Bulut, S.; Cicek, B.; Coban, T.A.; Demir, O.; Suleyman, H. Effect of adenosine triphosphate on methylphenidate-induced oxidative and inflammatory kidney damage in rats. *Drug Chem. Toxicol.* **2025**, *48*, 1284–1292. [[CrossRef](#)]
70. Karatas, E.; Yavuzer, B.; Demir, O.; Sezgin, E.T.; Hendem, E.; Cinici, E.; Coban, T.A.; Suleyman, H. Protective Role of Thiamine Pyrophosphate Against Erlotinib-Induced Oxidative and Inflammatory Damage in Rat Optic Nerve. *Biomedicines* **2025**, *13*, 2614. [[CrossRef](#)]
71. Goth, L. A simple method for determination of serum catalase activity and revision of reference range. *Clin. Chim. Acta* **1991**, *196*, 143–151. [[CrossRef](#)]
72. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.