

Original Research Article

Metformin attenuates paracetamol-induced hepatorenal damage and oxidative stress in rats

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Sent for review: 23 November 2025

Revised accepted: 18 April 2026

Abstract

Purpose: To evaluate the protective effect of metformin against hepatorenal damage induced by acute paracetamol toxicity in rats.

Methods: Twenty Wistar rats of both sexes were divided into four groups ($n = 5$ each). Groups 1 and 2 received distilled water orally, while groups 3 and 4 received metformin (500 mg/kg) and silymarin (100 mg/kg), respectively, orally once daily for six days. On day 7, paracetamol (3 g/kg) was administered orally to groups 2 – 4 to induce toxicity. After 24 h, blood samples were collected for the biochemical assessment of liver enzymes (alanine transaminase - ALT, aspartate transaminase - AST, alkaline phosphatase - ALP, and lactate dehydrogenase - LDH), kidney markers (urea and creatinine), and oxidative stress parameters (superoxide dismutase - SOD, catalase - CAT, glutathione - GSH, glutathione peroxidase - GPx, malondialdehyde - MDA, and nitrite). Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test.

Results: Paracetamol administration significantly ($p < 0.05$) elevated liver enzymes, total bilirubin, urea, creatinine, MDA, and nitrite concentrations, while significantly ($p < 0.05$) reducing antioxidant enzymes and albumin as seen in group 2 compared to group 1. Pretreatment with metformin markedly ($p < 0.05$) attenuated these alterations, restored liver and kidney biomarkers, and improved antioxidant status. These effects were comparable to those of the silymarin treatment group.

Conclusion: Metformin demonstrates significant protective effects against paracetamol-induced hepatorenal toxicity, likely through antioxidant enhancement and reduction of oxidative stress, thus supporting its potential for repurposing to mitigate drug-induced organ injury.

Keywords: Metformin, Paracetamol toxicity, Hepatotoxicity, Nephrotoxicity, Oxidative stress

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INTRODUCTION

Paracetamol (acetaminophen or N-acetyl-p-aminophenol) is one of the most widely used over-the-counter medications used as an analgesic and antipyretic agent [1]. Paracetamol

acts via a few metabolic pathways that primarily include the inhibition of cyclooxygenase enzymes (COX-1, COX-2, and COX-3), and activation of the serotonergic and endocannabinoid systems which are essential for its analgesic action [2]. Compared to other non-steroidal anti-

inflammatory drugs NSAIDs, such as ibuprofen and naproxen, paracetamol does not have significant peripheral anti-inflammatory properties, as it crosses the blood-brain barrier and only acts primarily on the central nervous system [3]. At the therapeutic dose, paracetamol is considered safe but is toxic at higher doses [4]. Paracetamol intake at high doses is widely used as a model for studying oxidative and toxic injury to the kidneys and liver because of its well-defined pattern of overdose toxicity [2,5].

Metformin (a biguanide) is one of the most commonly used oral hypoglycemic agents as a first-line treatment for type 2 diabetes mellitus [6]. Recent studies have revealed additional effects of metformin beyond its glucose-lowering effects, such as antioxidant, anti-inflammatory, and mitochondria-modulating effects, in multiple tissues [7-9]. Although preclinical studies suggest potential benefits, evidence supporting metformin as a direct protector against paracetamol-induced liver and kidney injuries remains limited [10].

Given the significant clinical burden of drug-induced organ injury, it is important to explore how commonly used drugs such as metformin might modify this risk. Therefore, carefully designed experimental studies are necessary in which direct comparison of metformin with established hepatoprotective agents, such as silymarin, in controlled Wistar rat models could help clarify both the magnitude and mechanisms of protection. Such work may also reveal whether widely available drugs such as metformin may be repurposed as adjunctive therapies to prevent liver or kidney injury. In this study, we investigated the protective effects of metformin against hepatic and renal damage induced by acute paracetamol toxicity in Wistar rats.

EXPERIMENTAL

Drugs

Acetaminophen (paracetamol) 500 mg tablet (Emzor Pharmaceutical Industries Ltd., Lagos, Nigeria; purity $\geq 99\%$); Silymarin 100 mg tablet (Micro Labs Ltd., Goa, India; standardized to contain 80 % silymarin flavonoids); Metformin hydrochloride 500 mg tablet (Swiss Pharma Ltd., Lagos, Nigeria; purity $\geq 98\%$).

Animals

Male Wistar rats weighing 145 - 180 g were procured from the Animal House of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria. The animals were acclimatized

for a period of two weeks prior to the study and were placed on rat feed and clean water *ad libitum*. During acclimatization and throughout the experiment, the rats were housed in polypropylene cages under standard laboratory conditions: temperature of $22 \pm 2^\circ\text{C}$, relative humidity of $55 \pm 10\%$ and a 12-hour light/dark cycle. The guidelines were followed for the handling of animals in accordance with the ethical standards of the Institutional Animals Ethics (IAEC), as adopted by the ethical committee of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria. Ethical approval was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria (RBC/FBMC/DELSU/24/363).

Design and grouping

The rats were divided into four groups of five animals each: group 1 (normal control) and group 2 (paracetamol control) orally received distilled water (10 ml/kg) daily, while groups 3 and 4 were treated with metformin (MET) 500 mg/kg and silymarin (SLY) 100 mg/kg once daily for 6 consecutive days. On the 7th day, groups 2 - 4 were administered a single dose of paracetamol (3 g/kg) to induce acute liver toxicity [11]. All drugs were administered using an oral gastric cannula. Twenty-four hours (24 h) after toxicity induction, the rats were anesthetized using diethyl ether, and blood samples (about 5 mL) were collected via retro-orbital plexus puncture into plain tubes. The serum was separated by centrifugation at 3000 rpm for 15 min and stored at -20°C until analysis.

The body weights of the animals were measured on the commencement of the treatment (1st day) and the end (8th day) using an automated weighing scale. Biochemical assays for liver enzymes (alanine transaminase - ALT, aspartate transaminase - AST, alkaline phosphatase - ALP, and lactate dehydrogenase - LDH), kidney function markers (urea and creatinine), total cholesterol, total bilirubin and albumin and oxidative stress parameters (superoxide dismutase - SOD, catalase - CAT, glutathione - GSH, glutathione peroxidase - GPx, malondialdehyde - MDA, and nitrite) were performed using standardized commercial reagent kits [12-15].

Statistical analysis and data presentation

All data obtained were expressed as mean $\pm$ standard error of mean (SEM) and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Analyses were

performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA). Statistical significance was set at $p < 0.05$. Data are presented primarily as bar charts (mean $\pm$ SEM, $n = 5$), with body weights presented in a table.

RESULTS

Effects of treatment on body weight

The initial and final body weights of rats in the different groups are shown in Table 1. Paracetamol (3 g/kg) caused a reduction in weight gain compared to the normal control group. Pretreatment with metformin (500 mg/kg) and silymarin (100 mg/kg) produced slight increases in weight gain, although not significantly different from the paracetamol

control group. Overall, no severe weight loss or abnormal physical behavior was observed during the experiment.

Effect of metformin on serum liver enzymes

Paracetamol (3 g/kg) administration, as seen in the Paracetamol control, caused a significant ($p < 0.05$) increase in ALT, ALP, AST, and LDH levels compared to the normal control, confirming hepatic injury. Pretreatment with metformin significantly ($p < 0.05$) reduced the elevated enzyme levels in all cases, while silymarin produced a similar but slightly stronger reduction. Overall, both agents significantly ($p < 0.05$) attenuated the paracetamol-induced hepatocellular damage (Figure 1).

Table 1: Effects of metformin and silymarin on body weight in rats with acute paracetamol toxicity

Group	Initial weight (g)	Final weight (g)
Normal control	158.20 $\pm$ 6.42	175.60 $\pm$ 6.84
PCM control (3 g/kg)	155.08 $\pm$ 10.48	163.31 $\pm$ 12.27
MET (500 mg/kg)	150.24 $\pm$ 5.20	162.44 $\pm$ 4.73
Silymarin (100 mg/kg)	160.30 $\pm$ 8.02	171.52 $\pm$ 5.16

Values are expressed as mean $\pm$ standard error of mean (SEM), $n = 5$; * $p < 0.05$ vs normal control; # $p < 0.05$ vs PCM control. PCM, paracetamol; MET, metformin

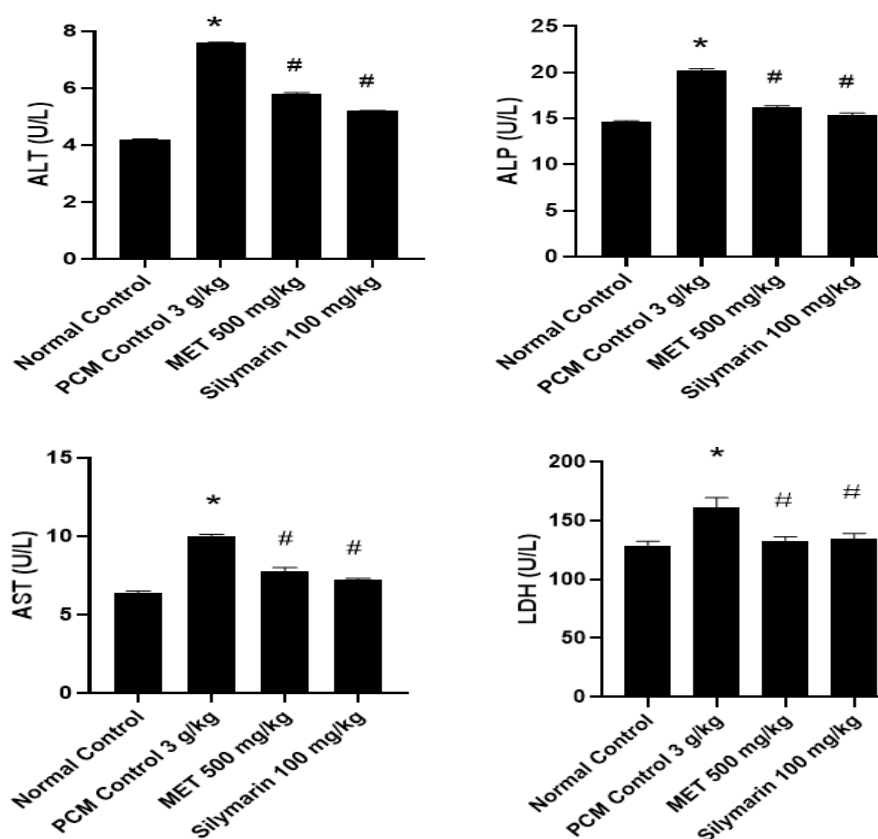


Figure 1: Impact of metformin on paracetamol-induced liver enzyme dysfunction (ALT, ALP, AST, LDH). All values are expressed as mean $\pm$ standard error of mean (SEM), $n = 5$; * $p < 0.05$ vs normal control; # $p < 0.05$ vs PCM control. PCM, paracetamol; MET, metformin

Effect of Metformin on serum levels of total cholesterol, total bilirubin and albumin

Administration of paracetamol (3 g/kg) as seen in group 2 produced a statistically significant ($p < 0.05$) increase in total cholesterol and bilirubin levels, accompanied by a significant ($p < 0.05$) decrease in serum albumin in comparison with the normal control. Figure 2 shows that pretreatment with metformin attenuated these alterations, leading to significant ($p < 0.05$) reductions in total cholesterol and total bilirubin and a significant ($p < 0.05$) increase in serum albumin, while silymarin elicited comparable effects.

Effect of Metformin on Kidney Function Markers (Urea and Creatinine)

Administration of paracetamol (3 g/kg) to group 2 resulted in a significant elevation in serum urea and creatinine levels compared with normal control group ($p < 0.05$), indicating impaired renal function (Figure 3). Pretreatment with metformin

attenuated the significant paracetamol-induced increases in both urea and creatinine ($p < 0.05$), while silymarin produced a further reduction in these markers; however, silymarin produced the greatest numerical decrease in urea and creatinine levels.

Effect of metformin on oxidative stress parameters

Administration of paracetamol (3 g/kg) produced marked oxidative stress, as evidenced by a significant reduction ($p < 0.05$) in the activities of catalase, superoxide dismutase (SOD), reduced glutathione (GSH), and glutathione peroxidase (GPx), along with a significant increase ($p < 0.05$) in malondialdehyde (MDA) levels and nitrite concentrations in the paracetamol control group compared with the normal control group. Pretreatment with metformin and silymarin significantly attenuated ($p < 0.05$) these paracetamol-induced alterations (Figure 4).

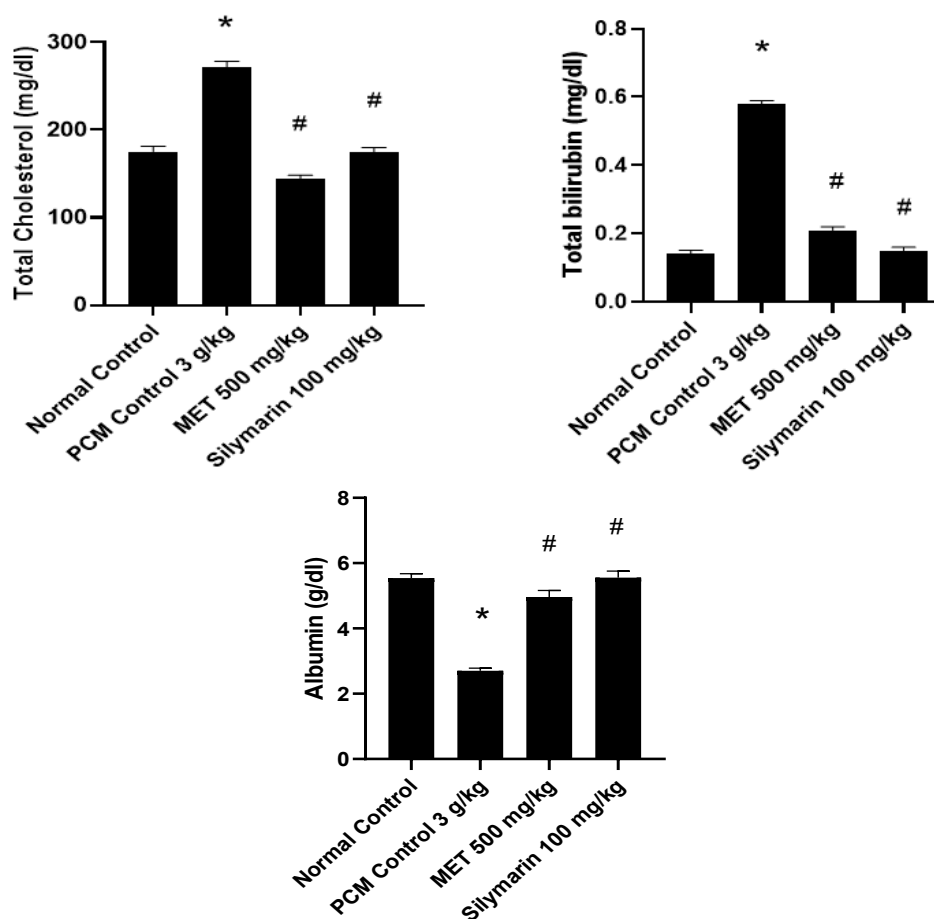


Figure 2: Effect of metformin on serum total cholesterol, total bilirubin and albumin of paracetamol-induced toxic rats. Values are expressed as mean $\pm$ standard error of mean (SEM), $n = 5$; * $p < 0.05$ vs normal control; # $p < 0.05$ vs PCM control. PCM, paracetamol; MET, metformin

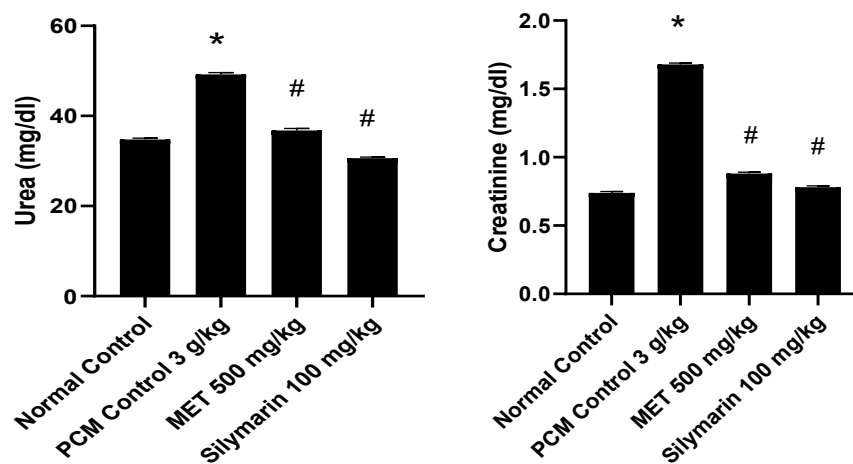


Figure 3: Effect of metformin on paracetamol-induced kidney dysfunction (urea and creatinine) in rats. All values are expressed as mean $\pm$ standard error of mean (SEM), $n = 5$; * $p < 0.05$ vs normal control; # $p < 0.05$ vs PCM control. PCM, paracetamol; MET, metformin

Both agents increased catalase and SOD activities towards normal values, with silymarin producing a slightly higher elevation in both enzymes. Similarly, metformin and silymarin significantly increased ($p < 0.05$) GSH and GPx activities relative to the paracetamol-only group. Metformin produced a marginally greater increase in GSH and GPx than silymarin, although these differences were not statistically significant.

In contrast, the elevated MDA levels observed in the paracetamol group were effectively normalized by both metformin and silymarin, with MDA concentrations in the treated groups approaching those in the normal control. Furthermore, the paracetamol-induced increase in nitrite concentration was significantly reduced by metformin and silymarin ($p < 0.05$), with metformin eliciting a slightly greater, albeit not statistically significant, reduction. Collectively, these findings indicate that metformin exerts a protective antioxidant effect, comparable to that of silymarin, against paracetamol-induced oxidative stress.

DISCUSSION

The results of this study showed that metformin significantly mitigated liver and kidney damage induced by acute paracetamol (acetaminophen) overdose in Wistar rats. Pretreatment with metformin reduced elevations in serum markers of liver injury (ALT, AST, ALP, and LDH), improved liver synthetic function (albumin), and normalized kidney markers (urea and creatinine). This protective effect was accompanied by the restoration of key antioxidant defenses (catalase, SOD, GSH, and GPx) and suppression of

oxidative stress (reduced MDA and nitrite concentration). These findings are consistent with the cytoprotective properties of metformin against oxidative stress-driven organ damage. Sadeghi *et al* [15] showed that metformin attenuates oxidative stress and hepatic injury in bile duct ligation models. The study revealed that metformin (250–500 mg/kg) reduced lipid peroxidation and protein oxidation and increased antioxidant reserves in rats with bile duct ligation (BDL)-induced cholestatic liver injury. Their work aligns with the current study, which reflects the potential of metformin to lower reactive oxygen species and preserve redox balance in a model of liver pathology.

Oxidative stress is a central mediator of organ damage [16]. Metformin inhibits oxidative-stress-induced apoptosis in primary hepatocytes. In vitro studies using menadione to induce reactive oxygen species (ROS) revealed that metformin blocked caspase-9, -6, and -3 activation, reduced Poly (ADP-ribose) polymerase (PARP) cleavage, and upregulated heme oxygenase-1 (HO-1) while suppressing Jun N-terminal kinase (JNK) phosphorylation [17]. This is particularly relevant because JNK activation is a well-known mediator of acetaminophen-induced hepatotoxicity. In addition, a key in vivo study demonstrated that metformin protects against acetaminophen-induced liver injury via Growth Arrest and DNA Damage-inducible, beta (Gadd45 β)-dependent regulation of JNK signaling [18]. In the absence of functional Gadd45 β , the protective effect of metformin was significantly diminished, suggesting that this pathway is critical for its action.

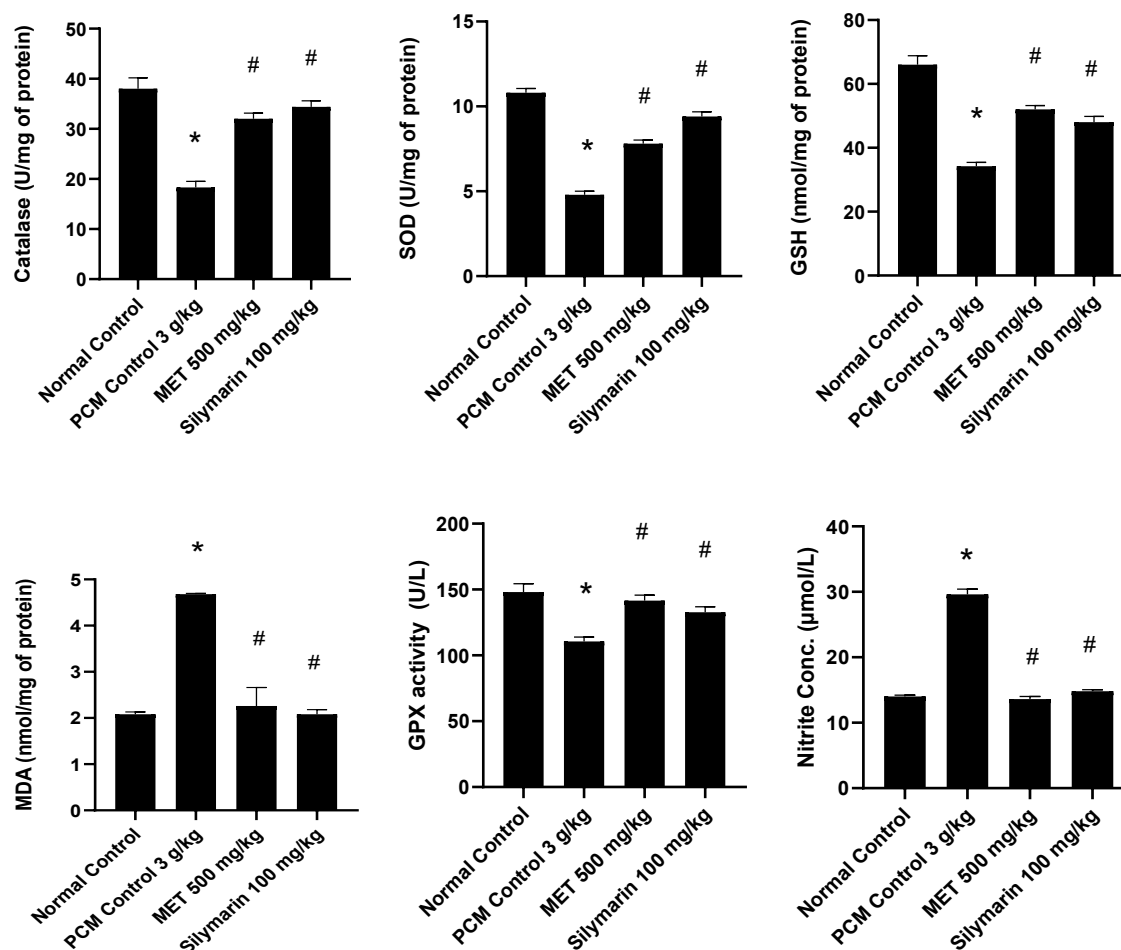


Figure 4: Metformin repressed paracetamol-induced oxidative stress (Catalase, SOD, GSH, MDA, GPX and nitrite concentration) in rats. Values are expressed as mean $\pm$ standard error of mean (SEM), $n=5$; * $p < 0.05$ vs normal control; # $p < 0.05$ vs PCM control. PCM, paracetamol; MET, metformin

Beyond signaling, metformin's protection may also involve direct modulation of mitochondrial oxidative stress. For instance, one of the main findings of a study showed that metformin, administered either before or after acetaminophen administration in mice, attenuated mitochondrial reactive oxygen species production and preserved mitochondrial function (Complex I activity), without necessarily preventing adduct formation or fully blocking JNK activation [19]. This indicates that metformin's benefit lies not only in upstream signaling but also in maintaining mitochondrial integrity, which prevents downstream injury. Such mitochondrial protection likely underlies the reduction in lipid peroxidation (MDA) and restored antioxidant enzyme function observed in our rat model.

In addition to its liver-protective effects, metformin has broader organ-protective actions. For example, in cholestasis-induced renal injury, metformin improves mitochondrial function and reduces oxidative stress in the kidneys [20]. This

suggests that metformin's ability to modulate both oxidative stress and mitochondrial dynamics extends beyond hepatocytes to renal tissue, providing a plausible basis for our observation that metformin reduces paracetamol-induced increases in urea and creatinine.

Moreover, the hepatoprotective properties of metformin have also been documented in other liver injury models. In rats exposed to thioacetamide (a classic hepatotoxin), metformin preserved the liver architecture, reduced fibrosis, lowered MDA levels, and protected antioxidant enzymes such as SOD [21]. These results further validate the notion that metformin can counteract chemical-induced liver injury via its antioxidative and anti-fibrotic effects.

Generally, the protective effects of metformin observed in this study likely resulted from multiple converging mechanisms. These include the reduction of mitochondrial ROS, stabilization of mitochondrial function, inhibition of apoptotic

signaling (JNK via Gadd45 β), and enhancement of endogenous antioxidant defenses. These mechanisms align closely with those reported in other experimental models, reinforcing the translational relevance of metformin's pleiotropic (beyond-glucose lowering) effects.

Clinically, these findings are promising, as metformin is widely used, well characterized, and inexpensive. If similar protective effects are observed in humans, metformin could potentially be repurposed as an adjunct therapy in settings of acetaminophen overdose or in populations at risk of drug-induced liver injury. However, careful consideration is needed because metformin has contraindications (e.g., in patients with renal impairment or a risk of lactic acidosis), and its safety in the context of acute overdose has not been established.

Nonetheless, this study had several limitations. First, our work was limited to a single high dose of paracetamol and metformin; we did not assess dose-response relationships or the optimal timing of metformin administration (i.e., preventive vs. therapeutic). In addition, we did not perform histopathological analyses, which could further validate biochemical protection with tissue-level evidence. Finally, the mechanisms we inferred (e.g., mitochondrial protection and JNK signaling) are based on previous literature rather than direct measurements in our model. Future studies should assess the signaling pathways, mitochondrial function, and gene expression.

CONCLUSION

This study provides new evidence that metformin significantly protects against acute paracetamol-induced hepatorenal toxicity, likely through antioxidant and mitochondrial-stabilizing mechanisms. These findings expand the therapeutic potential of metformin beyond glycemic control and support further investigation into its repurposing in drug-induced organ injury models.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

All procedures were approved by the Research and Ethical Committee of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria (RBC/FBMC/DELSU/24/363).

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. KKA and EGM conceptualized the study. KKA, EGM, EJJ, and NPI performed the experiments. PC and SEO performed the analyses. EGM, PC and NPI wrote the first draft, while all authors approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest with this study.

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