

ORIGINAL ARTICLE

PROTECTIVE ROLE OF EMPAGLIFLOZIN AGAINST
METHOTREXATE-INDUCED LIVER INJURYSalahuddin Shaikh, Sheraz Ansari*, Farzana Rahim Memon, Farheen Malik**,
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Background: Methotrexate (MTX), a commonly used chemotherapeutic and immunosuppressive agent, is associated with dose-dependent hepatotoxicity characterized by oxidative stress and liver tissue damage. Empagliflozin (EM), a sodium-glucose cotransporter-2 (SGLT2) inhibitor, has shown antioxidant properties in various organ systems. This study aimed to evaluate the hepatoprotective effects of EM against MTX-induced liver injury in rats. **Methods:** Forty adult Wistar rats were divided into four groups of 10 each. Group A (Control, 5% dimethyl sulfoxide as vehicle control), Group B (MTX 20 mg/Kg, i.p., on day 3), Group C (EM 10 mg/Kg/day for 7 days+MTX), and Group D (EM 30 mg/Kg/day for 7 days+MTX). Serum liver enzymes (ALT, AST), bilirubin, and oxidative stress markers (MDA, SOD, GPx, catalase) were measured. Liver tissues were also examined histologically using a semi-quantitative scoring system for cellular degradation, cytoplasmic vacuolization, sinusoidal dilatation, and inflammatory infiltration. **Results:** MTX administration resulted in significant elevations in ALT, AST, bilirubin, and MDA levels, along with reductions in SOD, GPx, and catalase activities ($p<0.001$). Histopathology confirmed severe hepatic damage in the MTX group. EM co-treatment, significantly increased antioxidant enzyme activity, reduced lipid peroxidation, and improved histological scores, with Group D showing better results ($p<0.001$). Biochemical and microscopic parameters showed a dose-dependent protective effect of EM. **Conclusion:** EM significantly attenuates MTX-induced hepatotoxicity by reducing oxidative stress and preserving liver histoarchitecture. These findings suggest a potential role for EM as a hepatoprotective agent against drug-induced liver injury.

Keywords: Empagliflozin, Hepatotoxicity, Liver injury, Methotrexate, Oxidative stress

Pak J Physiol 2026;22(1):12-5, DOI: <https://doi.org/10.69656/pjp.v22i1.1886>

INTRODUCTION

Drug-induced hepatic injury has become a widespread and significant global health issue.¹ This challenge not only hinders drug approval but also negatively impacts the therapeutic effectiveness of drugs and reduces patient compliance. Drug-induced liver toxicity may arise through several mechanisms, including direct interference with cellular processes, formation of toxic metabolites, or immune-mediated responses that provoke inflammation and hepatocellular damage.² In recent years, the occurrence of hepatotoxic reactions due to medications has increased, prompting growing concern in clinical and pharmaceutical research communities.³

Methotrexate (MTX) is an antimetabolite and folic acid analogue commonly used to manage malignancies like leukaemia and chronic inflammatory disorders such as psoriasis and rheumatoid arthritis.⁴ Its clinical application is limited by adverse effects, particularly hepatotoxicity. Although the precise pathways remain under investigation, oxidative stress and the generation of reactive oxygen species (ROS) are thought to play central roles in MTX-induced hepatic injury.^{5,6} By disrupting mitochondrial respiration, MTX promotes excessive ROS production, which can damage cellular structures, initiate lipid peroxidation, and trigger hepatocyte death.⁷ Therefore, minimizing oxidative

stress and ROS accumulation is a key target for preventing MTX-related liver damage.

Empagliflozin (EM) is a sodium-glucose cotransporter-2 (SGLT-2) inhibitor that lowers blood glucose by blocking glucose and sodium reabsorption in the proximal renal tubules.⁸ SGLT-2 facilitates the reabsorption of glucose and sodium from urine into the bloodstream. Although it is primarily expressed in the kidneys, it is also found in the liver, heart, thyroid, and skeletal muscle.⁹ In addition to its glucose-lowering effect, empagliflozin has demonstrated pleiotropic benefits, including reductions in body weight, blood pressure, arterial stiffness, lipid levels, systemic inflammation, insulin resistance, and uric acid concentration factors that contribute to cardiovascular protection.¹⁰ Emerging evidence indicates that EM has protective effects on the kidneys, liver, gastrointestinal system, brain, and peripheral nerves.¹¹⁻¹³ These protective effects are largely attributed to its ability to reduce oxidative stress. The objective of the current study was to evaluate the hepatoprotective effects of empagliflozin against methotrexate-induced liver injury.

MATERIAL AND METHODS

This study was conducted in the Department of Pathology, Isra University, Hyderabad, between February and July 2025, following approval from the

Institutional Ethical Review Committee. A total of 40 healthy male Wistar rats (weighing 200±20g) were procured. The sample size was determined using power analysis methods appropriate for animal research studies.¹⁴ Rats were kept in an appropriate standard habitat with a regular photoperiod (12/12 hours light/dark cycle).

All rats had regular food and unlimited access to water. The animals were randomly allocated into 4 groups of 10 each. Group A (Control): Received 5% dimethyl sulfoxide (DMSO) orally by gavage once daily for 7 days as the vehicle control. Group B (MTX-only): Received 5% dimethyl sulfoxide (DMSO) orally by gavage once daily for 7 days, and a single intraperitoneal injection of methotrexate (20 mg/Kg body weight) on day 3. Group C (EM 20 mg/Kg+MTX): Received empagliflozin at a dose of 20 mg/Kg body weight, administered orally by gavage once daily for 7 days. Methotrexate (20 mg/Kg body weight) was administered as a single intraperitoneal injection on day 3. Group D (EM 30 mg/Kg+MTX): Received empagliflozin at a dose of 30 mg/Kg body weight, administered orally by gavage once daily for 7 days, with methotrexate (20 mg/Kg body weight) given as a single intraperitoneal injection on day 3.

Blood was collected through cardiac puncture post-cervical dislocation, following standard protocols. Serum levels of liver function markers (Alanine Transaminase, Aspartate Transaminase, Bilirubin), along with oxidative stress markers (Superoxide Dismutase, Glutathione peroxidase, Catalase,

Malondialdehyde), were assessed using commercially available diagnostic kits.

Excision of liver tissue specimen, formalin fixation, and preparation of slides after H&E staining for microscopic evaluation was performed as per standard protocols. Histopathological evaluation was performed by an experienced pathologist who was blinded to the experimental groups. Liver sections were assessed using a semi-quantitative scoring system evaluating cellular degradation, cytoplasmic vacuolization, sinusoidal dilatation, and inflammatory cell infiltration, graded on a scale from 0 (normal) to 3 (severe).¹⁵ Inter-observer variability was not assessed in this study.

Data was analyzed using SPSS-25, and $p < 0.05$ was considered statistically significant.

RESULTS

Significant differences were observed among the groups for liver function markers. ALT levels were lowest in Group A (29.31±2.4 U/L) and highest in Group B (253.3±4.2 U/L), with Group C (130.2±4.9 U/L) and Group D (108.3±1.9 U/L) showing intermediate values ($p < 0.001$). AST levels were significantly elevated in Group B (170.57±8.4 U/L) compared to Group A (38.42±3.2 U/L), while Group C (113.33±5.8 U/L) and Group D (94.81±7.6 U/L) showed partial recovery ($p < 0.001$). Serum bilirubin was markedly increased in Group B (2.6±0.6 mg/dL) relative to Group A (0.8±0.1 mg/dL), whereas Group C (1.5±0.15 mg/dL) and Group D (1.1±0.11 mg/dL) demonstrated dose-dependent improvement ($p < 0.001$). (Table-1).

Table-1: Liver function parameters (ALT, AST, and Bilirubin) across experimental groups

Variables	Group A	Group B	Group C	Group D	<i>p</i>
ALT (U/L)	29.31±2.40 ^{bcd}	253.30±4.20 ^{acd}	130.20±4.90 ^{abd}	108.30±1.90 ^{abc}	<0.001*
AST (U/L)	38.42±3.20 ^{bcd}	170.57±8.40 ^{acd}	113.33±5.80 ^{abd}	94.81±7.60 ^{abc}	<0.001*
Bilirubin (mg/dL)	0.80±0.10 ^{bcd}	2.60±0.60 ^{acd}	1.50±0.15 ^{abd}	1.10±0.11 ^{abc}	<0.001*

*Statistically significant

Significant alterations were noted across all groups for antioxidant enzymes and lipid peroxidation. SOD levels were highest in Group A (418±32.57 U/mg) and significantly reduced in Group B (247.35±41.75 U/mg), while partial restoration was observed in Group C (330.14±24.71 U/mg) and Group D (367.78±18.97 U/mg) ($p < 0.001$). GPx activity followed a similar trend, with the lowest levels in Group B (4.1±0.34 U/mg) and progressive improvement in Group C (5.6±0.47 U/mg) and Group D (6.3±0.25 U/mg), compared to Group A

(7.2±0.52 U/mg) ($p < 0.001$). Catalase levels were significantly reduced in Group B (32.54±3.48 U/mg) compared to Group A (55.31±4.22 U/mg), but improved in Group C (42.28±4.67 U/mg) and Group D (48.87±5.48 U/mg) ($p < 0.001$). MDA levels, a marker of lipid peroxidation, were significantly elevated in Group B (330.57±21.34 nmol/mg) compared to Group A (160.44±20.41 nmol/mg), but decreased in Group C (261.72±16.18 nmol/mg) and further in Group D (202.62±21.88 nmol/mg) ($p < 0.001$), indicating a dose-dependent reduction in oxidative damage. (Table-2).

Table-2: Oxidative stress markers (SOD, GPx, Catalase, and MDA) across experimental groups

Variables	Group A	Group B	Group C	Group D	<i>p</i>
SOD (U/mg)	418.00±32.57 ^{bcd}	247.35±41.75 ^{acd}	330.14±24.71 ^{abd}	367.78±18.97 ^{abc}	<0.001*
GPx (U/mg)	7.20±0.52 ^{bcd}	4.10±0.34 ^{acd}	5.60±0.47 ^{abd}	6.30±0.25 ^{abc}	<0.001*
Catalase (U/mg)	55.31±4.22 ^{bcd}	32.54±3.48 ^{acd}	42.28±4.67 ^{abd}	48.87±5.48 ^{abc}	<0.001*
MDA (nmol/mg)	160.44±20.41 ^{bcd}	330.57±21.34 ^{acd}	261.72±16.18 ^{abd}	202.62±21.88 ^{abc}	<0.001*

*Statistically significant

Semi-quantitative analysis of liver tissue revealed significant differences in histopathological damage among the groups. Group A exhibited no signs of liver injury, with a score of 0 for all parameters, including cellular degradation, cytoplasmic vacuolization, sinusoidal dilatation, and inflammatory cell recruitment. Group B showed the most severe damage, scoring 3 for all four parameters, indicating extensive hepatocellular injury, vacuolization, sinusoidal congestion, and prominent inflammation. Group C showed moderate improvement, with scores of 2 across all parameters, reflecting partial hepatoprotection. Group

D demonstrated the most marked improvement among treated groups, with scores of 1 for cellular degradation, cytoplasmic vacuolization, and inflammation, and 0 for sinusoidal dilatation, suggesting near-complete restoration of liver architecture. (Table-3).

Table-3: Semi-quantitative histopathological scores of liver tissue damage across experimental groups

Variables	Group A	Group B	Group C	Group D
Cellular degradation	0	3	2	1
Cytoplasmic vacuolization	0	3	2	1
Sinusoidal dilatation	0	3	2	0
Inflammatory cell recruitment	0	3	2	1

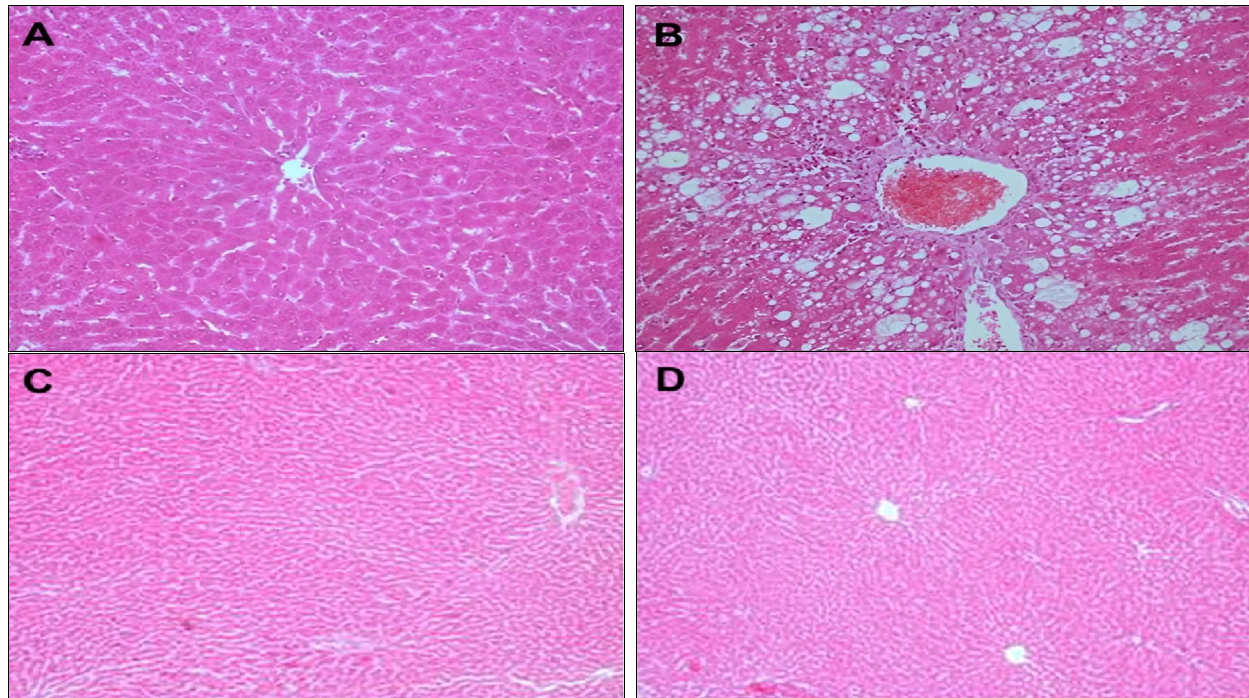


Figure-1: Histopathological evaluation of liver tissue in experimental groups A, B, C, D. (H&E stain, ×100)

DISCUSSION

Methotrexate, widely used in high doses for treating conditions such as acute leukaemia and severe psoriasis, is associated with hepatotoxicity, including acute liver injury, hepatic fibrosis, and cirrhosis.⁴ We investigated the protective effects of empagliflozin (EM) against MTX-induced liver damage. Our findings demonstrate that EM administration significantly attenuated hepatic injury, as reflected by improvements in both biochemical markers and histopathological features.

MTX-treated rats exhibited marked elevations in serum ALT, AST, and bilirubin levels —cytosolic chemicals that serve as sensitive indicators of hepatocellular damage and membrane integrity. This is consistent with the findings of Yanaşoğlu *et al*¹⁶, who reported MTX administration caused hepatotoxicity which was evident by the elevation of serum ALT, AST, and bilirubin levels. MTX significantly increased malondialdehyde (MDA) levels and reduced the activity

of antioxidant enzymes in liver tissue. Previous studies have shown that MTX promotes reactive oxygen species (ROS) formation, impairs NADPH availability, and depletes intracellular glutathione (GSH), thereby compromising the cellular antioxidant system and increasing susceptibility to oxidative damage.¹⁶ These biochemical findings were supported by histological evidence of MTX induced hepatocellular degeneration, cytoplasmic vacuolization, sinusoidal dilatation, and inflammatory infiltration, consistent with findings reported by Yanaşoğlu *et al*¹⁶ and Kalantari *et al*¹⁷.

EM pre-treatment notably reversed these alterations in a dose-dependent manner, with the higher dose demonstrating near-complete normalization of liver architecture and enzyme levels. EM's protective role may be attributed to its ability to enhance antioxidant enzyme activity and inhibit lipid peroxidation. In our study, EM significantly reduced MDA levels and restored GPx and SOD activities,

consistent with these prior findings.¹⁶ Histopathological improvements further confirmed the biochemical restoration, highlighting EM's ability to preserve hepatocyte integrity.

The reduction in ALT and AST levels in EM-treated groups aligns with earlier studies where EM attenuated liver enzyme elevations induced by MTX.¹⁷ Microscopic examination corroborated these results, revealing significant histological improvement in EM-treated rats compared to the MTX group. These findings suggest that EM may mitigate MTX-induced hepatotoxicity through antioxidant modulation.

A limitation of our study is the absence of inflammatory cytokine profiling, which could have further elucidated the anti-inflammatory effects of EM. Future studies should explore this aspect to better characterize the full spectrum of EM's hepatoprotective mechanisms.

CONCLUSION

Empagliflozin significantly protects rats' livers from damage caused by methotrexate. EM successfully reduced biochemical and histological indicators of hepatotoxicity by lowering oxidative stress and increasing antioxidant enzyme activity. These results demonstrate its potential as a preventative measure against drug-induced liver damage and call for additional research to determine its clinical suitability.

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Received: 19 Jul 2025

Reviewed: 7 Sep 2025

Accepted: 7 Sep 2025

Contribution of Authors:

All authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

SS: Concept, study design, drafting of script

FRM: Drafting the article, collection and assembly of data

UM: Collection and assembly of data

SA: Drafting of script, critical analysis

FM: Drafting the article, collection and assembly of data

AGM: Drafting of article, literature review

Conflict of Interest: None to declare

Funding: None received