ORIGINAL ARTICLE

# Erythropoietin's Therapeutic Value in Preventing Methotrexate's Adverse Renal Effects

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## Abstract

**Introduction:** Methotrexate may cause problems with the kidneys. Nonetheless, erythropoietin's advantageous extra-hematopoietic impact may protect against methotrexate-induced nephrotoxicity. **Objectives:** Clarifying the impact of renoprotective erythropoietin on renofunctional and renomorphological aspects in adult male albino rats will help determine its role in mitigating methotrexate-induced nephrotoxicity. **Materials and Methods:** 60 male albino rats were used in the investigation, and they were divided equally into 3 groups. The control one received normal saline 2 times a week for 9 weeks at a dosage of 0.5 mg/kg body weight (BW). Group 2 received 0.5 mg/kg BW intraperitoneal injections of methotrexate hydrate twice a week for 9 weeks. Group 3 received methotrexate hydrate intraperitoneally for the same amount of time and at the same dosage as group 2, along with a once-weekly subcutaneous injection (S.C) of 100 IU/kg recombinant human erythropoietin. Rats were slaughtered, renal sections were ready for histological analysis, and serum urea, creatinine, and albuminuria were determined at the conclusion of the study. **Results:** The methotrexate-treated group showed significantly higher levels of renal function assessed chemicals with worsening histological renal alterations when compared to the control or the methotrexate and erythropoietin co-treated group. The latter showed significantly improved microscopic renal alterations along with statistically significant reductions in the drug levels. Furthermore, there were negligible statistical differences in renal morphology and biochemistry between different groups. **Conclusion:** This study found that animals given with erythropoietin in addition to methotrexate provided a valuable and effective resistance against methotrexate-induced nephrotoxicity.

**Key words:** Cytoprotection, erythropoietin, kidney, methotrexate, oxidative stress

## INTRODUCTION

Damage to the kidneys caused by anticancer therapy might be demonstrated by abnormalities in tubular and/or glomerular function.[1] Chemotherapy for children's cancer frequently causes adverse renal effects, such as acute renal injury, acid-base disturbances, capillary endothelial damage, tubulointerstitial disease, and electrolyte imbalances.[2] Chemotherapeutic drug-induced renal problems are attributed to tubular blockage, inadequate renal parenchymal damage, renal blood supply, and microvascular structural damage.[3]

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Methotrexate is a commonly used anticancer medication that functions as a conventional antifolate. High-dose methotrexate is a large dose of methotrexate that is administered by delayed intravenous infusion to patients with normal renal function after an excess of fluid consumption and alkalinization to make the drug soluble in urine. Leucovorin co-administration significantly reduces the risk of fatal methotrexate poisoning.[4] Even with these precautions, methotrexate-induced nephrotoxicity still occurs, although rarely. Since methotrexate is eliminated by the kidney through excretion, methotrexate-induced renal dysfunction is linked to an interruption in methotrexate removal, which raises its plasma concentration continuously and causes methotrexate-toxic manifestations to manifest, primarily myelosuppression and inflammation of the liver, skin, and mucous membranes.[5] Furthermore, several medications, including non-steroidal anti-inflammatory drugs, penicillin, probenecid, and sulfisoxazole, have been linked to increased nephrotoxicity when taken with methotrexate because they interfere with the drug's excretion from the kidney and cause difficulties with renal tubular secretion. Abrupt elevation of serum creatinine combined with a marked rise in methotrexate plasma concentration signals the onset of methotrexate-induced renal impairment.[6] Furthermore, the development of renal failure has not always coincided with a severe hepatic impairment linked to high-dose methotrexate therapy. Considered a growth factor, erythropoietin plays a critical role in neovascularization and cell proliferation. Furthermore, biochemically speaking, erythropoietin is a glycoprotein that is secreted by interstitial cells, circulated in a soluble state, and stimulates erythropoiesis. It mostly treats individuals with transplant kidney disease and anemia caused by end-stage renal disease.[7,8]

Although the precise molecular pathophysiology of erythropoietin's self-protective impact is still unknown, new research has shown that erythropoietin performs a number of important biological functions that can preserve several organs, including the kidney in the event of ischemia renal injury.[9]

Nephrotoxicity, which is characterized by a reduced renal blood supply and glomerular filtration rate, is one of the most common adverse effects of oncotherapeutic medications.[10]

The study's goal was to identify erythropoietin's protective and ameliorative function against methotrexate-induced toxic renal damage in male rats.

## MATERIALS AND METHODS

The protocol (SCBR-139-2023) for this study was developed in accordance with the laboratory protection of animal rights' ethical guidelines. The albino rats weighing between 210 and 240 g were taken from the animal house at PSA University's pharmacy college laboratory. The rats were kept in a controlled environment. They were fed a regular rat chow and had unrestricted access to water while living in the animal house. All parameters are within the typical range for a laboratory, including cages and a 55% relative humidity environment, central ventilation with 15 air changes per hour and 20°C temperature. Following an acclimation period of 2 weeks, the animals were split evenly into three groups, each consisting of twenty rats. For 9 weeks, each group was fed a regular chow diet; for a total of 9 weeks, animals in the control group received intraperitoneal injections of normal saline twice a week (a dosage of 0.5 mg/kg body weight [BW]). Group 2: For a total of 9 weeks, animals in this group received intraperitoneal injections of methotrexate hydrate twice a week at a dose of 0.5 mg/kg BW. Group 3: For 9 weeks, animals received subcutaneous injections of 100 IU/kg recombinant human erythropoietin once a week for 9 weeks, in addition to intraperitoneal injections of methotrexate hydrate twice a week at a dosage of 0.5 mg/kg BW (total of nine injections).

Serum creatinine, serum urea, and urinary microalbuminuria concentrations were measured, along with renal function, by collecting blood and urine samples at the end of the trial. Samples of urine and blood were measured as follows: Measurement of blood urea level (mg/dL) using the modified Berthelot reaction enzymatic method. The level of serum creatinine (mg/dL) was determined using a colorimetric kinetic technique. The specific apparatus measures the amount of albumin in urine (mg/dL). Finally, an overdose of anesthetic resulted in the sacrifice of every rat in each of the three groups. Renal specimens from each rat used in the autopsy were removed, cleaned multiple times in normal saline, and then, fixed in formalin. The specimens were properly fixed, then rinsed with tap water, dried with ethyl alcohol in series, cleaned with xylol, imbedded, and cast in paraffin.

To perform a general morphological examination and detect histopathological changes, thin paraffin sections were prepared, placed on charged glass slides, and stained with hematoxylin and eosin, collagen fibers, collagen fibers, and glycogen content of renal tubular lining cells and basement membranes of renal parenchymal structures using periodic acid Schiff (PAS) with diastase and also Masson T. stain also used.

Version 17 of the Statistical Package for the Social Sciences software program was used to gather and examine data. The F-test (Analysis of Variance) was utilized to assess quantitative data and compare the various groups. Pearson's coefficient was employed to calculate the degree of correlation among various parameters. Furthermore, $P$ values obtained from the test of least significant difference $\leq 0.05$.

## RESULTS

When comparing the mean values of all renal function biochemically assessed components in the methotrexate-erythropoietin-managed group (G3) to the control group (G1), Table 1 reveals statistically insignificant changes ($P > 0.05$). When compared to the control group, the

**Table 1:** Findings from chemicals that were biochemically tested in every group

| Substances examined | Serum urea (mg/dL) | Serum creatinine (mg/dL) | Urine albumin (mg/dL) |
|---|---|---|---|
| G1 (control) | 40.19±5.212 | 1.24±0.137 | 25.39±6.081 |
| G2 (methotrexate) | 64.97±8.98 | 4.63±0.524 | 41.42±9.115 |
| G3 (methotrexate+erythropoietin) | 41.11±1.91 | 1.41±0.198 | 27.39±5.132 |

methotrexate-treated group (G2) had notable nephrotoxicity as evidenced by a highly statistically significant rise in the mean values of urine albumin level ($P < 0.05$), serum urea and creatinine, and both. Conversely, when the third group was exposed to the second group, there was a highly significant drop ($P < 0.05$) in the mean values of serum urea, creatinine, and urine albumin levels [Figure 1].

Under a microscope, the control renal sections showed typical parenchymal tissue, including renal tubules and glomeruli with regular lumens and cubical epithelial cells lining them [Figure 2].

On the other hand, methotrexate resulted in renal tubule degeneration and rupture of their basement membranes in group 2. The majority of renal tubules had flattened epithelial cell lining and cystic luminal dilatation. In addition, the glomeruli had deteriorated [Figure 3]. In addition, in the group 3 renal sections administered erythropoietin along with methotrexate, there were significantly fewer harmful renal structural alterations observed. Group 3's improved histological changes were demonstrated by a morphological return that was almost identical to the control group's appearance, with the exception of a small number of moderately dilated renal tubules [Figure 4].

Furthermore, the study found that, although group 2's lining cells showed no discernible change in morphology, PAS with diastase stain revealed a strong eosinophilic cytoplasmic reaction within the renal tubular lining cells and the integrity of tubular basement membranes in both the control and third [Figure 3d] groups.

In addition, the control group's intact glomerular Bowman's capsule and intact renal tubular and glomerular basement membranes were revealed by Massion's trichrome stain, together with normally distributed collagen in the renal parenchymal tissue [Figure 2d]. Nonetheless, group 2 showed comparatively higher levels of collagen fibers in the glomeruli and surrounding the renal tubules, along with damaged tubular and glomerular basement membranes. The structural alterations seen in group 2 were lessened and almost returned to the control group's typical look in group 3 [Figure 4d].

## DISCUSSION

Blood urea nitrogen (BUN) and creatinine, which are catabolic protein products mostly processed by the kidney, are the primary markers of renal function.

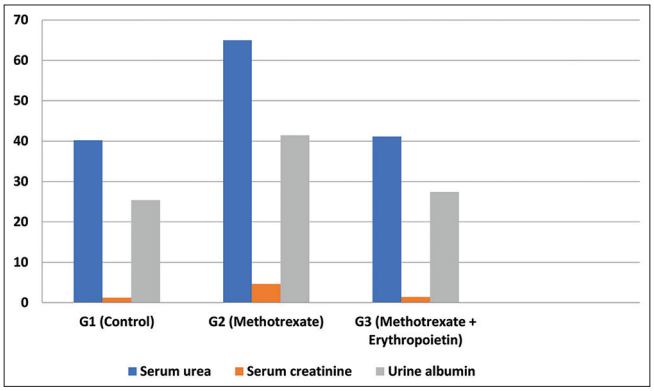


**Figure 1:** Renal function assessment in the groups under study

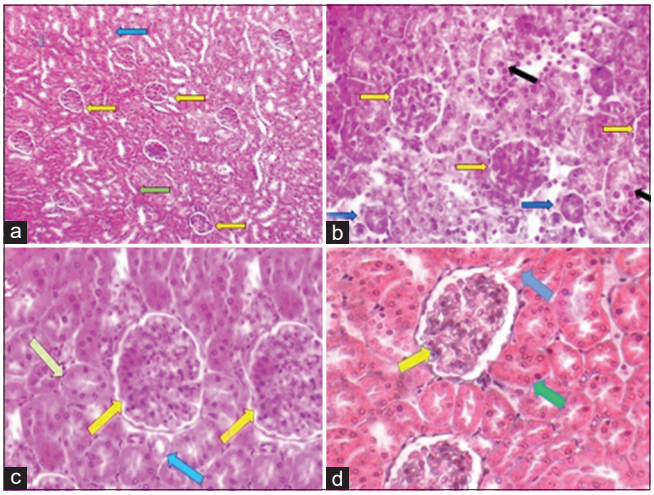


**Figure 2:** (a) Section in the kidney of control group, showing renal corpuscles formed of glomerulus (yellow arrow). The renal tubules are proximal (green arrow) and distal (blue arrow) **(**H&E, ×100**)**. (b) The renal corpuscles formed of glomerulus (yellow arrow). The renal tubules are proximal (blue arrow) and distal (black arrow) (H&E, ×200). (c) The control group showing normal glomeruli (yellow arrow), clear normal-sized subcapsular space (blue arrow), the Proximal convoluted tubule (PCT) (blue arrow), the Distal convoluted tubule (DCT) (yellow arrow) (H&E ×400). (d) Showing minimal amount of collagen (green color). And normal glomerular tuft of capillaries (yellow arrow), proximal (green arrow), and distal (blue arrow) convoluted tubules in the cortex with minimal amount of collagen around renal tubules Bowman's capsule (yellow arrow) (Masson T. ×400). H&E: Hematoxylin and eosin

When renal function is impaired beyond the capacity of the body to compensate, serum creatinine and BUN levels tend to increase.

The goal of our research was to prevent and treat methotrexate-induced nephrotoxicity to prevent the potentially fatal

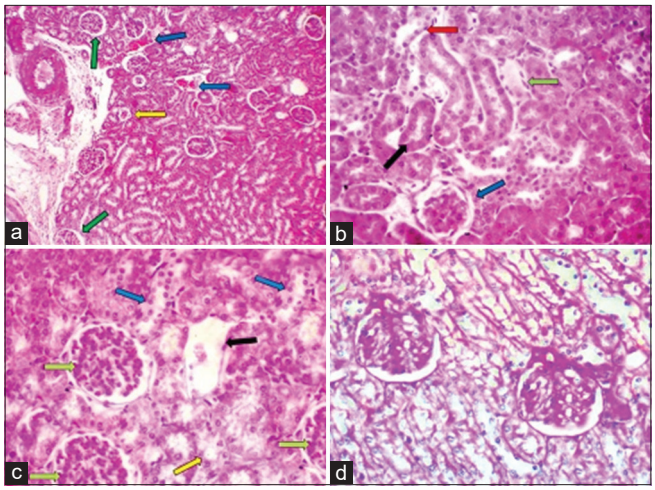


**Figure 3:** (a) A section in the kidney of methotrexate group showing interstitial hemorrhage (blue arrows), widening of the Bowman's space in most of the glomeruli (green arrow), and some glomeruli are atrophied (yellow arrow) (H&E ×100). (b) Showing interstitial hemorrhage (blue arrows), hemorrhagic vacuolated glomerulus (black arrow) with widening of the Bowman's space in the glomeruli (green arrow), and other atrophied and vacuolated glomerulus (red arrow**)** (H&E ×200). (c) Showing interstitial hemorrhage (yellow arrow), tubular vacuolation (yellow arrow). Widening of the Bowman's space (green arrow) hemorrhagic vacuolated glomerulus (black arrow) (H&E ×400). (d) Showing marked decrease of PAS reaction in the) tubules accompanied and glomeruli (PAS ×400**)**. PAS: Periodic acid Schiff, H&E: Hematoxylin and eosin

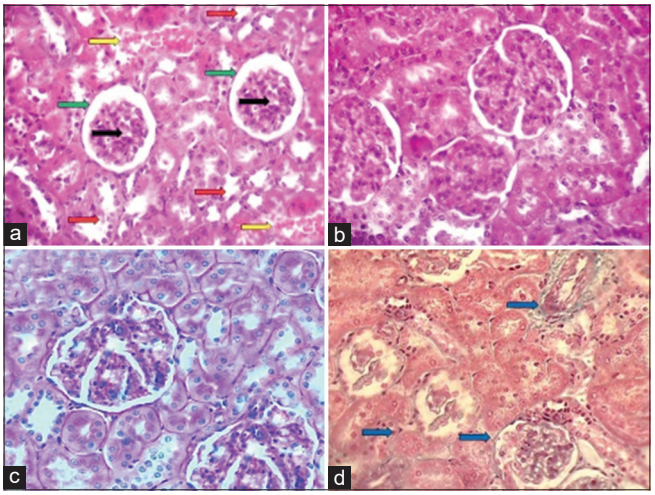


**Figure 4:** (a) Section in the kidney of erythropoietin treated group showing minimal loss of brush border and minimal vacuolizations in some cells of proximal tubules (red arrows). Also, the glomerular tuft of capillaries appeared to be minimally shrinked with minimal vacuolations (black arrow) and increased Bowman's space (green arrow), (H&E ×200). (b) Showing minimal vacuolizations in some cells of proximal tubules and the rest of tubules are normal shaped (H&E ×400). (c) Showing rise of glycogen content (PAS-positive reaction) in the basement membrane. In addition, there were some indications of recovery in the renal tubules, particularly in the brush boundary of the proximal tubular epithelial cells, which is where PAS +ve distal tubules occur (PAS ×400). (d) Showing minimal amount of collagen fibers in interstium (blue arrow**) (**Masson T. ×400). PAS: Periodic Acid Schiff, H&E: Hematoxylin and eosin

side effects of the drug, as reported by Widemann and Adamson. These side effects included hepatic inflammation, mucocutaneous irritation, and bone marrow suppression.[4]

In the methotrexate-treated group, we observed varying renal parenchymal structural alterations linked to markedly higher creatinine levels and serum urea together with elevated urine albumin levels.

Degeneration of the renal tubules and glomeruli, breakdown of their basement membranes, and a comparatively greater amount of collagen fibers in the peritubular and glomerular regions were among the morphological alterations.

Furthermore, despite the unremarkably altered glycogen content seen within their lining cells, the majority of renal tubules displayed a cystically dilated lumen with flattening of their lining epithelial cells.

These results were in line with those found by Sharbaf *et al*., who reported that the majority of the oncotherapeutic drug's absorption takes place in the proximal renal tubules and that the drug's concentration in the kidney's tubular cells is several times greater than its concentration in the extracellular compartment.[11]

Furthermore, Liao *et al*. observed a marked increase in blood BUN and creatinine levels in the ischemic rat kidney at 4, 12, and 24 h after renal reperfusion. They also reported renal parenchymal tissue damage following reperfusion of ischemia, which resulted in a significant reduction in the kidney's ability to excrete urea nitrogen and creatinine.[12]

According to Smeland *et al*., Messmann, and Allegra, methotrexate-induced renal nephrotoxicity can result from the drug's direct harmful action on the renal tubules or from the drug's precipitation of its metabolites in the renal tubules.[13]

In the third group of our investigation, the aberrant toxic renal structural and biochemical alterations were significantly reduced by co-administration of erythropoietin and methotrexate. With the exception of slightly dilated renal tubules, this group's histopathological alterations involving the amount of collagen fibers in the glomeruli and surrounding the renal tubules improved and almost completely restored the control group's normal appearance. Furthermore, group 3's biochemical renal analysis results showed a considerable drop, approaching the control normal range.[14]

Our findings paralleled those of Liao *et al*., who established the defensive effect of erythropoietin on renal ischemia by finding lower BUN and serum creatinine levels in the erythropoietin-treated group of rats within 4, 12, and 24 h of renal reperfusion than their values in the rat group suffering

renal ischemic injury. Furthermore, they confirmed that renal cellular apoptosis, endothelial damage to the renal arteries, oxidative stress on the renal tissue, and the inflammatory response inside the kidney have all been linked to the pathologic alterations associated with renal ischemia.[12]

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## CONCLUSION

In summary, our research validated the renoprotective and mitigating effects of erythropoietin on the structure and function of methotrexate-induced nephrotoxicity in male albino rats. As a result, taking erythropoietin supplements together with methotrexate treatment is crucial.

## ACKNOWLEDGMENTS

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## INFORMED CONSENT

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data are available upon request from the authors.

## ETHICS APPROVAL

All series of steps that were implemented in this study that included animal models were in compliance with the Ethics Committee of Prince Sattam Bin Abdulaziz University Institutional Review Board (SCBR-139-2023).

## REFERENCES

1. Denker B, Robles-Osorio ML, Sabath E. Recent advances in diagnosis and treatment of acute kidney injury in patients with cancer. Eur J Intern Med 2011;22:348-54.
2. Salahudeen AK, Doshi SM, Pawar T, Nowshad G, Lahoti A, Shah P. Incidence rate, clinical correlates, and outcomes of AKI in patients admitted to a comprehensive cancer center. Clin J Am Soc Nephrol 2013;8:347-54.
3. Salahudeen AK, Bonventre JV. Onconephrology: The latest frontier in the war against kidney disease. J Am Soc Nephrol 2013;24:26-30.
4. Widemann BC, Adamson PC. Understanding and managing methotrexate nephrotoxicity. Oncologist 2006;11:694-703.
5. Stark AN, Jackson G, Carey PJ, Arfeen S, Proctor SJ. Severe renal toxicity due to intermediate-dose methotrexate. Cancer Chemother Pharmacol 1989;24:243-5.
6. Lawrenz-Wolf B, Wolfrom C, Frickel C, Fengler R, Wehinger H, Henze G. Schwere renale methotrexat-ausscheidungsstörung nach hochdosis-therapie [Severe renal impairment of methotrexate elimination after high dose therapy]. Klin Padiatr 1994;206:319-26. [German].
7. Moore E, Bellomo R. Erythropoietin (EPO) in acute kidney injury. Ann Intensive Care 2011;1:3.
8. Yasari F, Nafar M, Alipour Abdei B, Ahmadpoor P, Pour-Reza-Gholi F, Samadian F, *et al*. Effect of erythropoietin on kidney allograft survival: Early use after transplantation. Iran J Kidney Dis 2012;6:44-8. Erratum in: Iran J Kidney Dis 2012;6:157.
9. Zou YR, Zhang J, Wang J, Peng L, Li GS, Wang L. Erythropoietin receptor activation protects the kidney from Ischemia/Reperfusion-induced apoptosis by activating ERK/p53 signal pathway. Transplant Proc 2016;48:217-21.
10. Launay-Vacher V, Rey JB, Isnard-Bagnis C, Deray G, Daouphars M, European Society of Clinical Pharmacy Special Interest Group on Cancer Care. Prevention of cisplatin nephrotoxicity: State of the art and recommendations from the European Society of Clinical Pharmacy Special Interest Group on Cancer Care. Cancer Chemother Pharmacol 2008;61:903-9.
11. Sharbaf FG, Farhangi H, Assadi F. Prevention of chemotherapy-induced nephrotoxicity in children with cancer. Int J Prev Med 2017;8:76.
12. Liao JG, Li MY, Wang XH, Xie Q. The protective effect of erythropoietin pretreatment on ischemic acute renal failure in rats. J Acute Dis 2016;5:408-12.
13. Smeland E, Fuskevåg OM, Nymann K, Svendesn JS, Olsen R, Lindal S, *et al*. High-dose 7-hydromethotrexate: Acute toxicity and lethality in a rat model. Cancer Chemother Pharmacol 1996;37:415-22.
14. Watowich SS. The erythropoietin receptor: Molecular structure and hematopoietic signaling pathways. J Investig Med 2011;59:1067-72.
15. Zafirov D, Petrusevska G, Sikole A, Trojacanec J, Labacevski N, Kostova E, *et al*. Erythropoietin reduces cumulative nephrotoxicity from cisplatin and enhances renal tubular cell proliferation. Prilozi 2008;29:167-83.

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