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Comparative study of Valsartan and Leucovorin alone or in combination for renal protection in methotrexate-induced acute kidney injury in rats

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ABSTRACT

Methotrexate (MTX) is a widely used chemotherapeutic and immunosuppressive agent that may induce acute kidney injury (AKI), posing a major clinical challenge. This study investigated the renoprotective effects of Valsartan, Leucovorin and their combination in mitigating methotrexate (MTX)-induced renal damage in Wistar rats. The aim was to evaluate these effects in a rat model of MTX-induced acute kidney injury (AKI). After approval by the Institutional Animal Ethics Committee (IAEC), forty male Wistar rats were divided into five groups: control (saline), MTX (single intraperitoneal injection of 20 mg/kg), Valsartan (10 mg/kg/day), leucovorin (10 mg/kg/day), and combination (Valsartan plus leucovorin). Drug treatment was initiated one day before MTX administration and continued orally for five days. Renal function was assessed by measuring serum creatinine and blood urea nitrogen (BUN). Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were evaluated as inflammatory markers, while malondialdehyde (MDA) and glutathione (GSH) were assessed as oxidative stress markers. Histopathological examination of renal tissue was also performed. Data were analyzed using GraphPad Prism version 6. The MTX group showed significant renal dysfunction ($p < 0.001$) and increased inflammatory and oxidative stress markers. Both Valsartan and leucovorin produced partial renoprotective effects, with Valsartan significantly reducing oxidative stress ($p < 0.001$) and leucovorin markedly decreasing inflammatory markers ($p < 0.001$). The combination therapy demonstrated the most pronounced renoprotective effect ($p < 0.001$), as evidenced by improved renal function and reduced oxidative damage and inflammation. Histopathological analysis revealed decreased tubular necrosis and inflammatory infiltration in the combination group. Valsartan and leucovorin exerted individual renoprotective effects; however, their combined administration provided highly significant protection against MTX-induced AKI. These findings suggest that combination therapy may represent a promising strategy for preventing renal injury in patients undergoing MTX treatment.

INTRODUCTION

Methotrexate (MTX), a folate antagonist, is widely used as a chemotherapeutic and immunosuppressive agent for various malignancies and autoimmune disorders [1]. Although MTX is a cornerstone of many treatment protocols due to its efficacy and broad clinical applications, it is often associated with serious adverse effects [2]. One of the most significant and life-threatening complications is nephrotoxicity, particularly acute kidney injury (AKI) [3]. MTX-induced AKI compromises renal function and limits the continuation of therapy, adversely affecting patient prognosis [4].

Despite advances in supportive care, the management of MTX-induced AKI remains a major clinical challenge [5]. The pathogenesis of MTX-induced renal injury involves multiple mechanisms, including direct tubular toxicity,

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oxidative stress, inflammatory responses, and disruption of folate metabolism. These processes collectively contribute to structural and functional damage of renal tissues . Consequently, the identification of effective pharmacological interventions capable of preventing or attenuating MTX-induced nephrotoxicity has become an important area of research [6]. In this context, Valsartan and Leucovorin have attracted attention because of their potential renoprotective properties.

Valsartan, an angiotensin II receptor blocker (ARB), is commonly prescribed to treat hypertension and various kidney diseases [7]. Its protective effect on the kidneys stems from its ability to interact with the renin-angiotensin-aldosterone system (RAAS), which is a key physiological mechanism involved in maintaining blood flow to the kidneys and regulating blood pressure. By reducing intraglomerular pressure, decreasing proteinuria, and suppressing inflammatory and fibrotic pathways, Valsartan contributes to the preservation of glomerular filtration [8]. These mechanisms collectively contribute to preserving renal tissue structure and function under stressful conditions, including drug-induced nephrotoxicity.

Leucovorin, also known as folinic acid, is a reduced form of folic acid that counteracts the inhibition of dihydrofolate reductase induced by MTX [9]. It functions as a "rescue" agent by directly supplying tetrahydrofolate to cells, thereby supporting critical biological processes such as DNA synthesis and repair in normal tissues. During MTX therapy, Leucovorin plays a crucial role in limiting off-target cytotoxicity, including renal toxicity, by restoring folate-dependent metabolic pathways and promoting cellular recovery and regeneration [10].

Although Valsartan and Leucovorin have individually demonstrated protective effects against renal injury, their combined therapeutic potential in MTX-induced AKI has not been extensively investigated. Given their distinct yet complementary mechanisms, Valsartan primarily targeting hemodynamic and inflammatory pathways, and Leucovorin supporting metabolic and cellular repair their co-administration may produce synergistic renoprotective effects.

Therefore, the present study aimed to evaluate and compare the efficacy of Valsartan, Leucovorin, and their combination in attenuating MTX-induced AKI in an experimental rat model. By analyzing renal function parameters, oxidative stress biomarkers, inflammatory mediators, and histopathological changes, this study seeks to elucidate the underlying mechanisms of protection and to identify effective strategies for mitigating MTX-associated nephrotoxicity.

MATERIALS AND METHODS

Drugs and chemicals

Methotrexate, Valsartan, and Leucovorin were obtained from the institutional medical pharmacy.

Experimental animals

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) under approval number BVDUMC/2214/2024/17. A total of forty healthy adult male Wistar rats, weighing between 150-200 g, were used in this study. All experimental procedures were conducted in accordance with the ethical guidelines of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India.

Experimental design and grouping

The forty rats were randomly divided into five groups, with each group comprising eight animals (n = 8), as follows:

- Group I (Control) – Received normal saline orally.
- Group II (MTX Group) – Received a single intraperitoneal (IP) injection of methotrexate (MTX) at a dose of 20 mg/kg to induce renal damage.
- Group III (MTX + Valsartan Group) – Received MTX (20 mg/kg, single IP dose) and oral Valsartan at 10 mg/kg/day for 5 consecutive days.
- Group IV (MTX + Leucovorin Group) – Received MTX (20 mg/kg, single IP dose) and oral Leucovorin at 10 mg/kg/day for 5 consecutive days.
- Group V (MTX + Valsartan + Leucovorin Group) – Received MTX (20 mg/kg, single IP dose) along with a combination of Valsartan and Leucovorin (each 10 mg/kg/day orally) for 5 consecutive days.

In Groups III, IV, and V, methotrexate was administered on the second day of treatment.

On the sixth day, blood samples were collected from the retro-orbital plexus under ketamine anesthesia. Serum was separated by centrifugation and stored at -20°C for subsequent biochemical evaluation. Following sample collection, the animals were humanely euthanized, and their kidneys were excised

Renal function was assessed by measuring serum creatinine, blood urea nitrogen (BUN), and uric acid levels using commercially available diagnostic kits according to the manufacturers' protocols. Pro-inflammatory markers were quantified in serum samples using enzyme-linked immunosorbent assay (ELISA) kits.

Lipid peroxidation in kidney tissue was evaluated by estimating malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) assay. Antioxidant capacity was assessed by measuring reduced glutathione (GSH) levels using Ellman's reagent method.

For histological examination, kidney tissues were fixed in 10% neutral-buffered formalin, processed using standard paraffin-embedding techniques, and sectioned into 5 µm slices. The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope to identify morphological alterations such as tubular necrosis, interstitial inflammation, and glomerular damage. Histopathological scoring was performed by a pathologist blinded to the treatment groups to ensure unbiased assessment.

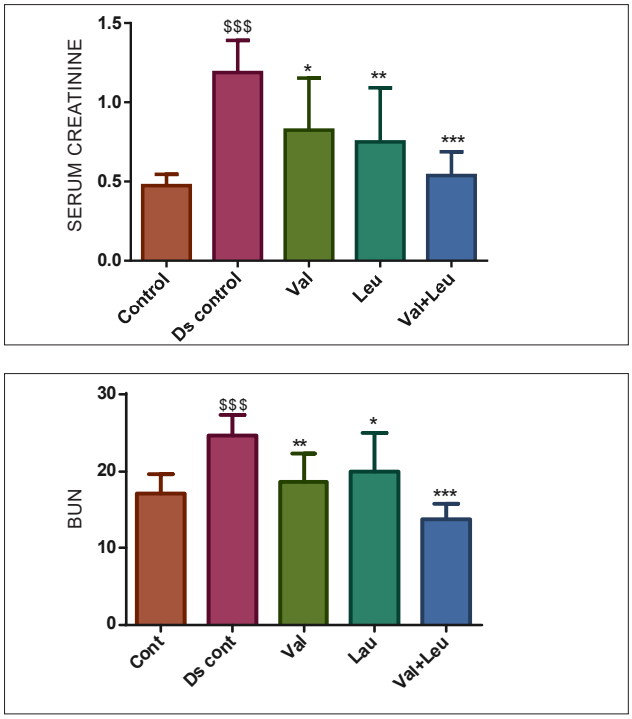
Statistical analysis

All results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant. Data processing and analysis were carried out using GraphPad Prism version 6.

RESULTS

Administration of methotrexate (MTX) resulted in a significant increase in serum creatinine and blood urea nitrogen (BUN) levels compared to the control group, indicating the successful induction of acute kidney injury ($p < 0.001$). Treatment with Valsartan significantly reduced serum creatinine levels ($p < 0.05$) and BUN levels ($p < 0.01$) when compared to the MTX group. Similarly, Leucovorin administration led to a significant decrease in serum creatinine ($p < 0.01$) and BUN ($p < 0.05$) levels.

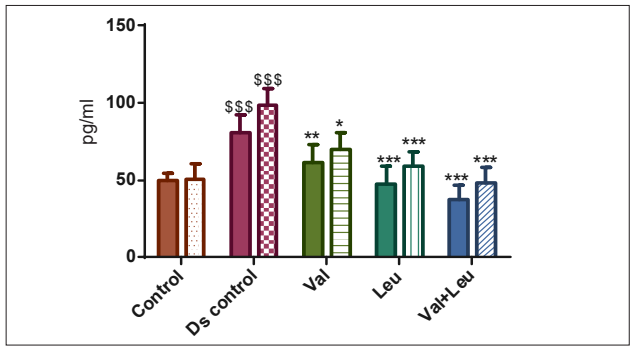
The combination therapy of Valsartan and Leucovorin produced a marked reduction in both serum creatinine ($p < 0.001$) and BUN ($p < 0.001$) levels, restoring them close to normal values, with highly significant improvements compared to the MTX group.



DS - Disease, Val - Valsartan, Leu - Leucovorin
Results are expressed as mean ± SD
n = 8; One way ANOVA followed by Tukey's test
$$$ p<0.001 in comparison with control, *p<0.05 & **p<0.01, ***p<0.001 in comparison with disease control

Figure 1. Effect of Valsartan and Leucovorin on serum creatinine and BUN levels in MTX-induced AKI

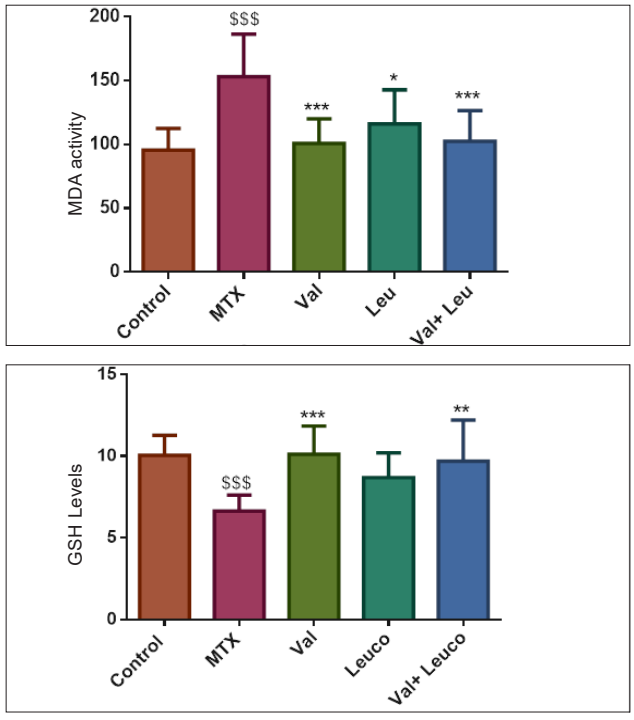
MTX administration led to a significant increase in pro-inflammatory cytokine levels, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), compared to the control group ($p < 0.001$ for both), indicating the induction of acute kidney injury. Valsartan treatment significantly attenuated this inflammatory response, as evidenced by reductions in IL-6 ($p < 0.01$) and TNF-α ($p < 0.05$) levels. Leucovorin exhibited a more potent anti-inflammatory effect, significantly lowering both IL-6 and TNF-α concentrations ($p < 0.001$ for both) compared with the MTX-treated group. The combination therapy group, receiving both Valsartan and Leucovorin, showed the greatest decline in these inflammatory markers, with IL-6 and TNF-α levels significantly reduced ($p < 0.001$ for both), approaching those observed in the control animals.



DS - Disease, Val - Valsartan, Leu - Leucovorin
Results are expressed as Mean ± SD
n = 8; One way ANOVA followed by Tukey's test
$$$ p<0.001 in comparison with control, *p<0.05 & **p<0.01, ***p<0.001 in comparison with disease control

Figure 2. Effect of Valsartan and Leucovorin on inflammatory markers

Methotrexate (MTX) administration resulted in a significant elevation of malondialdehyde (MDA) levels ($p < 0.001$) and a notable decline in reduced glutathione (GSH) levels ($p < 0.001$) compared to the control group, indicating heightened oxidative stress associated with MTX-induced acute kidney injury. Valsartan treatment effectively mitigated this oxidative damage, as demonstrated by a significant reduction in MDA levels ($p < 0.001$) and a significant restoration of GSH levels ($p < 0.001$) relative to the MTX group. Leucovorin therapy also led to a significant decrease in MDA levels ($p < 0.05$), suggesting a moderate reduction in lipid peroxidation. Although GSH levels increased with Leucovorin, the improvement did not reach statistical significance compared with the MTX group. The combination therapy with Valsartan and Leucovorin exhibited



MTX - Methotrexate, Val - Valsartan, Leuco - Leucovorin
Results are expressed as Mean ± SD
n = 8; One way ANOVA followed by Tukey's test
$$$ p<0.001 in comparison with control, *p<0.05 & **p<0.01, ***p<0.001 in comparison with disease control

Figure 3. Effect of Valsartan and Leucovorin on oxidative stress markers

the most pronounced antioxidant effect, evidenced by a highly significant decrease in MDA ($p < 0.001$) and a significant rise in GSH levels ($p < 0.001$), indicating strong protection against oxidative kidney damage.

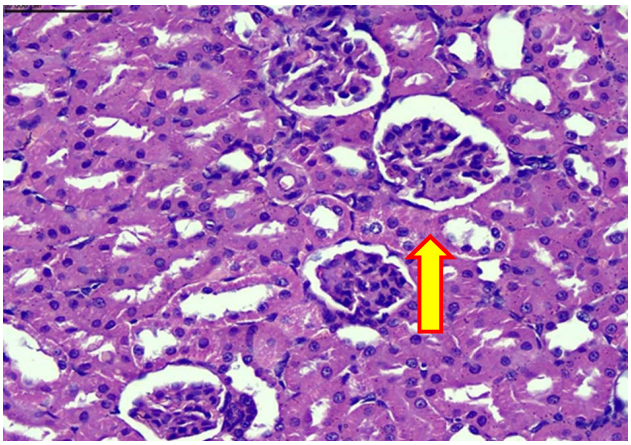
The control group exhibited normal renal architecture with intact glomeruli, well-preserved tubular structures, and no signs of inflammation, necrosis, or edema.

The MTX group showed severe histopathological alterations, including widespread tubular epithelial cell degeneration, tubular necrosis, glomerular atrophy, interstitial edema, and significant inflammatory cell infiltration, confirming the induction of acute kidney injury.

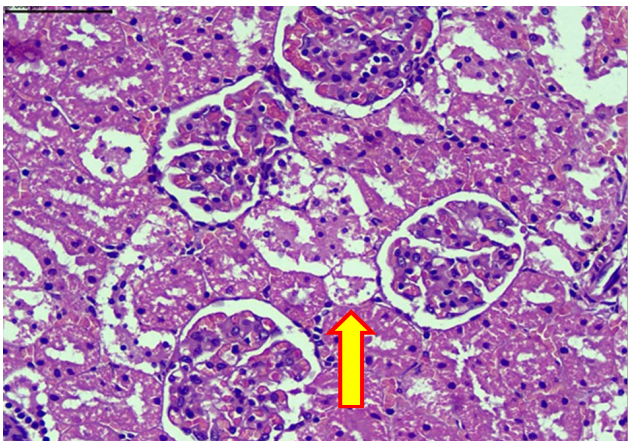
The Valsartan-treated group demonstrated moderate improvement in renal histology, with reduced tubular degeneration and necrosis and fewer inflammatory infiltrates compared to the MTX group. Glomerular structure appeared relatively preserved, indicating partial protection.

The Leucovorin-treated group also showed attenuation of MTX-induced damage, with mild to moderate improvement in tubular and glomerular architecture. There was a noticeable reduction in necrosis and interstitial inflammation, though the effect was less pronounced than in the Valsartan-treated group.

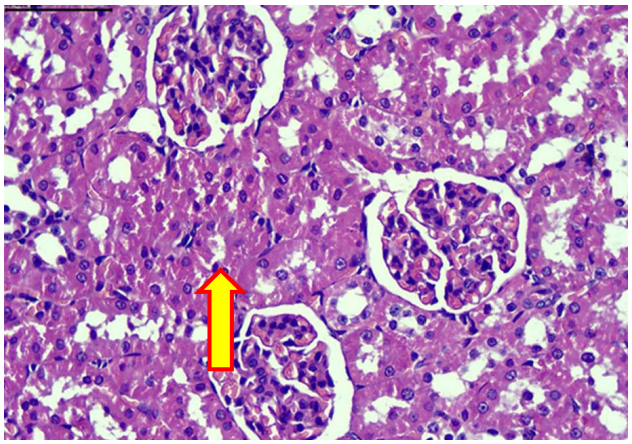
The combination group exhibited the the greatest histological protection. Kidney sections revealed nearly normal renal architecture with minimal tubular injury, preserved glomeruli, and negligible inflammatory cell infiltration.



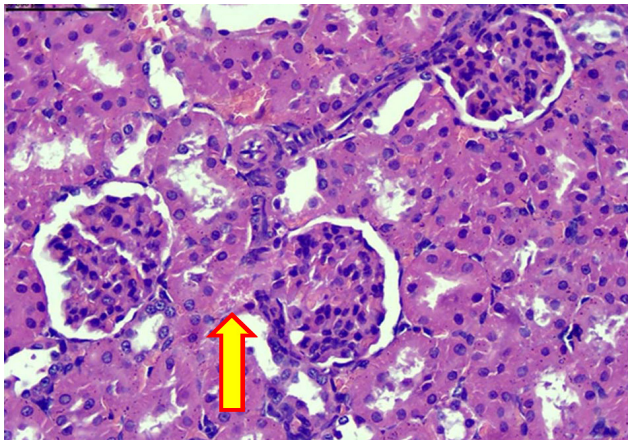
4a Group I (control): Shows normal glomerulus, renal tubules in cortex and medulla. 40×, H & E Stain



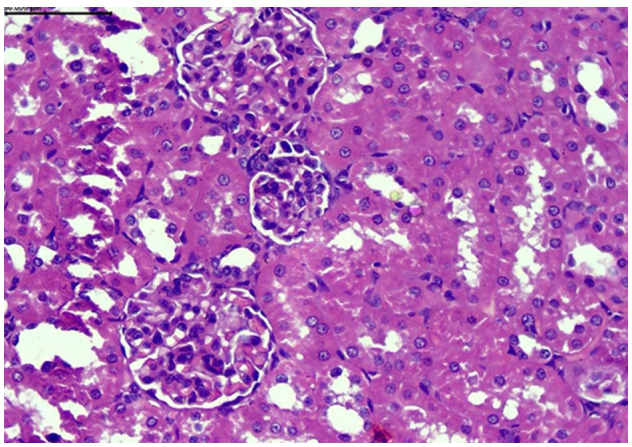
4b Group II (methotrexate): Shows vacuolar degeneration of renal tubules in cortex and medullae



4c Group III (Valsartan): Shows vacuolar degeneration of renal tubules in cortex and medullae multifocal, minimal, mild



4d Group IV (Leucovorin): Shows degeneration of tubules in cortex & medullae multifocal, minimal, mild



4e Group V (Leucovorin & Valsartan): Shows no abnormality

Figure 4. Effect of Valsartan and Leucovorin on histopathology of renal tissue

DISCUSSION

Methotrexate (MTX), a folate antagonist, is extensively used in the management of various neoplastic and autoimmune disorders because of its potent antiproliferative properties. However, its clinical utility is frequently limited by dose-dependent toxicities, among which acute kidney injury (AKI) represents one of the most serious complications. As the kidney is the primary route of MTX excretion, high intratubular concentrations may induce direct tubular toxicity, crystal nephropathy, oxidative damage, and inflammation, ultimately leading to impaired renal function [11,12].

In the present study, MTX administration resulted in a significant elevation of serum creatinine and blood urea nitrogen (BUN) levels (Figure 1), which are well-established indicators of renal dysfunction. These biochemical alterations were accompanied by pronounced histopathological damage, including tubular degeneration, necrosis, and interstitial inflammation (Figure 4), confirming severe renal injury. These findings are consistent with previous reports demonstrating that MTX-induced nephrotoxicity is mediated by oxidative stress and activation of pro-inflammatory cytokine pathways [13,14].

Valsartan, an angiotensin II receptor blocker (ARB), exhibited marked renoprotective effects in this experimental model. Treatment with Valsartan significantly reduced serum creatinine and BUN levels, decreased oxidative stress as evidenced by reduced MDA and increased GSH concentrations (Figure 3), and attenuated the inflammatory response by lowering IL-6 and TNF-α levels (Figure 2). These observations are in agreement with earlier studies indicating that ARBs ameliorate renal injury by modulating the renin-angiotensin system, reducing intraglomerular hypertension, and suppressing inflammatory and fibrotic pathways [15,16]. Furthermore, Valsartan has been reported to stabilize mitochondrial function, limit reactive oxygen species (ROS) generation, and improve endothelial function, thereby contributing to renal protection [17].

Leucovorin (folinic acid), a reduced form of folic acid, exerts its protective effects by bypassing the dihydrofolate reductase blockade induced by MTX, thereby replenishing intracellular tetrahydrofolate pools and rescuing normal cells from MTX-related toxicity [18]. In the present study, Leucovorin significantly decreased serum creatinine and BUN levels and demonstrated a more pronounced effect on inflammatory markers compared with Valsartan. This anti-inflammatory activity may be attributed to its role in facilitating DNA repair and preserving cellular integrity under oxidative and inflammatory stress conditions [19]. Although the increase in GSH levels did not reach statistical significance, the observed reduction in MDA levels supports the antioxidative potential of Leucovorin.

Importantly, combined treatment with Valsartan and Leucovorin produced synergistic renoprotective effects. This group exhibited the most significant improvements across all evaluated parameters, including renal function indices, inflammatory cytokines, oxidative stress markers, and histopathological alterations. The combined therapy not only restored biochemical parameters toward normal values but also preserved renal architecture more effectively than either agent alone. These findings suggest that Valsartan primarily mitigates hemodynamic disturbances and oxidative stress, whereas Leucovorin complements these effects by supporting cellular metabolism and repairing MTX-induced DNA damage.

Histopathological evaluation further confirmed these observations. Kidneys from MTX-treated animals displayed characteristic features of nephrotoxicity, such as tubular necrosis, inflammatory cell infiltration, and glomerular damage (Figure 4a). These pathological changes were markedly attenuated in the combination group, which showed near-normal renal morphology (Figure 4e), indicating superior preservation of tissue integrity. These results are consistent with previous studies suggesting that multi-targeted therapeutic strategies are more effective in managing drug-induced toxicities than monotherapies [20].

The present study represents one of the limited comparative investigations evaluating the individual and combined effects of Valsartan and Leucovorin in an MTX-induced AKI model. The findings support the hypothesis that co-administration of these agents provides enhanced protection, likely due to their complementary mechanisms of action. Valsartan primarily modulates the renin-angiotensin system and suppresses inflammation, whereas Leucovorin restores folate metabolism and promotes cellular repair.

CONCLUSIONS

Current research indicates that Valsartan and Leucovorin significantly alleviate methotrexate (MTX)-induced kidney damage due to their anti-inflammatory and antioxidant properties. Combining the two provides maximum protection, suggesting a promising therapeutic strategy against MTX-induced nephrotoxicity. These findings may pave the way for improved supportive therapies for patients undergoing high-dose methotrexate treatment, potentially enhancing treatment tolerance and improving patient outcomes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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