OnLine Journal of Biological Sciences

Original Research Paper

Evaluation of the Renoprotective Effects of Valsartan in Methotrexate-Induced Acute Kidney Injury in Rats

Dina Kutbi

Department of Pharmacy, King Fahad Armed Forces Hospital, Jeddah, Saudi Arabia
Department of Pharmacology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia

Article history
Received: 22-01-2024
Revised: 23-03-2024
Accepted: 01-04-2024

Email: dina.t.kutbi@gmail.com

Abstract: Methotrexate (MTX) is a widely used chemotherapeutic agent known for its nephrotoxic effects, which can lead to acute kidney injury in hospitalized patients. However, the mechanism by which MTX induces nephrotoxicity is not entirely clear, but it is believed to involve oxidative stress and direct toxic effects on renal tubules. Valsartan, an angiotensin II receptor blocker, has been shown to exert renoprotective effects in various contexts, including diabetic nephropathy. In this study, the renoprotective potential of Valsartan to mitigate MTX-induced acute kidney in rats was explored. A total of twenty-four Wistar rats were randomly assigned to three groups. The Control Group (GRP 1) was administered an oral dose of 1 ml/kg of normal saline. The second group (GRP 2) received a single Intraperitoneal (IP) dose of 20 mg/kg MTX for a duration of 5 consecutive days. The third Group (GRP 3) was subjected to a single IP dose of 20 mg/kg MTX followed by an oral dose of 30 mg/kg Valsartan (Val) over the same 5-day period. At the end of the experiment, the levels of serum biochemical parameters, renal damage markers, and inflammatory and oxidative stress markers were accessed. Additionally, the impact of MTX on kidney tissue histology was investigated. The results indicate that MTX administration in rats led to a significant increase in serum biochemical parameters, renal damage markers, inflammatory markers, and oxidative stress markers, as well as changes in kidney histology. However, the administration of Val following MTX treatment to rats attenuated these effects. Conclusions: Val shows therapeutic potential in the treatment of MTX-induced kidney injury and its associated consequences.

Keywords: Acute Kidney Injury, Acute Renal Damage, Methotrexate, Oxidative Stress, Valsartan

Introduction

Acute Kidney Injury (AKI) is a prevalent clinical condition characterized by the abrupt reduction or loss of kidney function. AKI not only leads to significant illness and death but also elevates the likelihood of developing chronic kidney disease. The classic criteria for diagnosing and staging AKI involve evaluating serum creatinine levels and urine output rates (Turgut *et al*., 2023). AKI can arise from conditions like dehydration, sepsis, and low cardiac output, as well as renal artery obstructions and glomerulonephritis. Additionally, factors like acute tubular necrosis, interstitial nephritis, and postrenal obstructions in the urinary tract can also lead to AKI (Duga, 2016). In the United States, 1% of all hospital admissions involve acute kidney injury upon admission. Additionally, the incidence rate of AKI during hospitalization varies between 2 and 5%, with up to 67% of patients admitted to the intensive care unit developing AKI (Winther-Jensen *et al*., 2018; Kirkley *et al*., 2019).

Methotrexate (MTX), which is an analog of and counteracts the effects of folic acid, finds common use in treating a wide spectrum of both cancerous and non-cancerous ailments (Chan and Cronstein, 2013). Originally formulated as an anticancer drug, MTX now serves as the primary choice among disease-modifying anti-rheumatic drugs for conditions like rheumatoid arthritis, juvenile idiopathic arthritis, and psoriasis.

Additionally, it demonstrates its utility in the management of inflammatory bowel diseases, multiple sclerosis, vasculitis, systemic lupus erythematosus, and other connective tissue diseases and for transplant patients, courtesy of its valuable anti-inflammatory and immunomodulatory properties (Chan and Cronstein, 2010; Bedoui *et al*., 2019). The use of high-dose methotrexate MTX carries the risk of acute kidney injury, which can result in delayed removal of MTX from the body. Extended exposure to elevated MTX levels poses serious dangers like severe mucositis, hematopoietic suppression, and even potential mortality. Addressing high-dose MTX-related renal issues and delayed MTX elimination is a medical emergency. Various interventions have been utilized to manage this critical complication, including high-dose leucovorin, glucarpidase, and thymidine (Flombaum *et al*., 2018). However, these drugs, for example, glucarpidase, costs a lot, has a short half-life and it is not readily available (Domingo-González *et al*., 2021).

Valsartan is an angiotensin II receptor blocker widely used to treat hypertension. Its mechanism of action involves inhibiting angiotensin II, a hormone that constricts blood vessels and raises blood pressure (Ecder, 2014). This results in the dilation of glomerular artery blood vessels, lowering glomerular pressure and reducing protein filtration, thus inhibiting proteinuria (Jacobsen *et al*., 2003). Valsartan may decrease renal vasoconstriction and inflammation by blocking the AT1 receptor, resulting in a decrease in kidney damage (Siragy *et al*., 2000). Additionally, Val downregulates endothelin and cytokines, curbing inflammation and glomerular cell proliferation, promoting extracellular matrix degradation, and suppressing collagen synthesis, which collectively delay glomerular sclerosis and maintain renal function (Jacobsen *et al*., 2003). Notably, it has been found to reduce the incidence of microalbuminuria without causing renal dysfunction in individuals with glucose intolerance. In diabetic mouse models, Val has shown potential in alleviating renal podocyte damage and reducing profibrotic growth factors, proinflammatory cytokines, lipid accumulation, and glomerulosclerosis (Currie *et al*., 2017).

Earlier studies suggest that Val effectively decelerates the progression of renal function decline in patients with advanced chronic kidney disease and hypertension (Sun *et al*., 2021). Yet, little is known about the renoprotective ability of Val in MTX-induced kidney injury. In this study, Valsartan's kidney-protective potential in a rat model of methotrexate-induced acute kidney injury was explored, with the aim of expanding our understanding of its renoprotective effects during acute kidney injury and its possible mechanism.

Materials and Methods

Drugs and chemical: Methotrexate (50 mg/2mL) injections were purchased from Hospira Pharmaceutical Co., Illinois, United States with a Lot number (G024412AB). Valsartan 80 mg strength tablets were purchased from Novartis Pharmaceutical, Basel, Switzerland with a Lot number (BTX09).

Animals: Twenty-four male Wistar rats weighing between 170 and 190 g were bought from the animal house at the King Abdulaziz University's faculty of pharmacy in Jeddah, Saudi Arabia. All animals were given a week to acclimatize at a temperature of 22°C, a relative humidity range of 35-75%, and a 12-h light/dark cycle.

Experimental design: Rats were randomly placed into three groups with n = 8 in each group. GRP 1 was the control group and received 1 mL/kg of normal saline. GRP 2 was given an Intraperitoneal (IP) injection of MTX at a dose of 20 mg/kg. GRP 3 was given an IP of 20 mg/kg of MTX and a 30 mg/kg oral dose of Val. All animal treatments lasted for five days. Finally, the animals were euthanized after being sedated with isoflurane. Following that, blood was taken through the retro-orbital plexus and kidneys were retrieved, cleaned with PBS, and weighed. The left kidneys were frozen at -80°C and used for tissue oxidative stress parameters, whereas the right kidneys were maintained in 10% buffered formalin for histology. All animal experiments were carried out in accordance with the declaration of Helsinki and were approved by the ethics committee of the faculty of pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia, with the reference number (PH-1442-75).

Determination of biochemical parameters: After centrifugation at 3000 rpm for 10 min at 4°C, serum samples were recovered from whole blood. An ELISA kit (MyBioSource, California, USA) was used to measure serum urea, creatinine, albumin, serum Lactate Dehydrogenase (LDH), Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), Neutrophil Gelatinase-Associated Lipocalin (NGAL) and kidney injury molecule-1 (KIM-1), according to the manufacturer’s guide.

Measurement of kidney tissue levels of superoxide dismutase, glutathione, and lipid peroxidation in tissue homogenate: 10% of kidney homogenate was made by weighing 100 mg of frozen kidney tissue in an Eppendorf tube and mixed with 1 mL of 100 mm phosphate buffer (pH 7.4) containing 1mm EDTA and spun at 14,000 rpm for 15 min at 4°C. The amounts of Superoxide Dismutase (SOD), Glutathione (GSH), and Malondialdehyde (MDA) were tested in the supernatant using a commercial kit (MyBioSource, California, USA).

Quantification of GFR: The glomerular filtration rates of the experiment animals’ kidneys were estimated according to the formulas below.

When serum creatinine <52 μmol/mL (0.588 mg/d):

1. GFR (mL/min) = 880× body weight (g) 0.695× creatinine concentration (µmol/mL)$^{-0.660}$× urea concentration (mmol/mL)$^{-0.391}$
2. When serum creatinine ≥52 µmol/mL (0.588 mg/dL)
3. GFR (mL/min) = 5862× body weight (g) 0.695× creatinine concentration (µmol/mL)$^{-1.15}$× urea (mmol/mL)$^{-0.391}$

Histopathology: Following a 10% neutral-buffered formalin fixation, the kidney tissues were dehydrated in graded alcohol, cleared in xylene, and embedded in paraffin for 12 h. Following the sectioning of the paraffin blocks at a thickness of 5 µm, the slides were heated for an hour at 60°C. Then the slides were deparaffinized in xylene and then rehydrated in graded alcohol before being washed in distilled water for 2 min. Slides were then stained with a solution of hematoxylin and eosin and finally, images were captured using a light microscope.

Statistical analysis: GraphPad prism V6.0 software (GraphPad Software, San Diego, CA) was used for the graphs. Statistical analysis was done using one-way ANOVA and Sidak's multiple comparisons. A significant difference is defined as $p<0.05$.

Results

Effects of MTX and Val on serum biochemical parameters: Rats administered with MTX recorded an elevated level in the serum levels of urea, creatinine, uric acid, and urea in comparison to the untreated control animals in GRP 1. However, rats treated with 30 mg/kg body weight of Val following MTX administration revealed a significant decrease in the serum biochemical parameter tested when compared to the MTX-only administered rats (Fig. 1, Table S1).

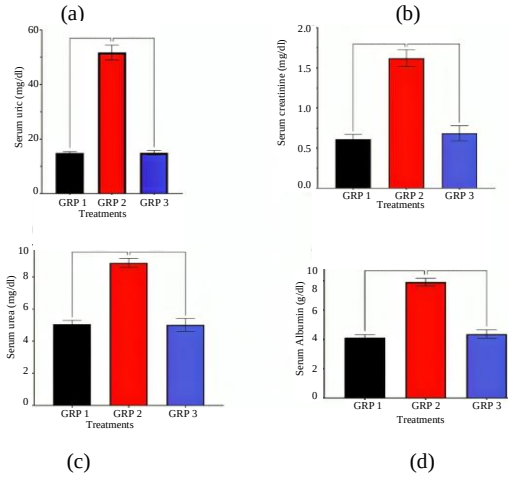

Fig. 1: Effects of MTX and Val on serum biochemical parameters. Serum urea; (a) Serum creatinine; (b) Serum uric acid; (c) Serum albumin; (d). Results are presented as mean ± SEM, (n = 8). Data was analyzed by one-way ANOVA followed by Sidak's multiple comparisons. ****p<0.0001 compared with the MTX group

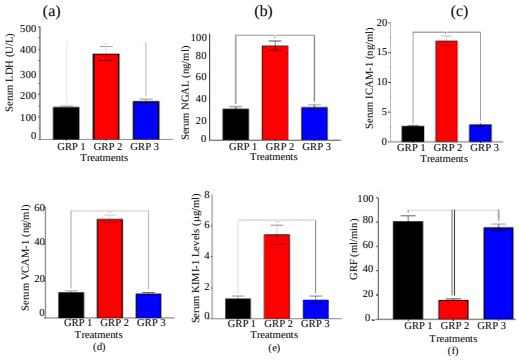

Fig. 2: Effects of MTX and Val on renal damage markers. Serum LDH; (a) Serum NGAL; (b) Serum ICAM-1; (c) Serum VCAM-1 (d), Serum KIM-1; (e) GFR; (f). Results are presented as mean ± SEM, (n = 8). Data was analyzed by one-way ANOVA followed by Sidak's multiple comparisons. ****p<0.0001 compared with the MTX group

Table S1: Effects of MTX and Val on serum biochemical parameters

Parameter/group	Control	MTX	MTX + Val
Serum urea level (mg/dL)	14.860±0.6070	51.750±2.730*	14.970±0.823#
Serum creatinine level (mg/dL)	0.604±0.0520	1.594±0.095*	0.643±0.090#
Serum uric acid level (mg/dL)	5.063±0.2370	8.875±0.283*	5.014±0.395#
Serum albumin level (g/dL)	4.135±0.1680	7.850±0.231*	4.410±0.241#

Values are presented as mean ± SEM (n = 8). *p<0.05, vs control; #p<0.05, vs MTX

Table S2: Effects of MTX and Val on renal damage markers

Parameter/group	Control	MTX	MTX + Val
Serum LDH (U/L)	148.500±7.141	384.600±26.77*	170.900±8.601#
Serum NGAL (ng/L)	29.640±1.930	89.130±4.418*	31.100±2.454#
Serum ICAM-1 (ng/mL)	2.655±0.121	16.970±0.810*	2.923±0.294#
Serum VCAM-1 (ng/mL)	13.850±0.720	53.610±1.859*	13.240±0.671#
Serum KIM1 (µg/L)	1.296±0.162	5.438±0.613*	1.237±0.236#
GFR (mls/min)	80.700±4.416	16.100±0.676*	75.710±2.723#

Values are presented as mean ± SEM (n = 8). *p<0.05, vs control; #p<0.05, vs MTX

Effects of MTX and Val on renal damage markers: Animals in the GRP 2 treated with MTX showed a significant increase in the levels of serum LDH, serum NGAL, serum ICAM-1, serum VCAM-1, serum KIM-1 and the Glomerular Filtration Rate (GFR) when compared to the untreated rats in the control group, GRP 1. Interestingly, the treatment of animals in GRP 3 with Val after being administered with MTX ameliorated the MTX-induced increase in the kidney injury markers when compared to the MTX-only treated rats (Fig. 2, Table S2).

Effects of MTX and Val on Serum Inflammatory Markers: The intraperitoneal administration of MTX to rats significantly elevated and caused the release of inflammatory markers TNF-α and IL-6 in comparison to the untreated rats in the control group. However, rats treated with 30 mg/kg of Val, following the intraperitoneal administration of MTX showed a significant reduction in the inflammatory marker levels when compared to the MTX-only administered rats and this reduction was near normal (Fig. 3, Table S3).

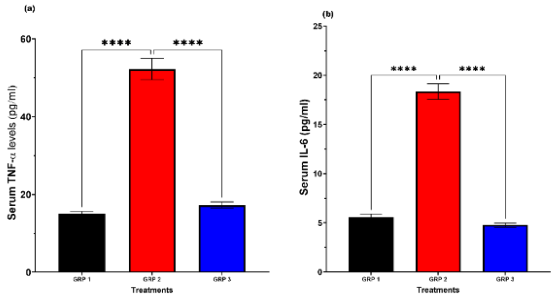

Fig. 3: Effects of MTX and Val on serum inflammatory markers. Serum TNF-α; (a) Serum IL-6; (b) Results are presented as mean ± SEM, (n = 8). Data was analyzed by One-way ANOVA followed by Sidak's multiple comparisons. ****p<0.0001 compared with the MTX group

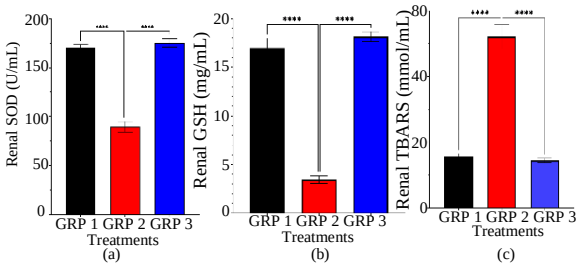

Fig. 4: Effects of MTX and Val on oxidative stress markers. Renal superoxide dismutase level; (a) Renal glutathione level; (b) Renal lipid peroxidation levels, quantified as TBARS level; (c) Results are presented as mean ± SEM, (n = 8). Data was analyzed by one-way ANOVA followed by Sidak's multiple comparisons. ****p<0.0001 compared with the MTX group

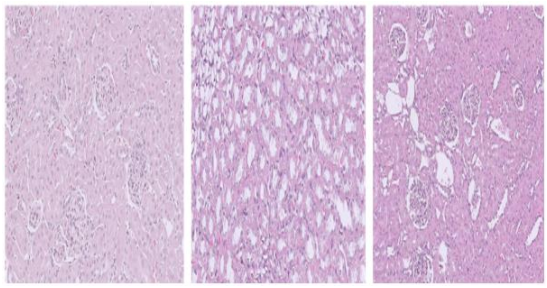

Fig. 5: Effects of MTX and Val on kidney histology. H and E staining from the kidney section of animals in the control group, demonstrating normal histological structures of the kidneys, including renal corpuscles, glomerular capillaries and renal tubules; (a) H and E staining from the kidney section of animals administered with MTX only, demonstrating a marked aggregation of infiltrating unviable cells, smaller renal corpuscles and glomeruli and an enlarged tubular lumen; disorganization of renal parenchyma accompanied by malformation and atrophy of renal corpuscles; (b) H and E staining from the kidney section of animals administered with MTX and treated with Val, demonstrating an improvement in the renal structure of the rat kidney; (c)

Table S3: Effects of MTX and Val on serum inflammatory markers.

Parameter/Group	Control	MTX	MTX + Val
Serum TNF-α (pg/mL)	15.090±0.547	52.25±2.750*	17.290±0.810$^{\#}$
Serum IL-6 (pg/mL)	5.575±0.322	18.35±0.794*	4.767±0.211$^{\#}$

Values are presented as mean ± SEM (n = 8). *p<0.05, vs control; #p<0.05, vs MTX

Table S4: Effects of MTX and Val on oxidative stress markers

Parameter/Group	Control	MTX	MTX + Val
Renal Tissues SOD (U/mL)	170.60±2.9700	89.630±5.378*	175.30±4.363$^{\#}$
Renal Tissues GSH (mg/mL)	16.98±1.0340	3.450±0.403*	18.16±0.516$^{\#}$
Renal Tissues TBARS (mmol/mL)	15.69±0.8530	52.130±3.632*	14.46±0.698$^{\#}$

Values are presented as mean ± SEM (n = 8). *p<0.05, vs control; #p<0.05, vs MTX

Effects of MTX and Val on Oxidative Stress Markers: Then, we explored the anti-oxidative stress potentials of Val in MTX-induced kidney-injured rats. Figure 4, the administration of MTX to rats led to a significant decrease in the levels of renal superoxide dismutase and glutathione when compared to the untreated animals in GRP 1. In addition, MTX administration resulted in a significant increase in renal lipid peroxidation as revealed by the TBARS levels when compared to the untreated control rats in GRP 1. Thus, signifying a condition of oxidative stress. However, the treatment of rats with Val after the administration of MTX ameliorated the MTX-induced oxidative stress (Fig. 4, Table S4).

Effects of MTX and Val on Kidney Histology: Finally, we examined the effects of MTX and Val on kidney histology. Figure 5, the histological sections from the H and E-stained kidneys of the untreated animals in GRP 1 showed normal histology, with clearly visible renal tubules, capillaries, and glomerular capillaries (Fig. 5a). In contrast, the stained kidney of rats administered with MTX in GRP 2, showed a noticeable accumulation of invading, non-viable cells, a reduction in the size of the renal corpuscles, cell aggregation and tubular lumen dilation (Fig. 5b). However, rats administered with MTX and treated with 30 mg/kg Val in GRP 3 showed amelioration in the structural deformation to the kidney arising from MTX administration (Fig. 5c).

Discussion

The present study investigated the renoprotective potentials of valsartan against methotrexate-induced kidney injury in rats. MTX is a widely used chemotherapeutic agent known for its nephrotoxic effects, which can lead to acute kidney injury. The mechanism by which MTX induces nephrotoxicity is not entirely clear, but it is believed to involve oxidative stress and direct toxic effects on renal tubules (Devrim *et al*., 2005; Saka and Aouacheri, 2017).

Valsartan, an angiotensin II receptor blocker, has been shown to exert renoprotective effects in various contexts, including diabetic nephropathy (Suzuki *et al*., 2002). In this study, the potential renoprotective effects of Val to mitigate MTX-induced AKI in a rat model were explored.

These results indicate that MTX administration in rats leads to a significant increase in serum biochemical parameters, renal damage markers, inflammatory markers, and oxidative stress markers, as well as changes in kidney histology. These findings are consistent with previous research indicating that MTX, particularly at high doses, results in renal toxicity (May *et al*., 2014; Hamed *et al*., 2022).

MTX is primarily excreted by the kidneys, making nephrotoxicity a common adverse effect (El-Agawy *et al*., 2022). In addition, MTX is known to influence cytokine production by T cells and macrophages, which could explain the elevation in inflammatory markers following MTX administration in rats (Fig. 3) and is consistent with a previous report (Chan and Cronstein, 2002; Bilginaylar *et al*., 2022).

Interestingly, the administration of Val following MTX treatment appears to attenuate these effects. Val treatment resulted in a considerable drop in serum biochemical parameters, renal damage markers, and inflammatory markers and it also alleviated MTX-induced oxidative stress. This is in conformity with earlier reports suggesting that Val has renoprotective benefits (Suzuki *et al*., 2002), can lower inflammatory and oxidative stress markers (Kuboki *et al*., 2007), and possesses neuroprotective effects by inhibiting activation of pro-inflammatory cytokine and oxidative stress (Hadi *et al*., 2015).

Lactate Dehydrogenase (LDH) is an enzyme that is involved in energy production exists in almost all cells in the body and has one of its highest levels in the kidneys. It is an inflammatory marker (Poggiali *et al*., 2020; Zhang and Shi, 2021). Our results demonstrated that the treatment of MTX caused an increase in the LDH level and agrees with the study conducted by Tunali-Akbay *et al*. who showed that Wistar rats who received a single dosage of MTX (20 mg/kg IP.) had greater levels of LDH than the untreated animals in the control group (Tunalı-Akbay *et al*., 2010). In our study, the treatment of animals with Val significantly attenuated this increase in LDH levels (Fig. 2). To further explore the renoprotective potentials of Val, we examined its effects on the levels of several AKI biomarkers including kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin.

The Kidney Injury Molecule-1 (KIM-1) is a transmembrane glycoprotein. Recent studies on both mice and humans have shown that an increase in the serum KIM-1 level can serve as a biomarker of kidney injury (Sabbisetti *et al*., 2014). In this study, the serum KIM-1 level increased in the MTX-tested group which is similar to what was observed by Younis *et al*. (Younis *et al*., 2021). where the authors examined the effect of Geraniol on MTX-induced AKI in rats. The Kim-1's elevated concentration induced by MTX may function as a compensatory strategy, acting as an adhesion molecule to lessen epithelial loss (Arun, 2013). This increase in the KIM-1 level was significantly reversed with the administration of Val.

The Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a siderophore-binding protein that is part of the lipocalin protein family (Clerico *et al*., 2012; Itenov *et al*., 2014). NGAL has been proven to be a strong biomarker for early AKI detection. An increased NGAL level usually occurs as early as 2 h after injury to the kidney (Hang *et al*., 2017). In this study, MTX caused an increase in NGAL which agrees with a previous study (Fouad and Al-Melhim, 2018) which examined the effects of the administration of vanillin to mitigate the adverse effects of cisplatin and MTX treatment on rat kidneys and also found an increase in NGAL level due to MTX treatment. The increase in the levels of NGAL is due to the MTX-induced inhibition of kidney reabsorption of NGAL in the renal tubule (Oh *et al*., 2017). Furthermore, the elevated level of NGAL may function as a compensating mechanism, causing tissue re-epithelization and a decrease in tubular cell apoptosis (Mohamed *et al*., 2017). Interestingly, in our study, the administration of Val decreased the increased NGAL values caused by MTX, suggesting that the drugs mediated this effect through the increase in kidney reabsorption of NGAL in the renal tubule.

The GFR describes the rate of flow of filtered fluid through the kidneys. It is the measurement of filtered volume through the glomerular capillaries into the Bowman's capsule per unit time. The amount of creatinine present in the blood serum and urine is mostly used to determine the GFR (Shahbaz and Gupta, 2023). A decrease in the GFR is a major definition of AKI. A decrease in GFR usually occurs as a result of an increase in the serum urea and creatinine level which was also noted in this study. The GFR diminished with the administration of MTX which was subsequently attenuated with the administration of Val.

The limitation of this study is that the animals used were normal animals and not cancerous and as such, the anti-tumor effects of Val were not determined. Nevertheless, this does not affect the overall effects of Val on the MTX-administered rats.

Conclusion

The data presented in this study demonstrate that MTX administration can lead to kidney toxicity, inflammation, and oxidative stress, but these effects can be considerably prevented by subsequent treatment with Val. This underlines the potential therapeutic benefit of Val, a drug frequently used in the treatment of hypertension, in treating MTX-induced renal damage and associated consequences. As an angiotensin II receptor blocker, valsartan inhibits the angiotensin II type 1 receptor, which is implicated in inflammation, salt retention, and vasoconstriction. Vasoconstriction and inflammation in the kidneys caused by MTX may be prevented by valsartan by blocking the AT1 receptor, which would

reduce kidney damage. However, further study is needed to fully understand the mechanism behind these effects and to define the optimal dose and timing of Val administration following MTX treatment.

Acknowledgment

The author would like to thank King Fahad Armed Forces Hospital, Jeddah, Saudi Arabia, and King Abdulaziz University, Jeddah, Saudi Arabia for their moral support.

Funding Information

The author has not received any financial support or funding to report.

Ethics

All animal experiments were carried out in accordance with the Declaration of Helsinki and were approved by the ethics committee of the Faculty of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia, with the reference number (PH-1442-75).

References

Arun, M. (2013). *Acute Kidney Injury in the Medical Wards of Government Rajaji Hospital, Madurai: A Prospective study* (Doctoral dissertation, Madurai Medical College, Madurai). http://repository-tnmgrmu.ac.in/id/eprint/6381

Bedoui, Y., Guillot, X., Sélambarom, J., Guiraud, P., Giry, C., Jaffar-Bandjee, M. C., ... & Gasque, P. (2019). Methotrexate is an old drug with new tricks. *International Journal of Molecular Sciences*, *20*(20), 5023. https://doi.org/10.3390/ijms20205023

Bilginaylar, K., Aykac, A., Sayiner, S., Özkayalar, H., & Şehirli, A. Ö. (2022). Evaluation of the antiapoptotic and anti-inflammatory properties of chitosan in methotrexate-induced oral mucositis in rats. *Molecular Biology Reports*, *49*(4), 3237-3245. https://doi.org/10.1007/s11033-022-07158-x

Chan, E. S., & Cronstein, B. N. (2002). Molecular action of methotrexate in inflammatory diseases. *Arthritis Research and Therapy*, *4*, 1-8. https://doi.org/10.1186/ar419

Chan, E. S., & Cronstein, B. N. (2010). Methotrexate-how does it really work?. *Nature Reviews Rheumatology*, *6*(3), 175-178. https://doi.org/10.1038/nrrheum.2010.5

Chan, E. S., & Cronstein, B. N. (2013). Mechanisms of action of methotrexate. *Bulletin of the NYU Hospital for Joint Diseases*, *71*(suppl 1), S5. https://www.proquest.com/openview/6317635d9d4740f9b0574c26a11be355/1?pq-origsite=gscholar&cbl=1646346

Clerico, A., Galli, C., Fortunato, A., & Ronco, C. (2012). Neutrophil Gelatinase-Associated Lipocalin (NGAL) as biomarker of acute kidney injury: A review of the laboratory characteristics and clinical evidences. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *50*(9), 1505-1517. https://doi.org/10.1515/cclm-2011-0814

Currie, G., Bethel, M. A., Holzhauer, B., Haffner, S. M., Holman, R. R., & McMurray, J. J. (2017). Effect of valsartan on kidney outcomes in people with impaired glucose tolerance. *Diabetes, Obesity and Metabolism*, *19*(6), 791-799. https://doi.org/10.1111/dom.12877

Devrim, E., Çetin, R., Kılıçoğlu, B., Imge Ergüder, B., Avcı, A., & Durak, İ. (2005). Methotrexate causes oxidative stress in rat kidney tissues. *Renal Failure*, *27*(6), 771-773. https://doi.org/10.1080/08860220500244823

Domingo-González, A., Osorio, S., Landete, E., Monsalvo, S., & Díez-Martín, J. L. (2021). A second administration of glucarpidase in a different cycle of high-dose methotrexate: Is it safe and effective in adults?. *Journal of Oncology Pharmacy Practice*, *27*(3), 734-738. https://doi.org/10.1177/1078155220946464

Duga, A. L. (2016). Acute kidney Injury: Prevalence, Diagnosis, Causes and treatment. *Journal of Clinical Research*, *3*, 134-148. https://doi.org/10.21522/TIJCR.2014.03.02.Art011

Ecder, T. (2014). Renal and metabolic effects of valsartan. *The Anatolian Journal of Cardiology*, *14*, S14. https://doi.org/10.5152/akd.2014.00003

El-Agawy, M. S. E. D., Badawy, A. M. M., Rabei, M. R., Elshaer, M. M. A., El Nashar, E. M., Alghamdi, M. A., ... & Elsayed, H. R. H. (2022). Methotrexate-induced alteration of renal Aquaporins 1 and 2, oxidative stress and tubular apoptosis can be attenuated by Omega-3 fatty acids supplementation. *International Journal of Molecular Sciences*, *23*(21), 12794. https://doi.org/10.3390/ijms232112794

Flombaum, C. D., Liu, D., Yan, S. Q., Chan, A., Mathew, S., Meyers, P. A., ... & Muthukumar, T. (2018). Management of patients with acute methotrexate nephrotoxicity with high-dose leucovorin. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *38*(7), 714-724. https://doi.org/10.1002/phar.2145

Fouad, A. A., & Al-Melhim, W. N. (2018). Vanillin mitigates the adverse impact of cisplatin and methotrexate on rat kidneys. *Human and Experimental Toxicology*, *37*(9), 937-943. https://doi.org/10.1177/0960327117745694

Hadi, N. R., Hashim, T., Raheem, K. J., Kadhim, K. A., & Taher, A. (2015). Valsartan modulates the inflammatory response and apoptosis and protects from cerebral ischemia Reperfusion injury. *Journal of Advanced Pharmacy Education and Research Jan-Mar*, *5*(1). https://api.semanticscholar.org/CorpusID:52029300

Hamed, K. M., Dighriri, I. M., Baomar, A. F., Alharthy, B. T., Alenazi, F. E., Alali, G. H., ... & Alzahrani, S. (2022). Overview of methotrexate toxicity: A comprehensive literature review. *Cureus*, *14*(9). https://doi.org/10.7759/cureus.29518

Hang, C. C., Yang, J., Wang, S., Li, C. S., & Tang, Z. R. (2017). Evaluation of serum neutrophil gelatinase-associated lipocalin in predicting acute kidney injury in critically ill patients. *Journal of International Medical Research*, *45*(3), 1231-1244. https://doi.org/10.1177/0300060517709199

Itenov, T. S., Bangert, K., Christensen, P. H., Jensen, J. U., Bestle, M. H., & Procalcitonin and Survival Study (PASS)-study group. (2014). Serum and plasma Neutrophil Gelatinase Associated Lipocalin (NGAL) levels are not equivalent in patients admitted to intensive care. *Journal of Clinical Laboratory Analysis*, *28*(2), 163-167. https://doi.org/10.1002/jcla.21662

Jacobsen, P., Andersen, S., Jensen, B. R., & Parving, H. H. (2003). Additive effect of ACE inhibition and angiotensin II receptor blockade in type I diabetic patients with diabetic nephropathy. *Journal of the American Society of Nephrology*, *14*(4), 992-999. https://doi.org/10.1097/01.ASN.0000054495.96193.BF

Kirkley, M. J., Boohaker, L., Griffin, R., Soranno, D. E., Gien, J., Askenazi, D., ... & Neonatal Kidney Collaborative (NKC). (2019). Acute kidney injury in neonatal encephalopathy: An evaluation of the AWAKEN database. *Pediatric Nephrology*, *34*, 169-176. https://doi.org/10.1007/s00467-018-4068-2

Kuboki, K., Iso, K., Murakami, E., Abe, S., Araki, E., Ueshiba, H., & Yoshino, G. (2007). Effects of valsartan on inflammatory and oxidative stress markers in hypertensive, hyperglycemic patients: An open-label, prospective study. *Current Therapeutic Research*, *68*(5), 338-348. https://doi.org/10.1016/j.curtheres.2007.10.008

May, J., Carson, K. R., Butler, S., Liu, W., Bartlett, N. L., & Wagner-Johnston, N. D. (2014). High incidence of methotrexate associated renal toxicity in patients with lymphoma: A retrospective analysis. *Leukemia and Lymphoma*, *55*(6), 1345-1349. https://doi.org/10.3109/10428194.2013.840780

Mohamed, A. F., Safar, M. M., Zaki, H. F., & Sayed, H. M. (2017). Telluric acid ameliorates endotoxemic kidney injury in mice: involvement of TLR4, Nrf2 and PI3K/Akt signaling pathways. *Inflammation*, *40*, 1742-1752. https://doi.org/10.1007/s10753-017-0617-2

Oh, S. M., Park, G., Lee, S. H., Seo, C. S., Shin, H. K., & Oh, D. S. (2017). Assessing the recovery from prerenal and renal acute kidney injury after treatment with single herbal medicine via activity of the biomarkers HMGB1, NGAL and KIM-1 in kidney proximal tubular cells treated by cisplatin with different doses and exposure times. *BMC Complementary and Alternative Medicine*, *17*, 1-9. https://doi.org/10.1186/s12906-017-2055-y

Poggiali, E., Zaino, D., Immovilli, P., Rovero, L., Losi, G., Dacrema, A., ... & Terracciano, C. (2020). Lactate dehydrogenase and C-reactive protein as predictors of respiratory failure in CoVID-19 patients. *Clinica Chimica Acta*, *509*, 135-138. https://doi.org/10.1016/j.cca.2020.06.012

Sabbisetti, V. S., Waikar, S. S., Antoine, D. J., Smiles, A., Wang, C., Ravisankar, A., ... & Bonventre, J. V. (2014). Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. *Journal of the American Society of Nephrology*, *25*(10), 2177-2186. https://doi.org/10.1681/ASN.2013070758

Saka, S., & Aouacheri, O. J. J. B. A. (2017). The investigation of the oxidative stress-related parameters in high doses methotrexate-induced albino Wistar rats. *J Bioequiv Availab*, *9*(02), 372-376. https://www.researchgate.net/publication/315943187

Shahbaz, H., & Gupta, M. (2023). Creatinine Clearance. *StatPearls*. https://www.ncbi.nlm.nih.gov/books/NBK544228/

Siragy, H. M., de Gasparo, M., & Carey, R. M. (2000). Angiotensin type 2 receptor mediates valsartan-induced hypotension in conscious rats. *Hypertension*, *35*(5), 1074-1077. https://doi.org/10.1161/01.HYP.35.5.1074

Sun, F., Jiang, D., & Cai, J. (2021). Effects of valsartan combined with α-lipoic acid on renal function in patients with diabetic nephropathy: A systematic review and meta-analysis. *BMC Endocrine Disorders*, *21*(1), 178. https://doi.org/10.1186/s12902-021-00844-0

Suzuki, K., Souda, S., Ikarashi, T., Kaneko, S., Nakagawa, O., & Aizawa, Y. (2002). Renoprotective effects of low-dose valsartan in type 2 diabetic patients with diabetic nephropathy. *Diabetes Research and Clinical Practice*, *57*(3), 179-183. https://doi.org/10.1016/S0168-8227(02)00098-0

Tunalı-Akbay, T., Sehirli, O., Ercan, F., & Sener, G. (2010). Resveratrol protects against methotrexate-induced hepatic injury in rats. *Journal of Pharmacy and Pharmaceutical Sciences*, *13*(2), 303-310. https://doi.org/10.18433/J30K5Q

Turgut, F., Awad, A. S., & Abdel-Rahman, E. M. (2023). Acute kidney injury: Medical causes and pathogenesis. *Journal of Clinical Medicine*, *12*(1), 375. https://doi.org/10.3390/jcm12010375

Winther-Jensen, M., Kjaergaard, J., Lassen, J. F., Køber, L., Torp-Pedersen, C., Hansen, S. M., ... & Hassager, C. (2018). Use of renal replacement therapy after out-of-hospital cardiac arrest in Denmark 2005-2013. *Scandinavian Cardiovascular Journal*, *52*(5), 238-243. https://doi.org/10.1080/14017431.2018.1503707

Younis, N. S., Elsewedy, H. S., Shehata, T. M., & Mohamed, M. E. (2021). Geraniol averts methotrexate-induced acute kidney injury via Keap1/Nrf2/HO-1 and MAPK/NF-κB pathways. *Current Issues in Molecular Biology*, *43*(3), 1741-1755. https://doi.org/10.3390/cimb43030123

Zhang, D., & Shi, L. (2021). Serum lactate dehydrogenase level is associated with in-hospital mortality in critically Ill patients with acute kidney injury. *International Urology and Nephrology*, 1-8. https://doi.org/10.1007/s11255-021-02792-z