Vol. 2, No. (9) oct. 2026

ISSN: 4556-3489

Arab Journal of Advanced Research and Development (AJARD)

المجلة العربية للبحوث المتقدمة والتنمية

The Possible Ameliorative Effect of Dulaglutide on Methotrexate-Induced Nephrotoxicity in Rats: via Oxidative Stress, eGFR, KIM-1 and AMPK Markers

Omar W. Maher*

***Department of Pharmacology and Toxicology, Faculty of Pharmacy,**

Tobruk University, Libya

Abstract

Background and Objectives: Nephrotoxicity results from the impairment of the cellular antioxidant defense system. When the antioxidant defense system is compromised, reactive oxygen species proliferate and induce oxidative harm in renal cells. Methotrexate (MTX), an agent used in chemotherapy and immunosuppression, is often connected to nephrotoxicity, which is a prominent side effect with unclear implications. Consequently, the present research examines the potential safeguarding role of dulaglutide (DUL) in relation to MTX-induced nephrotoxicity. **Methods**: Group 1 consisted of control rats; group 2 received MTX at a dose of 14 mg/kg/week administered intraperitoneally for 2 weeks; group 3 received a combination of MTX and DUL at a dose of 0.1 mg/kg/week administered subcutaneously and group 4 received just DUL (0.1 mg/kg/week, s.c.) throughout treatment. On day 15, animals were slaughtered for biochemical evaluations of oxidative stress indicators (MDA and GSH), renal function tests (eGFR and KIM-1) and protein kinase (AMPK). **Results**: Our findings indicated that MTX significantly elevated some oxidative stress indicators and renal function assessments. **Conclusions**: DUL provides considerable defense against MTX-induced kidney damage, presumably via its antioxidant characteristics, renal function assessment, and protein kinase pathway. This underscores its medicinal promise in averting drug-induced kidney damage.

Keywords: Methotrexate; Dulaglutide, Nephrotoxicity, Renal function test, Oxidative stress, protein kinase.

1. Introduction

Kidney is located retroperitoneally on the rear wall of the abdomen, nestled between the transverse processes of T12 and L3, the kidneys are tiny organs weighing around 165 ± 40 [g] in males and 122 ± 29 [g] in females. Despite receiving more than 20% of the cardiac output, the human kidneys only make up around 0.2-0.5% of the total body weight. In spite of their little size, the kidneys play a crucial role in maintaining life by filtering out xenobiotics—substances introduced to the organ in high concentrations that might cause harm—and their extensive renal blood circulation. As a result, it's not unreasonable to assume that the urinary system and kidneys are the most common places where foreign substances might cause harm (Kalucki et al., 2020). Furthermore, the kidneys and the urinary tract play a crucial role in the excretion of xenobiotics, such as

pharmaceuticals. Kidney function, including glomerular filtration and tubular secretion, aid in the excretion of parent chemicals and their metabolites via urine as part of the detoxification process for these medications. Moreover, the kidneys' excretory function arises from their capacity to metabolize pharmaceuticals and other foreign substances, however this capability is restricted in comparison to the biotransformation occurring in the liver.

Nephrotoxicity is a prevalent clinical issue. It is estimated that it will influence between 14–26% of adults and 16% of children (Siddama and Suneel, 2017). Nonetheless, the prevalence of nephrotoxicity associated with drugs may be as high as 66%. In the elderly population, the prevalence is elevated due to the presence of numerous comorbidities and polypharmacy, leading to various diagnostic and therapeutic interventions that may harm the kidneys (e.g., the administration of contrast agents in imaging studies). Renal impairment associated with drugs is often reversible and decreases with the cessation of the causative medication; yet, it may also lead to permanent structural harm to renal tissue. Nephrotoxicity is evident throughout an extensive range, showing harm to several nephron segments, including glomerular and tubular areas. In conclusion, nephrotoxic effects of pharmaceuticals are a contributing element to acute renal impairment and chronic kidney disease (CKD) (Naughton, 2008).

There are many pathways that drugs might take that lead to kidney damage: The inflammatory and immune-mediated processes that cause glomerulonephritis, interstitial nephritis, acute kidney injury, tubular cell toxicity, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy. The kidney is a significant site of MTX toxicity; yet, hepatotoxicity, neurotoxicity, as well as testicular and intestinal toxicities have also been linked to MTX (Hassanein et al., 2021).

Methotrexate, a competitive antagonist of dihydrofolate reductase, is used in the management of certain autoimmune diseases and malignancies (osteosarcoma, acute lymphoblastic leukemia, Hodgkin’s lymphoma). It infiltrates cells by several transporters depending on the cell type, particularly OATP1B1 (Mikkelsen et al., 2011). The primary route of excretion is via the kidneys; renal impairment induced by MTX results in a protracted elimination of MTX, leading to sustained increases in plasma MTX concentrations (Howard et al., 2016). Methotrexate toxicity seems to manifest more often in individuals with pre-existing comorbid conditions or discernible risk factors. Elderly individuals, diminished renal excretion, chronic kidney disease (CKD), and medication miscalculations or inconsistent compliance have all been linked to a heightened risk of toxicity (Asaduzzaman et al., 2024). The clinical presentations of MTX toxicity are diverse and may include mucocutaneous lesions, gastrointestinal damage, bone marrow suppression leading to myelosuppression, and involvement of hepatic, renal, pulmonary, and neurological systems, some of which can be severe or potentially fatal (Delyon et al., 2015). Nephrotoxicity associated with methotrexate has been linked to oxidative and

ISSN: 4556-3489
Arab Journal of Advanced Research and Development (AJARD)
المجلة العربية للبحوث المتقدمة والتنمية

inflammatory stress, mitochondrial impairment, and programd cell death (Heidari et al., 2018). Documented side effects of MTX include pneumonitis, hepatic impairment, gastrointestinal complications, and nephrotoxicity; the primary mechanisms behind MTX-induced nephrotoxicity are inflammation and oxidative stress (Dalia et al., 2025).

The research consistently demonstrates that oxidative stress, pro-inflammatory responses, apoptosis, and several complex signaling pathways contribute to the pathophysiology of MTX-induced nephrotoxicity (Ahmed et al., 2021). Research has shown that MTX induces the production of free radicals in the kidneys, resulting in renal oxidation, lipid peroxidation, a reduction in renal antioxidant capacity, and nephrotoxicity (Sayed et al., 2021).

The glucagon gene is located in the L cells of the small intestine following food ingestion, and dulaglutide is an analog of GLP-1. According to Cechin et al. (2012), it improves the generation of insulin that is reliant on glucose by attaching to certain GLP-1 receptors in the pancreas. Treataskis et al. (2012) found that glucagon-like peptide-1 receptor agonists (GLP-1RAs) are useful in the management of type 2 diabetes mellitus. Semaglutide, dulaglutide, exenatide, lixisenatide, liraglutide, albiglutide, and both short-acting and long-acting GLP-1 receptor agonists are all part of this class of drugs (Nauck et al., 2016). Several cell types and organs, including pancreatic beta cells, endothelial cells, the lungs, kidneys, and heart, exhibit neuroprotective, anti-inflammatory, and vasoprotective capabilities in GLP-1, in addition to its glycemic effects. Pancreatic beta cells, hypothalamus, endothelial cells, neurons, microglia, and astrocytes are among the many cells and tissues that benefit from GLP-1R activation's anti-inflammatory, antioxidative, neurogenerative, and vascular protective characteristics (Arakawa et al., 2010).

When antioxidant defenses are inadequate or ROS generation is excessive, it leads to a cascade of molecular, cellular, and tissue abnormalities collectively known as "oxidative stress" (Cheresh et al., 2013). Damage to tissues and malfunctioning cells are the results of oxidative stress, which occurs when the body's antioxidant defenses are outnumbered by the generation of oxidants. Malondialdehyde (MDA), an aldehyde that is formed during lipid peroxidation and shows the redox status, is a prominent human biomarker for this condition (França et al. 2013). Furthermore, the enzyme glutathione reductase (GSH) is recognized for its essential function in preventing lipid peroxidation, hence safeguarding cells from harm induced by oxidative stress (Flohé 1999).

The glomerular filtration rate (GFR) was first established to assess glomerular function by quantifying the volume of fluid filtered through the renal glomeruli during a certain time interval. It does not assess the function of individual nephrons; instead, it quantifies the cumulative filtration of all glomerular capillaries inside the human kidney (Zsom et al., 2022).

0096893649662 | info@ajard.org | www.ajard.org

One kind of glycoprotein that crosses cell membranes is KIM-1, which stands for kidney injury molecule 1 and is sometimes called TIM-1 (Han et al., 2002). Parikh et al. (2013) found that KIM-1 is a marker for proximal tubular injury, tubular regeneration, and renal tubular apoptotic body clearance. Human and rat urine may include fragments of the extracellular domain of KIM-1 that are broken down by matrix metalloproteinases after injury to the proximal tubules (Vaidya et al., 2010). Recent studies have linked increased circulating concentrations of KIM-1 in the bloodstream to both acute and chronic renal impairment (Sabbisetti et al., 2014).

High levels of intracellular AMP activate a protein kinase called adenosine 5'-monophosphate (AMPK), which is an essential energy sensor (Kahn et al., 2005). Different tissues have different AMPK subunit expression and activity profiles. According to Stapleton et al. (1996), the AMPK α1 subunit was found in a fairly even distribution across various organs in rats, including the heart, liver, kidneys, brain, spleen, lung, and skeletal muscle. In contrast, the AMPK α2 subunit was mainly found in skeletal muscle, with lower amounts in the heart, liver, brain, and kidneys. It was also detectable in the lung. Alterations in the cellular metabolic condition resulting from heightened ATP utilization, as observed during physical activity or elevated cellular metabolism, or from the suppression of ATP production, including hypoxia, glucose scarcity, thermal stress, or diminished mitochondrial oxidative phosphorylation, initiate AMPK activation. Beyond its role as an intracellular energy sensor and regulator, AMPK functions as a universal stress sensor essential for maintaining intracellular homeostasis under oxidative stress and other detrimental conditions (Kukidome et al., 2006).

The objective of the present research is to examine the potential mitigating effects and underlying mechanisms of dulaglutide in reducing the adverse effects of methotrexate in rats, based on the biological properties of dulaglutide as antioxidants.

2. Materials and Methods

2.1. Animals

Research supplied the 170–200 gram adult male Wistar rats. Before the studies began, the animals spent two weeks adjusting to the facility's surroundings. In a typical laboratory setting, the animals had unrestricted access to food and water, a 12-hour light/dark cycle, and a relative humidity of 60%. We followed all applicable ethical rules when I conducted any technique that required the use of animals in the lab. This research was given the go light by the Research Ethics Committee of Faculty of Pharmacy -Tobruk University.

2.2. Drugs

The Egyptian company Hikma Specialized Pharmaceutics supplied the methotrexate (MTX). The Indianapolis, USA-based Eli Lilly and Company generously supplied the dulaglutide (DUL).

2.3. Experimental design

Study used forty adult male wistar rats, with 10 rats in each of the four groups. As a control, rats in Group 1 received oral saline. Group 2 rats were administered 14 mg/kg of methotrexate intravenously once weekly for a duration of two weeks by the researchers who opted for this method. Previous studies have shown that methotrexate reliably produces kidney damage in such a little time frame, which is why this dosage was determined. Three group of rats were given MTX intraperitoneally at a dose of 14 mg/kg/week and DUL subcutaneously at a dose of 0.1 mg/kg/week for two weeks, whereas group four rats got DUL subcutaneously at a dose of 0.1 mg/kg/week on its own.

2.4. Sample preparation

The rats were given an intraperitoneal injection of 50 mg/kg of thiopental sodium anesthetic to make them sleepy after the experiment was over. After the sacrifice, the kidneys were carefully taken out and rinsed with salt water. In preparation for an upcoming Enzyme-Linked Immunosorbent Assay (ELISA), the kidney lobes were quickly frozen at -80 °C.

2.5. Biochemical Assays

In accordance with the guidelines given by the manufacturers of the enzyme-linked immunosorbent assay (ELISA) kits, the kidney tissue homogenates were examined for oxidative stress, renal function tests, and protein kinase. The following substances were used: malondialdehyde (MBS268427), reduced glutathione (GSH; Shang Hai Blue Gene Biotech, Cat. No. E02G0367), renal function tests, epidermal growth factor receptor (eGFR; MyBioSource, Cat. No. MBS269875), kidney injury molecule-1 (KIM-1; Elabscience, Cat. No. E-EL-M3039), and AMP-activated protein kinase (AMPK; My Life Span Bio Sciences, Cat. No. LS-F36060).

2.6. Statistical analysis

The data is shown using the mean plus or minus the standard deviation (SD). For our statistical analyzes, I relied on GraphPad Prism 8, which was created by GraphPad Software Inc. of La Jolla, California, USA. To compare the groups, we first used a one-way ANOVA, and then we used the Tukey Kramer multiple comparison post hoc test to see how they differed. A statistically significant result was defined as one with a p-value below 0.05.

Arab Journal of Advanced Research and Development (AJARD)
المجلة العربية للبحوث المتقدمة والتنمية
ISSN: 4556-3489
Vol. 2, No. (9) oct. 2026

3. Result

3.1. Effect on Oxidative Stress Marker

3.1.1. Effect of Dulaglutide on MDA in Methotrexate-Induced Nephrotoxicity

There was a significant rise in malondialdehyde (MDA) concentrations in renal tissue following 14 days of weekly intraperitoneal methotrexate (MTX) treatment compared to the control group, as shown in Figure 1 ($P < 0.05$). A significant reduction that successfully mitigated these oxidative changes was seen after fourteen days of weekly therapy with DUL at a dosage of 0.1 mg/kg. In the group that did not get MTX, MDA levels decreased by 61%. At a dosage of 0.1 mg/kg in particular, dulaglutide protects against oxidative stress in MTX-induced nephrotoxicity..

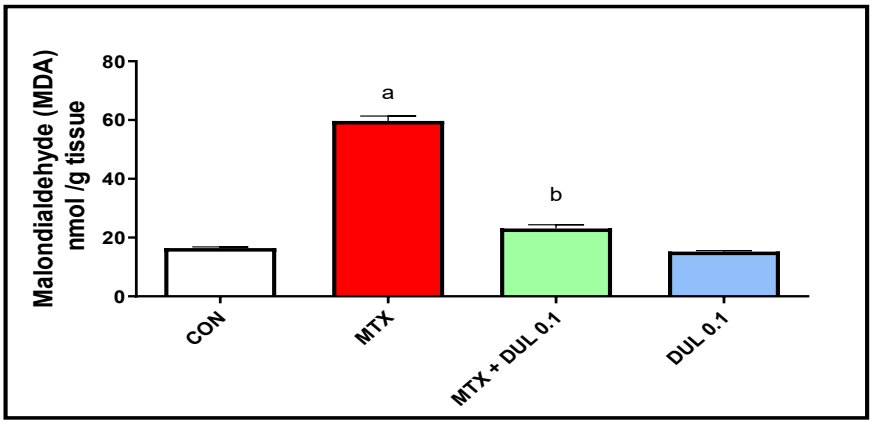

Figure 1. Effect of Dulaglutide on MDA in MTX-Induced nephrotoxicity in rats. a vs. control group, b vs. MTX group. Values are expressed as mean ± SD of 10 rats per group. (one-way ANOVA followed by Tukey multiple comparisons test; $p < 0.05$). CON ; Control, MTX; Methotrexate, DUL; Dulaglutide, MDA; Malondialdehyde.

3.1.2. Effect of Dulaglutide on GSH in Methotrexate-Induced Nephrotoxicity

Results from Figure 2 demonstrate that the group administered intraperitoneal methotrexate (MTX) at a dosage of 14mg/kg weekly for 14 days had substantially lower levels of reduced glutathione (GSH) in renal tissue compared to the control group ($P < 0.05$). Using a weekly dosage of 0.1 mg/kg of DUL for 14 days greatly improved the mitigation of these oxidative changes. There was a 141% increase in GSH levels as compared to the MTX group.

0096893649662 | info@ajard.org | www.ajard.org

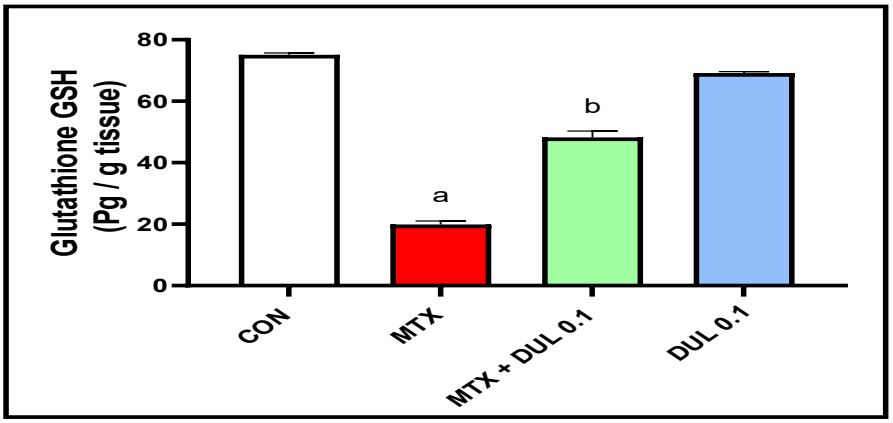

Figure 2. Effect of Dulaglutide on GSH in MTX-Induced nephrotoxicity in rats. a vs. control group, b vs. MTX group. Values are expressed as mean ± SD of 10 rats per group. (one-way ANOVA followed by Tukey multiple comparisons test; p <0.05). CON ; Control, MTX; Methotrexate, DUL; Dulaglutide, GSH; Glutathione.

3.2. Effect on Kidney Functional Marker

3.2.1 Effect of Dulaglutide on eGFR in Methotrexate-Induced Nephrotoxicity

Substantial reductions in eGFR values in renal tissue were seen following 14 days of weekly intraperitoneal methotrexate (MTX) treatment, as compared to the control group, according to the results in Table 1 ($P < 0.05$). Following fourteen days of weekly dosing with DUL at a dosage of 0.1 mg/kg, there was a 38.11% increase in eGFR values when contrasted with the MTX group. At doses of 0.1 mg/kg, dulaglutide preserves eGFR in cases of MTX-induced nephrotoxicity.

Table 1. Effect of Dulaglutide on eGFR in Methotrexate-Induced Nephrotoxicity Rats.

Groups	eGFR
Control	83.08 ± 0.37
Methotrexate (14 mg/kg)	26.50 ± 1.69 a
Methotrexate + Dulaglutide (0.1 mg/kg)	36.60 ± 1.41 b
Dulaglutide (0.1 mg/kg)	75.10 ± 0.28

Evaluating Dulaglutide's Effect on The Glomerular Filtration Rate in Rats with Renal Damage Induced by Methotrexate. an in comparison to the placebo group, and b in comparison to the group given MTX. The data is shown as the average plus or minus the

ISSN: 4556-3489
Arab Journal of Advanced Research and Development (AJARD)
المجلة العربية للبحوث المتقدمة والتنمية

standard deviation for ten rats in every category. When $p < 0.05$, Tukey's multiple comparison test succeeded after one-way ANOVA. eGFR, methotrexate (MTX), dulaglutide (DUL), and control (CON) are all acronyms for "estimated glomerular filtration rate."

3.2.2 Effect of Dulaglutide on KIM-1 in Methotrexate-Induced Nephrotoxicity

There was a significant rise in KIM-1 levels in renal tissue following 14 days of weekly intraperitoneal methotrexate (MTX) injection, as shown in Table 2 ($P < 0.05$), when contrasted with the control group. After 14 days of weekly dosing at 0.1 mg/kg, DUL caused a 58.85% decrease in KIM-1 levels, as compared to the MTX group. At doses as 0.1 mg/kg, dulaglutide effectively decrease KIM-1 in methotrexate-induced nephrotoxicity.

Table 2. Effect of Dulaglutide on KIM-1 in Methotrexate-Induced Nephrotoxicity rats.

Groups	KIM-1
Control	12.28 ± 0.44
Methotrexate (14 mg/kg)	48.20 ± 0.42 a
Methotrexate + Dulaglutide (0.1 mg/kg)	19.83 ± 0.46 b
Dulaglutide (0.1 mg/kg)	13.60 ± 0.36

Rats suffering from MTX-induced nephrotoxicity were compared to two groups: a compared to control group and b compared to receiving MTX. The results demonstrate that dulaglutide had an impact on KIM-1. The data is shown as the mean ± SD ($p < 0.05$) after a one-way ANOVA and Tukey's multiple comparisons test, with 10 rats per group serving as the basis. A acronym for "dulaglutide," "control," "methotrexate," and "kidney injury molecule" (KIM-1).

3.3 Effect of Dulaglutide on AMPK in Methotrexate-Induced Nephrotoxicity

Table 3 shows that when methotrexate (MTX) was given intraperitoneally once weekly for 14 days, there was a significant reduction in AMPK levels in renal tissue as compared to the control group ($P < 0.05$). After 14 days of therapy at a dosage of 0.1 mg/kg weekly, the AMPK levels in the DUL group were 192% higher than in the MTX group.

0096893649662 | info@ajard.org | www.ajard.org

ISSN: 4556-3489
Arab Journal of Advanced Research and Development (AJARD)
المجلة العربية للبحوث المتقدمة والتنمية

Table 3. Effect of Dulaglutide on AMPK in Methotrexate-Induced Nephrotoxicity Rats.

Groups	AMPK
Control	49.18 ± 0.41
Methotrexate (14 mg/kg)	12.80 ± 1.04 a
Methotrexate + Dulaglutide (0.1 mg/kg)	37.50 ± 1.87 b
Dulaglutide (0.1 mg/kg)	46.10 ± 0.58

Analysis of Dulaglutide's Effect on AMPK in Rats With Methotrexate-Induced Renal Injury. (a) compared to those who received a control group, and (b) compared to those who received MTX. After a one-way ANOVA and a Tukey multiple comparisons test ($p < 0.05$), the results are shown as the average plus or minus the standard deviation for 10 rats in each group. DUL denotes dulaglutide, AMPK denotes adenosine monophosphate kinase, CON means control, and MTX means methotrexate

Discussion

Methotrexate-induced nephrotoxicity is a significant and mostly unavoidable adverse effect that may arise from an allergic response or direct toxicity to the renal tubules (Ramamoorthy and Hephziba, 2013). Consequently, the therapeutic oversight of methotrexate, promoting its elimination and pharmaceutical mitigation of its nephrotoxic effects, is very significant (Green and Chamberlain, 2009). The optimal therapeutic effectiveness of chemotherapy remains unattainable due to its harmful toxicities, with substantial expert studies indicating that these toxicities are mostly linked to secondary carnitine deficiency (Sayed-Ahmed. 2004). Furthermore, a shortage in carnitine has been shown as a contributing element to several toxicities generated by chemotherapeutic agents; hence, its supplementation mitigates and cures the bad effects of the treatment without diminishing the anti-tumor efficacy (Sayed-Ahmed et al., 2004).

Researchers found that compared to rats given a placebo, animals given MTX (14 mg/kg/week) had significantly lower GSH levels and much higher MDA levels in their renal tissue. Methotrexate may induce significant nephrotoxicity via two primary mechanisms. Initially, the deposition of MTX and its metabolites inside the renal tubules might result in direct tubular toxicity. The likelihood increases in an acidic urine environment, given that MTX and some metabolites exhibit limited solubility at reduced pH levels. Secondly, vasoconstriction of the afferent arterioles generated by MTX may lead to a temporary decline in the glomerular filtration rate (Howard et al., 2016). Oxidative stress results in renal impairment, which may present as diminished renal function and subsequent elevations in uric acid, creatinine, and urea concentrations

0096893649662 | info@ajard.org | www.ajard.org

(Famurewa et al., 2017).The significance of oxidative stress in relation to MTX-induced renal damage has been shown in several studies, and our findings align with prior research that indicated oxidative stress occurs in renal tissue after MTX treatment. Furthermore, prior research indicated that MTX therapy increased renal reactive oxygen species levels while diminishing tissue antioxidant capacity. Antioxidant medications have been shown to significantly reduce kidney damage associated with MTX (Abraham et al., 2010; Selimoglu-Şen et al., 2014). According to a previous study (Famurewa et al., 2017), increased levels of malondialdehyde (MDA) are a biochemical indicator of oxidative stress and free radical generation caused by MTX.

On the other hand, those administered DUL at a dosage of 0.1 mg/kg/week exhibited significantly reduced MDA levels and elevated GSH levels in comparison to the MTX group. An expanding body of evidence supports the theory that GLP-1 receptor agonists provide renal protection not just by facilitating weight reduction and enhancing glycemic regulation but also by direct engagement with kidney cells. The presence of GLP-1R in the renal tubule and glomerulus has been shown by several human experiments. The sodium-hydrogen exchanger 3, which is located on the brush edge of proximal tubular cells, is directly inhibited and phosphorylated by GLP-1 receptor agonists, which promote diuresis and natriuresis, hence reducing glomerular hyperfiltration (Crajoinas et al., 2011). Shi et al. (2018) found that dulaglutide, via GLP-1-dependent signaling pathways, decreases ROS accumulation and oxidative stress-induced endothelial cell failure. Krasner et al. (2014) identified a potential mechanism by which GLP-1 might help reduce oxidative stress.

In this study, the administration of MTX (14 mg/kg/week) markedly decreased the levels of eGFR in renal tissue relative to the control group. In prior research, a modest dosage of MTX may result in a gradual decline in eGFR (Kremer et al., 1995). Conversely, Dulaglutide administered at 0.1 mg/kg/week significantly elevated eGFR expression levels in comparison to the MTX cohort. In preliminary research, dulaglutide, a GLP1, is said to enhance eGFR and promote weight reduction (Ishigo et al., 2018).

In the present investigation, the administration of MTX (14 mg/kg/week) markedly decreased the levels of AMPK in pulmonary tissue relative to the control group. MTX has a deleterious impact on renal tubular cells owing to its propensity to crystallize and form precipitates (Pazhayattil and Shirali, 2014). Kim-1 serves as a precise and sensitive indicator of nephrotoxicity (Bonventre, 2008). The nephrotoxic impact of MTX was validated by the heightened expression of Kim-1 in the renal tissue of rats (Mahmoud et al., 2019). Conversely, Dulaglutide administered at 0.1 mg/kg/week significantly reduced KIM-1 expression levels relative to the MTX group. Crucially, the findings of the current investigation indicate that DUL alleviates renal tubular damage, as seen by the markedly reduced renal injury biomarker KIM-1 in renal tissue. These findings are corroborated by a prior investigation (Mohamad et al., 2022).

ISSN: 4556-3489
Arab Journal of Advanced Research and Development (AJARD)
المجلة العربية للبحوث المتقدمة والتنمية

Treatment with MTX (14 mg/kg/week) considerably decreased AMPK levels in lung tissue as compared to the control group. In line with the observed reduction in AMPK activity produced by MTX, Atia et al. (2025) discovered that hepatic tissue DNA synthesis is hindered by methotrexate metabolites, which further lower AMPK activity. However, AMPK expression levels were significantly higher in the group that received Dulaglutide at a dose of 0.1 mg/kg/week as compared to the MTX group. By activating AMPK, GLP-1 inhibits the development of vascular smooth muscle cells (Jojima et al., 2017). Pharmacological activation of AMPK has been associated with improved organ structure and function by reducing oxidative stress, inflammation, and apoptosis (Li et al., 2022). In addition, Jäger et al. (2007) found that activating AMPK increased the expression of antioxidant enzymes, which in turn decreased ROS formation.

Limitations of the Study

This self-financed research was constrained by monetary and temporal limitations, hindering further inquiries and extended monitoring. Although the results in rats are encouraging, they may not immediately apply to humans, highlighting the need for forthcoming clinical studies to assess long-term kidney outcomes and therapeutic significance.

References

Abraham, P., Kolli, V.K., Rabi, S. (2010). Melatonin attenuates methotrexate-induced oxidative stress and renal damage in rats. Cell Biochem Funct, 28(5):426-33. doi: 10.1002/cbf.1676.

Ahmed, Z.S.O., Hussein, S., Ghandour, R.A., Azouz, A.A., El-Sakhawy, M.A. (2021). Evaluation of the effect of methotrexate on the hippocampus, cerebellum, liver, and kidneys of adult male albino rat: Histopathological, immunohistochemical and biochemical studies. Acta Histochem, 123(2): 151682. doi: 10.1016/j.acthis.2021.151682.

Arakawa, M., Mita, T., Azuma, K., Ebato, C., Goto, H., Nomiyama, T., Fujitani, Y., Hirose, T., Kawamori, R., Watada, H. (2010). Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4. Diabetes, 59 (4): 1030–1037. doi: 10.2337/db09-1694.

Asaduzzaman, M., Karim, M.R., Saha, P., Islam, M.M., Zaman, S.S., Roy, S., Roy, R.K., Alam, M.M.J. (2024). Accidental methotrexate overdose leading to multisystem toxicity: A case report. Toxicol Rep, 13:101821. doi: 10.1016/j.toxrep.2024.101821.

0096893649662 | info@ajard.org | www.ajard.org

Atia, H.A., Elariny, H.A., Abdallah, M.H., Khalifa, A.M., Estfanous, R.S., Abd Elmaaboud, M.A., Kabel, A.M. (2025). Repositioning Perindopril for Mitigation of Methotrexate-Induced Hepatotoxicity in Rats. J Pharm, 18 (3): 358. doi: 10.3390/ph18030358.

Bonventre, J.V. (2008). Kidney Injury Molecule-1 (KIM-1): a specific and sensitive biomarker of kidney injury. Scand J Clin Lab Invest Suppl, 241:78-83. doi: 10.1080/00365510802145059.

Brigelius-Flohé, R. (1999). Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med, 27(9-10):951-65. doi: 10.1016/s0891-5849(99)00173-2.

Cechin, S.R., Pérez-Álvarez, I., Fenjves, E., Molano, R.D., Pileggi, A., Berggren, P.O., Ricordi, C., Pastori, R.L. (2012). Anti-inflammatory properties of exenatide in human pancreatic islets. Cell Transplant, 21(4):633-48. doi: 10.3727/096368911X576027.

Cheresh, P., Kim, S.J., Tulasiram, S., Kamp, D.W. (2013). Oxidative stress and pulmonary fibrosis. Biochim. Biophys. Acta Mol. Basis Dis, 1832 (7): 1028–1040. doi: 10.1016/j.bbadis.2012.11.021.

Crajoinas, R.O., Oricchio, F.T., Pessoa, T.D., Pacheco, B.P., Lessa, L.M., Malnic, G., Girardi, A.C. (2011). Mechanisms mediating the diuretic and natriuretic actions of the incretin hormone glucagon-like peptide-1. Am J Physiol Renal Physiol, 301(2):F355-63. doi: 10.1152/ajprenal.00729.2010.

Delyon, J., Ortonne, N., Benayoun, E., Moroch, J., Wolkenstein, P., Sbidian, E., Chosidow, O. (2015). Low-dose methotrexate-induced skin toxicity: Keratinocyte dystrophy as a histologic marker. J Am Acad Dermatol, 73(3):484-90. doi: 10.1016/j.jaad.2015.06.015.

Famurewa, A.C., Aja, P.M., Maduagwuna, E.K., Ekeleme-Egedigwe, C.A., Ufebe, O.G., Azubuike-Osu, S.O. (2017). Antioxidant and anti-inflammatory effects of virgin coconut oil supplementation abrogate acute chemotherapy oxidative nephrotoxicity induced by anticancer drug methotrexate in rats. Biomed Pharmacother, 96:905-911. doi: 10.1016/j.biopha.2017.12.008.

França, B.K., Alves, M.R.M., Souto, F.M.S., Tiziane, L., Boaventura, R.F., Guimarães A., Jr A. A. (2013). Lipid peroxidation and obesity: Methods to measure the oxidative stress of the obese paciente's plasma. GE J Port Gastrenterol, 20(5):199–206. doi 10.1016/j.jpg.2013.04.002.

Gamal, D.M., Maged, G., Abdo, M., Elsharabasy, R.M., Samaan, S.F. (2025). Risk of dose-dependent methotrexate therapy on renal functions in patients with rheumatoid arthritis. The Egyptian Rheumatologist, 47(4): 182-186. 10.1016/j.ejr.2025.06.004

Green, M.R., Chamberlain, M.C. (2009). Renal dysfunction during and after high-dose methotrexate. Cancer Chemother Pharmacol, 63(4): 599-604. doi: 10.1007/s00280-008-0772-0.

Han, W.K., Bailly, V., Abichandani, R., Thadhani, R., Bonventre, J.V. (2002). Kidney Injury Molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. Kidney Int, 62(1):237-44. doi: 10.1046/j.1523-1755.2002.00433.x.

Hassanein, E.H.M., Kamel, E.O., Ali, F.E.M., Ahmed, M.A. (2021). Berberine and/or zinc protect against methotrexate-induced intestinal damage: Role of GSK-3β/NRF2 and JAK1/STAT-3 signaling pathways. Life Sci. 281:119754. doi: 10.1016/j.lfs.2021.119754.

Heidari, R., Ahmadi, A., Mohammadi, H., Ommati, M.M., Azarpira, N., Niknahad, H. (2018). Mitochondrial dysfunction and oxidative stress are involved in the mechanism of methotrexate-induced renal injury and electrolytes imbalance. Biomed Pharmacother, 107:834-840. doi: 10.1016/j.biopha.2018.08.050.

Howard, S.C., McCormick, J., Pui, C.H., Buddington, R.K., Harvey, R.D. (2016). Preventing and Managing Toxicities of High-Dose Methotrexate. Oncologist, 21(12):1471-1482. doi: 10.1634/theoncologist.2015-0164.

Ishigo, T., Kondo, F., Tateishi, R., Nonoyama, M., Fujii, S., Kimyo, T., Nakata, H., Noda, N., Miyamoto, A. (2018). Effects of dulaglutide on glucose control and renal function. Jpn J Nephrol Pharmacother, 7:201-209.

Jäger, S., Handschin, C., St-Pierre, J., Spiegelman, B.M. (2007). AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha Proc. Natl Acad Sci, 104 (29): 12017-12022. doi: 10.1073/pnas.0705070104.

Jojima, T, Uchida, K., Akimoto, K., Tomotsune, T., Yanagi, K., Iijima, T., Suzuki, K., Kasai, K., Aso, Y. (2017). Liraglutide, a GLP-1 receptor agonist, inhibits vascular smooth muscle cell proliferation by enhancing AMP-activated protein kinase and cell cycle regulation, and delays atherosclerosis in ApoE deficient mice. Atheroscler, 261:44–51. doi: 10.1016/j.atherosclerosis.2017.04.001

Kahn, B.B., Alquier, T., Carling, D., Hardie, D.G. (2005). AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab, 1 (1): 15-25. doi: 10.1016/j.cmet.2004.12.003.

Kalucki, S.A., Lardi, C., Garessus, J., Kfoury, A. Grabherr, S., Burnier, M., Pruijm, M. (2020). Reference values and sex differences in absolute and relative kidney size. A Swiss autopsy study. BMC Nephrol. 21(1):289. doi: 10.1186/s12882-020-01946-y.

Krasner, N.M., Ido, Y., Ruderman, N.B., Cacicedo, J.M. (2014). Glucagon-like Peptide-1 (GLP-1) analog liraglutide inhibits endothelial cell inflammation through a calcium and AMPK dependent mechanism. PLoS ONE, 9 (5): e97554.

Kremer, J.M., Petrillo, G.F., Hamilton, R.A. (1995). Pharmacokinetics and renal function in patients with rheumatoid arthritis receiving a standard dose of oral weekly methotrexate: association with significant decreases in creatinine clearance and renal clearance of the drug after 6 months of therapy. J Rheumatol, 22(1):38-40. PMID: 7699678.

Kukidome, D., Nishikawa, T., Sonoda, K., Imoto, K., Fujisawa, K., Yano, M., Motoshima, H., Taguchi, T., Matsumura, T., Araki, E. (2006). Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. Diabetes, 55 (1): 120-127. PMID: 16380484

Li, J., Li, J., Fang, H., Yang, H., Wu, T., Shi, X., Pang, C. (2022). Isolongifolene alleviates liver ischemia/reperfusion injury by regulating AMPKPGC1α signaling pathway-mediated inflammation, apoptosis, and oxidative stress. Int Immunopharmacol, 113:109185. doi:10.1016/j.intimp.2022.109185

Mahmoud, A.M., Hussein, O.E., Abd El-Twab, S.M., Hozayen, W.G.(2019). Ferulic acid protects against methotrexate nephrotoxicity via activation of Nrf2/ARE/HO-1 signaling and PPARγ, and suppression of NF-κB/NLRP3 inflammasome axis. Food Funct, 10(8):4593-4607. doi: 10.1039/c9fo00114j.

Mohamad, H.E., Abdelhady, M.A., Abdel Aal, S.M., Elrashidy, R.A. (2022). Dulaglutide mitigates high dietary fructose-induced renal fibrosis in rats through suppressing epithelial-mesenchymal transition mediated by GSK-3β/TGF-β1/Smad3 signaling pathways. Life Sci, 309:120999. doi: 10.1016/j.lfs.2022.120999.

Mikkelsen, T.S., Thorn, C.F., Yang, J.J., Ulrich, C.M., French, D., Zaza, G., Dunnenberger, H.M., Marsh, S., McLeod, H.L., Giacomini, K., Becker, M.L., Gaedigk, R., Leeder, J.S., Kager, L., Relling, M.V., Evans, W., Klein, T.E., Altman, R.B. (2011). PharmGKB summary: methotrexate pathway. Pharmacogenet Genomics. 21(10): 679-86. doi: 10.1097/FPC.0b013e328343dd93.

Nauck, M. (2016). Incretin therapies: highlighting common features and differences in the modes of action of glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. Diabetes Obes Metab,18(3):203-16. doi: 10.1111/dom.12591.

Naughton, C.A. (2008). Drug-induced nephrotoxicity. Am Fam Physician, 78(6):743-50. PMID: 18819242.

Parikh, C.R., Thiessen-Philbrook, H., Garg, A.X., Kadiyala, D., Shlipak, M.G., Koyner, J.L., Edelstein, C.L., Devarajan, P., Patel, U.D., Zappitelli, M., Krawczeski, C.D., Passik, C.S., Coca, S.G.(2013). Performance of kidney injury molecule-1 and liver fatty acid-binding protein and combined biomarkers of AKI after cardiac surgery. Clin J Am Soc Nephrol, 8(7):1079-88. doi: 10.2215/CJN.10971012.

Pazhayattil, G.S., Shirali, A.C. (2014). Drug-induced impairment of renal function. Int J Nephrol Renovasc Dis, 7:457-68. doi: 10.2147/IJNRD.S39747.

Ramamoorthy, S.K., Hephziba, R. (2013). Acute renal failure post high dose methotrexate infusion successfully managed with high dose folinic Acid and high flux dialysis. Indian J Hematol Blood Transfus, 29(2): 90-2. doi: 10.1007/s12288-011-0143-6.

Sabbisetti, V.S., Waikar, S.S., Antoine, D.J., Smiles, A., Wang, C., Ravisankar, A., Ito, K., Sharma, S., Ramadesikan, S., Lee, M., Briskin, R., De Jager, P.L., Ngo, T.T., Radlinski, M., Dear, J.W., Park, K.B., Betensky, R., Krolewski, A.S., 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. J Am Soc Nephrol, 25(10):2177-86. doi: 10.1681/ASN.2013070758.

Sayed, A.M., Hassanein, E.H.M., Ali, F.E.M., Omar, Z.M.M., Rashwan, E.K., Mohammedsaleh, Z.M., Abd-El-Ghafar, O.A.M. (2021). Regulation of Keap-1/Nrf2/AKT and iNOS/NF-κB/TLR4 signals by apocynin abrogated methotrexate-induced testicular toxicity: Mechanistic insights and computational pharmacological analysis. Life Sci., 284:119911. doi: 10.1016/j.lfs.2021.119911.

Sayed-Ahmed, M.M. (2010). Role of carnitine in cancer chemotherapy-induced multiple organ toxicity. Saudi Pharm J, 18(4):195-206. doi: 10.1016/j.jsps.2010.07.008.

Sayed-Ahmed, M.M., Eissa, M.A., Kenawy, S.A., Mostafa, N., Calvani, M., Osman, A.M. (2004). Progression of cisplatin-induced nephrotoxicity in a carnitine-depleted rat model. Chemotherapy, 50(4):162-70. doi: 10.1159/000080689.

Selimoglu-Şen, H., Sen, V., Bozkurt, M., Turkcu, G., Guzel, A., Sezgi, C., Abakay, Ö., Kaplan, I. (2014). Carvacrol and pomegranate extract in treating Methotrexate induced lung oxidative injury in rats. Med. Sci. Monit, 20, 1983-1990. doi: 10.12659/MSM.890972.

Shi, J.X., Huang, Q. (2018). Glucagon-like peptide-1 protects mouse podocytes against high glucose-induced apoptosis, and suppresses reactive oxygen species production and proinflammatory cytokine secretion, through sirtuin 1 activation in vitro. Mol Med Rep, 18(2):1789-1797. doi: 10.3892/mmr.2018.9085.

Siddama, A., Suneel, I.M. (2017). Drug induced kidney disease. Open Acc. J. Toxicol, 2(1): 555576. DOI: 10.19080/OAJT.2017.02.555576

Stapleton, D., Mitchelhill, K.I., Gao, G., Widmer, J., Michell, B.J., The, T., House, C.M., Fernandez, C.S., Cox, T., Witters, L.A., Kemp, B.E. (1996). Mammalian AMP-activated protein kinase subfamily. J Biol Chem, 271(2):611-4. doi: 10.1074/jbc.271.2.611.

Trevaskis, J.L., Griffin, P.S., Wittmer, C., Neuschwander-Tetri, B.A., Brunt, E.M., Dolman, C.S., Erickson, M.R., Napora, J., Parkes, D.G., Roth, J.D. (2012). Glucagon-like peptide-1 receptor agonism improves metabolic, biochemical, and histopathological indices of nonalcoholic steatohepatitis in mice. Am J Physiol Gastrointest Liver Physiol, 302(8):G762-72. doi: 10.1152/ajpgi.00476.2011.

Vaidya, V.S., Ozer, J.S., Dieterle, F., Collings, F.B., Ramirez, V., Troth, S., Muniappa, N., Thudium, D., Gerhold, D., Holder, D.J., Bobadilla, N.A., Marrer, E., Perentes, E., Cordier, A., Vonderscher, J., Maurer, G., Goering, P.L., Sistare, F.D., Bonventre, J.V. (2010). Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. Nat Biotechnol. 28(5):478-85. doi: 10.1038/nbt.1623.

Zsom, L., Zsom, M., Salim, S.A., Fülöp, T. (2022). Estimated Glomerular Filtration Rate in Chronic Kidney Disease: A Critical Review of Estimate-Based Predictions of Individual Outcomes in Kidney Disease. Toxins (Basel), 14(2):127. doi: 10.3390/toxins14020127.