

Hepatoprotective Effects of Dulaglutide against Methotrexate-Induced Liver Injury in Rats

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ABSTRACT

Background and Objectives: The cellular antioxidant defense system is disrupted, which results in hepatotoxicity. When the antioxidant defense system is weakened, hepatocyte oxidative damage results from an increase in reactive oxygen radicals. The chemotherapeutic and immunosuppressive drug methotrexate (MTX) is often linked to hepatotoxicity, a serious side effect with uncertain consequences. Therefore, the current study looks into the potential preventive effect of dulaglutide (DUL) against liver damage caused by MTX. **Methods:** The experiment included forty adult male wistar rats, who were divided into four groups: Group 2 was given 14 mg/kg/week of oral MTX for two weeks. On top of the oral MTX, Group 3 also got 0.1 mg/kg/week of subcutaneous DUL. Distributed under the skin, Group 4 was given 0.1 mg/kg/week of DUL. One group served as a control. The animals were sacrificed on the fifteenth day so that histopathological and biochemical testing could be carried out. In these investigations, liver function tests (ALT and AST) and markers of oxidative stress (MDA, GSH, CAT, and SOD) were included. **Results:** Our findings showed that MTX significantly raised a number of oxidative stress markers and liver function tests, employing hematoxylin and eosin (H&E) staining to support biochemical data with histological findings. **Conclusions:** Due to its antioxidant qualities and liver function, DUL significantly protects against MTX-induced hepatotoxicity. This demonstrates its medicinal potential in avoiding hepatotoxicity caused by drugs.

Keywords: Methotrexate; Dulaglutide, Hepatotoxicity, liver function, oxidative stress..

INTRODUCTION

A decrease in liver function is associated with hepatocytotoxicity, which occurs when drugs harm the liver's structure or function (Daly, 2010). The condition when the liver is damaged or becomes dysfunctional due to an excessive amount of a pharmaceutical is known as hepatotoxicity (Navarro and Senior, 2006). Although it is dose-dependent and leads patients to discontinue treatment, hepatotoxicity is a prevalent and short-lived side effect that negatively impacts cancer therapy (Al-Ali et al., 2005). It is thought that oxidative stress is the culprit responsible for the liver damage. According to previous studies, oxidative stress is a known mechanism of MTX's hepatotoxicity. The study was conducted by Devimet al. in 2005.

In order to prevent DNA synthesis, methotrexate (MTX) binds to dihydrofolic acid reductase and stops it from making tetrahydrofolate. Like dihydrofolic acid, this chemical has a floral aroma. Maeda et al. (2010) states that MTX causes an overproduction of reactive oxygen species (ROS), which in turn triggers other detrimental processes. Because it reduces methotrexate's (MTX) efficacy, hepatotoxicity is a crucial factor to think about. According to Mahmoud et al. (2017), oxidative stress is linked to MTX-induced hepatotoxicity. Blood levels of bilirubin, cholesterol, triglycerides, ALT, and AST were elevated because MTX severely damaged the liver, throwing off the equilibrium between oxidants and antioxidants. These findings are consistent with prior research. The same year also saw the work of Abo-Haded et al. The therapeutic use of MTX is often restricted due to its very high toxicity, as stated by Duman et al. (2013). Even at very low doses, MTX may cause the devastating liver diseases cirrhosis and hepatic fibrosis. Researchers Walker et al. found that 8% of psoriasis patients given low doses of MTX had transaminase elevations up to three times normal, and 7% of those individuals were at risk for cirrhosis (2000). Nevertheless, Conway and Carey (2017) found that high doses of MTX can lead to a significant spike in liver function test results. An estimated 15–50% of patients taking low to moderate dosages of MTX over an extended period of time would have elevated blood levels of alanine aminotransferase (ALT) or aspartate aminotransferase (AST). These anomalies often don't pose a major health risk and typically go away on their own without the need for medical treatment. Researchers Khan et al.

The small intestine L cells' glucagon gene creates a GLP-1 analog (Dulaglutide) once food is ingested. According to Cechin et al. (2012), it enhances the secretion of insulin that is reliant on glucose by binding to specific GLP-1 receptors in the pancreas. An agonist that binds to the glucagon-like peptide-1 receptor is used to treat type 2 diabetes (Trevaskis et al., 2012). Nauck et al. (2016) included exenatide, lixisenatide, liraglutide, albiglutide, semaglutide, and dulaglutide as GLP-1RAs that may be categorized as either short-acting or long-acting. In addition to its effects on blood sugar, GLP-1 exerts anti-inflammatory, antioxidant, neuroprotective, and vasoprotective effects across various organs and cell types, including the kidneys, heart, lungs, endothelial cells, and pancreatic β -cells.

An overabundance of reactive oxygen and nitrogen species (ROS/RNS) and insufficient antioxidant defenses lead to oxidative stress. When reactive oxygen species (ROS) are overproduced, they may damage biological macromolecules including DNA, lipids, and proteins, which can cause cells to die. Reactive oxygen species (ROS) are byproducts of several metabolic processes and enzymatic activities that include molecular oxygen. Their potential association with several human diseases, especially those affecting the liver, is intriguing. An example of an external source might be ionizing radiation, metals, food, or pesticides. The liver is an essential organ because of all the work it performs. Its primary functions in metabolizing and detoxifying alcoholic and narcotic drugs lead to the production of reactive oxygen species (ROS) (Chiang, 2014).

Mucins, which are metal-binding proteins like transferrin, lactoferrin, and metallothionein; superoxide dismutases (SOD) both intracellularly and extracellularly; catalase (CAT), an enzyme that decreases hydrogen peroxide (H_2O_2); glutathione-S-transferases, an enzyme system for detoxification; and other redox regulatory thiol proteins are all examples of small-molecular-weight antioxidants (Fattman et al., 2003).

Comparing blood AST to ALT levels is the primary way to identify liver injury. Such severe parenchymal damage to the liver is evident. Research has shown that the AST/ALT ratio may be used to evaluate cirrhosis, acute hepatitis, and chronic hepatitis (Wang and Kong 2020).

Indicators of liver damage, both histological and clinical, may be brought on by hepatotoxins. It is possible to identify liver disease using biochemical indicators like AST and ALT. While elevated levels of total and conjugated bilirubin suggest healthy liver function, elevated levels of certain enzymes in the blood are critical indicators of liver disease. When bilirubin and transaminase levels are two times the usual upper limit, it is suggested that there is hepatotoxicity (Reuben, 2004).

2. MATERIALS AND METHODS

2.1 Animals

The National Center for Research provided the adult male wistar rats, which weighed 170 to 200 grams. The animals spent two weeks in a controlled setting before the trial began. Twelve hours of light and darkness, sixty percent humidity, and a plenty of food and drink were all hallmarks of the laboratory environment. All aspects of the study adhered to ethical guidelines for the treatment of animals used in experiments. Methods for the study were greenlit by the faculty of pharmacy's research ethics council at Tobruk University.

2.2 Drugs

Methotrexate (MTX) was obtained from Hikma Specialized Pharmaceuticals, Cairo, Egypt. Dulaglutide (DUL) was generously provided by Eli Lilly and Company, Indianapolis, USA.

2.3 Experimental design

Each of the six groups consisted of 10 adult male Wistar rats, for a total of forty rats. As a control, Group 1 received oral saline. The second group endured two weeks of 14 mg/kg/week oral methotrexate (MTX). This dosage regimen is based on the strong evidence that MTX may induce liver damage and early-stage fibrosis in only two weeks. Group 3 subjects received 14 mg/kg/week of MTX orally and 0.1 mg/kg/week of dulaglutide subcutaneously. Previous studies on rats demonstrated the medication's antioxidant potential. When deciding on a dosage, the tissue-preserving properties of Dulaglutide in animals free of diabetes and hypoglycemia were considered. A weekly subcutaneous dose of 0.1 mg/kg is recommended. The fourth set of subjects was given 0.1 mg/kg of DUL orally once weekly for a duration of two weeks.

2.4 Sample preparation

At the end of the experimental period, the rats were anesthetized with thiopental sodium (50 mg/kg, intraperitoneally [IP]).

Careful saline extraction of the lung tissues followed sacrifice. Without delay, I transferred the first liver lobe to a deep freezer pre-set to -80 °C in preparation for the subsequent ELISA. Paraaffin was used to fix the liver's second lobes, which were then kept in 10% neutral-buffered formalin for histological investigation. Following that, they were sliced into 5 µm pieces using a rotary microtome.

2.5 Histopathological examination of the liver tissues

Liver slices from different experimental groups were deparaffinized, stained with hematoxylin and eosin (H&E), and then mounted on glass slides for a general histological evaluation. We used a light microscope to look at the colored slices.

2.6 Biochemical Assays

Utilizing commercially available enzyme-linked immunosorbent assay (ELISA) kits, we conducted oxidative stress testing on liver tissue homogenates according to the manufacturer's instructions. Superoxide Dismutase (SOD), MyBioSource, Shang Hai Blue Gene Biotech, and MyBioSource all provided clients with a range of colorimetric methods for evaluating liver function. These catalog numbers are relevant: MBS268427, E02G0367, MBS036924, and MBS006963.

2.7 Statistical analysis

When we display the mean value, we can or can't see the standard deviation (SD). We used GraphPad Prism version 8, which is created and published by GraphPad Software Inc. of La Jolla, California, USA, for our statistical study. To compare the groups, we used a one-way ANOVA with the Tukey-Kramer multiple comparison post hoc test. When the p-value was less than 0.05, we know that there is statistical significance.

3. RESULT

3.1 Effect on oxidative stress marker

Table 1 shows that when the control group took the supplement orally once weekly for 14 days, their levels of antioxidant markers including GSH, CAT, and SOD were much lower, while their amounts of MDA in lung tissue were much greater. These oxidative changes were successfully counteracted by administering 0.1 mg/kg of DUL. Relative to the MTX group, MDA levels were 42% lower, which is an important discovery. There was a 141% rise in the levels of catalase (CAT) and glutathione (GSH). The SOD levels were also 183% greater than those in the MTX group. Dulaglutide prevents oxidative stress in MTX-induced liver damage at doses of 0.1 mg/kg and above.

Table 1. Effect of Dulaglutide on Oxidative Stress Markers in Methotrexate-Induced Hepatotoxicity.

Group	MDA (nmol/g tissue)	GSH (Pg / g tissue)	CAT (U/g tissue)	SOD (U/g tissue)
Control	12.95 ± 1.27	77.58 ± 5.51	61.11 ± 3.69	48.92 ± 2.81
Methotrexate 14 mg/kg	51.65 ± 1.53 a	14.38 ± 0.71 a	11.57 ± 0.44 a	8.41 ± 0.81 a
Methotrexate Dulaglutide (0.1 mg/kg) +	29.85 ± 0.18 b	34.61 ± 1.61 b	27.97 ± 0.95 b	23.81 ± 1.12 b
Dulaglutide (0.1 mg/kg)	13.97 ± 0.98	61.3 ± 3.42	49.58 ± 2.26	43.83 ± 3.18

Numbers are shown as the mean ± standard deviation of 10 rats per group. When comparing groups a and b, a one-way ANOVA and a Tukey multiple comparisons test were used, with a significance level of p < 0.05. Methotrexate, dulaglutide, malondialdehyde, catalase, controlling, reduced glutathione, and superoxide dismutase are some of the often used acronyms.

3.2 Effect on liver functional test

3.2.1 Effect of Dulaglutide on ALT in Methotrexate-Induced Hepatotoxicity

The results can be shown in Figure 1, where MTX injection significantly raised ALT levels in liver tissue when contrasted with the control group (P < 0.05). Group 1 treated with DUL 0.1 mg/kg had ALT expression levels that were 375% lower

than group 2 treated with MTX. Based on these results, it seems that dulaglutide may have prevented MTX-induced liver damage by lowering the ALT signal.

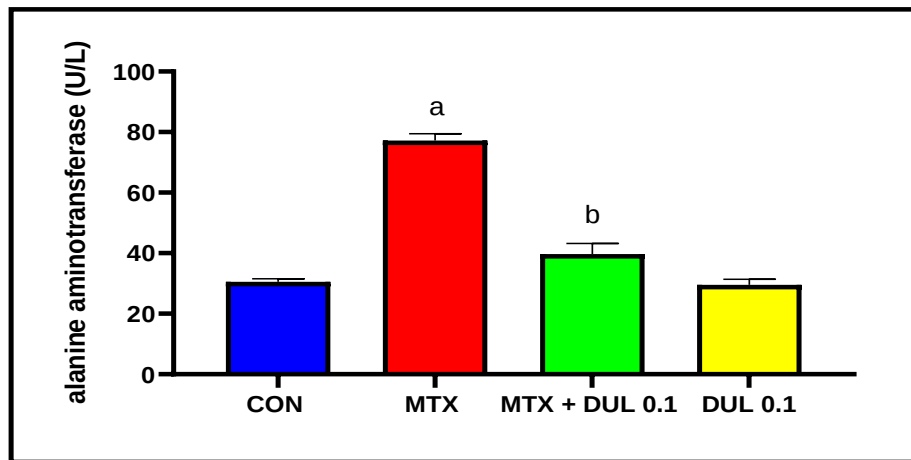


Figure 1. Effect of dulaglutide on ALT activity in methotrexate (MTX)-induced hepatotoxicity in rats. Values are expressed as mean $\pm$ SD (n = 10 rats per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple-comparisons test. aP < 0.05 vs. the control group; bP < 0.05 vs. the MTX group. DUL; Dulaglutide, MTX; methotrexate.

3.2.2 Effect of Dulaglutide on AST in Methotrexate-Induced Hepatotoxicity

Figure 2 shows that the AST levels in liver tissue were significantly higher in the MTX group when compared to the control group ($P < 0.05$). On the other hand, when comparing the groups treated with MTX and DUL at 0.1 mg/kg, the AST expression level in the latter was 375% lower. These studies suggest that dulaglutide, by reducing the AST signal, may protect the liver from MTX-induced damage.

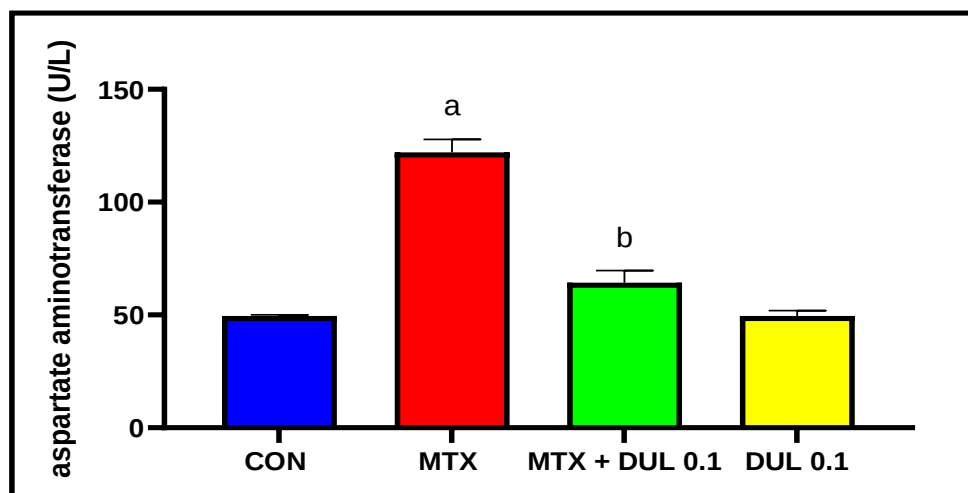


Figure 2. Effect of Dulaglutide on AST Activation in MTX-Induced hepatotoxicity in rats. a vs. control group, b vs. MTX group. Values are expressed as mean $\pm$ SD of 10 rats per group. (one-way ANOVA followed by Tukey

multiple comparisons test; $p < 0.05$). DUL; Dulaglutide, MTX; methotrexate.

3.3 Histopathological Hematoxylin and Eosin (H & E)

The mechanism by which dulaglutide protected the livers of rats from methotrexate-induced damage was determined by histopathological examination. The livers of the experimental groups had significant pathological changes when stained with hematoxylin and eosin (H&E), as shown in Figure 3. Either the protective/therapeutic response has been effective in preserving structural integrity, or the histological structure of the control group (A) is quite normal. Diffuse hepatocellular deterioration occurs in the MTX group (B) as a result of substantial toxic or metabolic stress. Hepatocytes in group C (MTX + DUL 0.1) show clear signs of metabolic disruption and significant cellular damage. There is a modest protective effect or restricted cellular harm in the DUL 0.1 group (D).

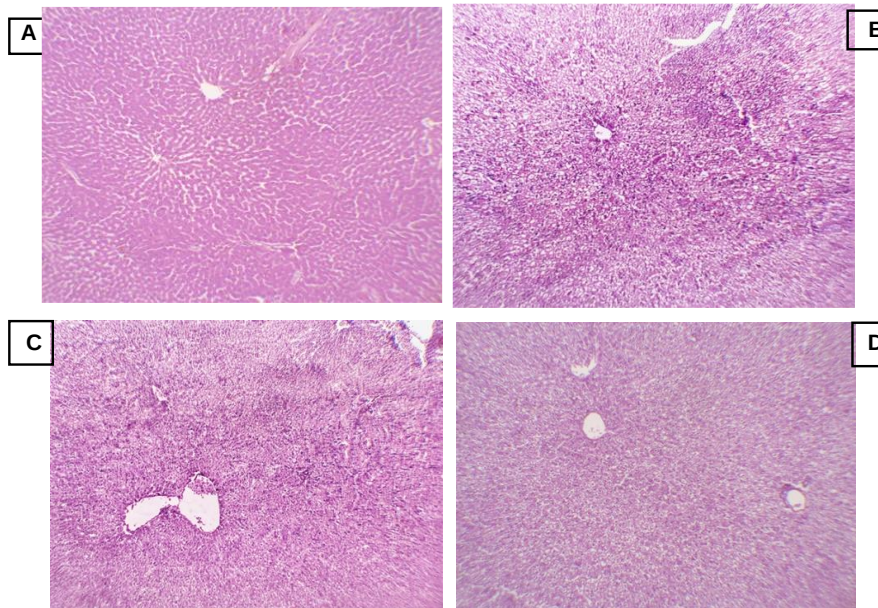


Figure 3. Photomicrographs of liver sections (H&E; 10×) showing the effect of Dulaglutide on methotrexate (MTX)–induced hepatotoxicity. A: control group showing Exhibits well-preserved hepatic parenchyma with regular cords of hepatocytes radiating orderly around the central vein and distinct cellular boundaries, B: MTX group showing marked cellular alterations characterized by extensive diffuse cytoplasmic vacuolation (hydropic degeneration/steatosis) affecting hepatocytes across the lobule, alongside a central vein and mild sinusoidal dilation, C: MTX + DUL 0.1 group showing Demonstrates prominent degenerative changes with widespread cellular swelling, indistinct cell borders, and extensive micro-vesicular cytoplasmic vacuolation radiating from the central area, D: DUL 0.1 group showing Reveals a relatively preserved hepatic architecture with mild, localized cytoplasmic vacuolation and clear, distinct vesicular nuclei in the majority of hepatocytes.

4. DISCUSSION

The potential harm to the liver from weekly dosing with 14 mg/kg of MTX has been shown in rat studies. While the hepatic levels of glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were markedly reduced, malondialdehyde (MDA) levels were much greater than those of the control group. According to research by Shahraman et al. (2013) and Mammadov et al. (2019), the imbalance between the formation of reactive oxygen species (ROS) and antioxidant defense systems is the root cause of oxidative stress in MTX-induced lung injury. Because it inhibits antioxidant enzymes, MTX may raise levels of reactive oxygen species (ROS), lipid peroxidation caused by oxidative stress, and overall lipid peroxidation, according to certain studies (Uzar et al., 2006). Superoxide dismutase (SOD), an enzyme that contains metals and is mostly found in mitochondria, converts radicals of superoxide into hydrogen peroxide (H_2O_2). Glutathione peroxidase (GPx) further converts H_2O_2 into water by oxidizing GSH, while catalase avoids the buildup of highly harmful hydroxyl radicals by converting it into water and oxygen (Aziz et al., 2020).

Nevertheless, DUL therapy significantly reduced MDA levels while increasing GSH, CAT, and SOD levels at a dose of 0.1 mg/kg/week. Administration of GLP-1 may increase GSH levels, according to further studies (Krause et al., 2017). The study

conducted by Bułdak et al. (2015) found that catalase and superoxide dismutase functioned better. Hydrogen peroxide (H_2O_2), which is mostly found in mitochondria, is produced when the metal-containing enzyme superoxide dismutase (SOD) converts superoxide radicals. While catalase decomposes H_2O_2 into oxygen and water, it prevents the development of very dangerous hydroxyl radicals (Kahraman et al., 2013). Glutathione peroxidase (GPx) oxidizes glutathione, further converting H_2O_2 into water. Researchers Zhang et al. (2017) and Shi et al. (2018) found that dulaglutide reduced ROS production and oxidative stress-induced cell dysfunction in endothelial cells via GLP-1-dependent signaling pathways. One possible mechanism by which GLP-1 mitigates oxidative stress was proposed by Krasner et al. (2014).

The results demonstrate that the MTX group's ALT and AST levels were much higher than the control group's. This points to a breakdown in the hepatic membrane's functional integrity as the cause of hepatotoxicity. Thus, previous studies have shown that MTX-treated rats had elevated ALT and AST levels (Türk et al., 2022). Many organ systems might experience acute toxicities as a result of cancer chemo treatments. Research has also shown that continuous or acutely high doses of MTX can potentially harm the liver. The liver converts MTX to hydroxymethotrexate. Polyglutamate, the intracellularly stored form of MTX, may be formed with prolonged use of the drug, increasing the risk of MTX-induced hepatotoxicity. (The study conducted by Kalantari et al. in 2024).

It is reasonable to assume that oxidative stress contributes to MTX-induced hepatotoxicity, as the medication produces reactive oxygen species (ROS) and reduces antioxidant capacity. Indicators of hepatotoxicity in clinical practice include lipid peroxidation, which causes free radical generation, and hepatocyte membrane damage, as well as an increase in intracellular markers like alanine transaminase (ALT) and aspartate aminotransferase (AST). In addition, oxidative stress creates inflammatory cytokines, which worsen cellular disease (Cure et al., 2015).

According to many studies, MTX harms the liver by blocking the synthesis of deoxyribonucleic acid, a molecule that relies on folate. The production of some nucleic and amino acids is hindered by this, which in turn causes enzyme leakage and impacts the organelles and plasma membranes of hepatic parenchymal cells (Hamed et al., 2022). Nevertheless, compared to a placebo, dulaglutide significantly reduced serum ALT and AST levels when taken once weekly, perhaps due to the medication's efficacy in managing blood sugar levels and lowering body weight (Gameil et al., 2020).

Histological examination revealed markedly greater hepatic injury in MTX-treated rats compared with control rats. Treatment with DUL at a dose of 0.1 mg/kg/week significantly attenuated the hepatic injury, as demonstrated by hematoxylin and eosin (H&E) staining.

When it came to improving overall physiological indicators like body weight and survival rates, as well as reestablishing antioxidant defense mechanisms, the medicine DUL performed very well. Because it may target several disease pathways, including oxidative stress and energy imbalance, it becomes an even more significant possibility for innovative therapeutics. Furthermore, the benefits of DUL as a possible alternative to methotrexate (MTX) for treating pulmonary toxicity are dose-dependent, particularly at the 0.1 mg/kg level.

CONCLUSION

An increase in oxidative stress in liver cells was shown to be strongly associated with MTX in this experiment. Giving someone MTX caused a lot of harm to their liver, including cirrhosis, necrosis, and tissue degeneration. Dulaglutide treatment ameliorated liver histology and restored antioxidant capacity at a dose of only 0.1 mg, hence reducing these degenerative effects. Results like these suggest that the widely used GLP-1 receptor agonist dulaglutide could be useful as a standalone or adjunctive therapy to mitigate the liver damage that methotrexate causes

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