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THE POTENTIAL BENEFICIAL EFFECT OF BEMPEDOIC ACID ON METHOTREXATE-INDUCED HEPATOTOXICITY IN RATS VIA THE ACTIVATION OF AMPK PATHWAY

[1]Omar W. Maher

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tobruk University, Libya

Corresponding Author:
Omarpharm638@gmail.com

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ABSTRACT

More than a thousand medications have been related to hepatotoxicity, A variety of clinical symptoms may be associated with it, or it may appear as a silent subclinical illness. Folic acid antagonist methotrexate (MTX) is frequently used as a cytotoxic medication to treat a range of illnesses, including leukaemia and inflammatory diseases like psoriasis and rheumatoid arthritis. However, because of its deadly adverse effects, particularly hepatotoxicity, its use in clinical practice has been restricted. This study aimed to evaluate the impact of bempedoic acid on the liver damage caused by MTX in rats. The rats were divided into four groups: bempedoic acid (BEM) (30 mg/kg/day p.o.), MTX (14 mg/kg/week i.p.), MTX with bempedoic acid, and control. The animals were killed on the fifteenth day so that histopathological and biochemical tests could be conducted. The liver damage was evaluated by looking at histopathologic changes. Tissue malondialdehyde (MDA), glutathione peroxidase (GSH), catalase (CAT), superoxide dismutase, and serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were also assessed. According to our findings, MTX treatment considerably alleviated the histopathologic changes and increased MDA, AST, and ALT levels brought on by MTX administration. Bempedoic acid therapy also markedly increased the activities of GSH, CAT, SOD, and AMPK. Nonetheless, a number of advantages were noted with the bempedoic acid dosage. According to our findings, bempedoic acid may protect liver tissue from MTX-induced hepatotoxicity by preventing oxidative stress.

KEY WORDS: *Hepatotoxicity, Methotrexate, bempedoic acid, oxidative stress.*

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1. INTRODUCTION

The liver is a vital organ that carries out several functions, including detoxifying and storing vitamins. Ekebas et al. ss(2019) state that exposure to dangerous chemicals, tainted water, bad eating habits, dirty environments, ingestion of toxic medicines, and excessive alcohol and painkiller usage are all common causes of liver illnesses. As a result, drug-induced hepatic damage has become more common during the last 10 years and has drawn a lot of interest from researchers, regulatory agencies, medical professionals, and pharmaceutical corporations. (Alempijevic, et al 2017).

According to Stevens et al. (2009), hepatotoxicity is the second most frequent reason for medication attrition during development and withdrawal after commercialization. Hepatocellular death, inflammation, and sudden liver failure can result from drug-induced hepatotoxicity. (Ayad, et al., 2014).

Methotrexate (MTX), an antagonist to folic acid, is frequently used to treat inflammatory conditions like multiple sclerosis and rheumatoid arthritis as well as a number of cancers, such as acute lymphoblastic leukaemia, sarcoma, lung, and bladder cancer (Levě et al., 2017). But the use of MTX in treatments has been restricted due to negative side effects, including hepatotoxicity (Shetty et al., 2017). The hepatotoxicity of high-dose methotrexate is explained by a number of interconnected processes (Ezhilarasan, 2021). Although oxidative stress and excessive production of reactive oxygen species (ROS) are the main causes of MTX-induced liver injury, (Mahmoud et al., 2017). Although oxidative stress and excessive production of reactive oxygen species (ROS) are the main causes of MTX-induced liver injury, (Naik and Panda, 2007). Therefore, minimizing oxidative stress and lowering ROS generation have been suggested as efficient strategies to protect the liver from MTX-induced damage.

Methotrexate induced severe liver damage by drastically altering the oxidant/antioxidant balance, This manifested as increased serum total bilirubin, cholesterol, triglycerides, ALT, and AST values. These results align with previous research (Abo-Haded et al., 2017). Recent research has shown that restoring AMPK activation decreased oxidative stress, stopped the generation of ROS, and increased enzymatic antioxidant activity (Wu et al., 2014; Liu et al., 2016). Moreover, pharmacological stimulation of AMPK has been demonstrated to reduce the inflammatory response both in vitro and in vivo. (Bai and others, 2010).

A non-statin medication that lowers cholesterol is bempedoic acid (ETC-1002, 8-hydroxy-2,2,14,14 tetra-methyl-penta-decanedioic acid). Because of its extended half-life (21 hours), it can be taken once daily. When given orally, it is easily absorbed and takes 3.5 hours to reach its maximum plasma concentration (Alqahtani et al., 2024). This new FDA-approved drug treats dyslipidemia by lowering cholesterol (Biolo et al. 2022). It modulates the metabolism of carbohydrates, reduces inflammation, and decreases the production of fatty acids and cholesterol via activating AMPK (Bilen and Ballantyne, 2016; Samsoondar et al., 2017). According to recent studies, restoring AMPK activation enhanced enzymatic antioxidant activity, reduced oxidative stress, and prevented the production of ROS. (Liu and others, 2016).

According to Niedernhofer et al. (2003), oxidative stress damages cellular biomembranes through radicals, leading to lipid peroxidation and the creation of oxidized chemicals that can change proteins, DNA, and other macromolecules. Normal pulmonary homeostasis requires a sufficient balance between intracellular and extracellular oxidants and antioxidants. Small-molecular-weight antioxidants include mucins, metal-binding proteins (like transferrin, lactoferrin, and metallothionein), intracellular and extracellular superoxide dismutases (SODs), H2O2-reducing enzymes (like catalase (CAT)), detoxification enzyme systems (like glutathione-S-transferases), and other redox regulatory thiol proteins (Fattman et al., 2003).

5'AMPK activation has long been hailed as a potential therapeutic approach for the treatment of metabolic diseases. AMPK is a crucial energy balance regulator (Carling, 2017). The well-known energy sensor adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) is triggered by elevated intracellular AMP levels (Kahn et al., 2005). Pharmacokinetic problems and safety concerns with direct kinase activation have hindered the clinical development of AMPK activators (Olivier et al., 2018). AMPK activation can result from alterations in the cellular metabolic state, such as increased ATP consumption during exercise or when cellular metabolism increases, or suppression of ATP synthesis, such as hypoxia, glucose deprivation, heat stress, or a decrease in mitochondrial oxidative phosphorylation. AMPK is a general stress sensor that is essential for maintaining intracellular homeostasis in the face of oxidative stress and other potentially hazardous situations, in addition to acting as an intracellular energy sensor and regulator (Kukidome et al., 2006). It has also been demonstrated to reduce acute inflammatory reactions, apoptosis, and cellular senescence (Hoogendijk et al., 2013).

The primary marker of liver damage is the ratio of serum levels of aspartate aminotransferase (AST) to alanine aminotransferase (ALT), which indicates the degree of liver parenchymal damage. Numerous studies have demonstrated the significance of the AST/ALT ratio in the diagnosis and severity evaluation of liver illnesses, including cirrhosis, acute hepatitis, and chronic hepatitis (Wang and Kong 2020).

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2. MATERIALS AND METHODS

2.1 ANIMALS

The National Center of Research provided adult male wistar rats weighing between 170 and 200 grams. The animals were acclimated to their housing for two weeks prior to the commencement of the study. With a 12-hour light/dark cycle, 60% humidity, and unlimited access to food and water, they were housed under conventional laboratory settings. The studies were conducted in compliance with the ethical guidelines for the care and use of experimental animals. The Tobruk University Faculty of Pharmacy's Research Ethics Committee authorized the study protocol.

2.2 DRUGS

Hikma Specialized Pharmaceutics, located in Cairo, Egypt, supplied the methotrexate (MTX). Daiichi-sankyo, Europe Gmbh, Munchen, Germany kindly provided bempedoic acid (BEM).

2.3 EXPERIMENTAL DESIGN

Four experimental groups were randomly selected from among forty mature male wistar rats (n = 10 per group). Rats in Group 1 were given saline orally (control negative). For two weeks, Group 2 rats were given methotrexate intraperitoneally (i.p.) at a dose of 14 mg/kg per week. This dosage plan was chosen since published research has demonstrated that it frequently causes liver damage in as little as two weeks. For two weeks, Group 4 rats were given BEM alone (30 mg/kg/day, p.o.), whereas Group 3 rats were given both BEM (30 mg/kg/day, p.o.) and MTX (14 mg/kg/week, p.o.).

2.4 SAMPLE PREPARATION

The rats were put to sleep at the conclusion of the experiment using thiopental sodium anaesthesia (50 mg/kg IP). The lung tissues were removed after sacrifice and carefully cleaned with saline. For the ELISA that followed, the first liver lobe was snap-frozen at -80 °C right away. For histological analysis, the second liver lobes were sectioned into 5 µm slices using a rotary microtome after being embedded in paraffin and preserved in 10% neutral-buffered formalin.

2.5 BIOCHEMICAL ASSAYS

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to analyse liver tissue homogenates for oxidative stress in compliance with the manufacturer's instructions. Malondialdehyde (MDA; MyBioSource, Cat. No. MBS268427), reduced glutathione (GSH; Shang Hai Blue Gene Biotech, Cat. No. E02G0367), catalase (CAT; MyBioSource, Cat. No. MBS036924), and superoxide dismutase (SOD; MyBioSource, Cat. No. MBS006963) were among the oxidative stress and antioxidant parameters. AMP-activated protein kinase (AMPK My Life Span Bio Sciences; LS-F36060) was one protein kinase parameter. Colorimetric techniques for liver test function included aspartate aminotransferase (AST; Teco Diagnostic) and alanine aminotransferase (ALT; Teco Diagnostic).

2.6 HISTOPATHOLOGICAL EXAMINATION OF THE LIVER TISSUES

For a general histological evaluation, liver slices from several experimental groups were deparaffinized, placed on glass slides, and haematoxylin and eosin (H&E) stained. A light microscope was used to examine the stained slices.

2.7 STATISTICAL ANALYSIS

The mean ± standard deviation (SD) of the data is displayed. GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analysis. Group differences were assessed using the Tukey Kramer multiple comparison post hoc test and one-way analysis of variance (ANOVA). Statistical significance was defined as a p-value of less than 0.05.

3. RESULTS

3.1 EFFECT ON OXIDATIVE STRESS MARKER

Figures 1-4 demonstrate that antioxidant markers like glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD) in liver tissue were significantly decreased ($P \leq 0.05$) and malondialdehyde (MDA) levels were significantly increased when methotrexate (MTX) was given orally once a week for 14 days. BEM 30 mg/kg therapy effectively corrected these oxidative alterations. Specifically, MDA levels were lower while GSH, CAT, and SOD levels were increased compared to the MTX group. In MTX-induced hepatotoxicity, BEM, especially at the 30 mg/kg dose, has a protective effect against oxidative stress.

3.1.1 EFFECT OF BEMPEDOIC ACID ON MDA IN METHOTREXATE-INDUCED HEPATOTOXICITY

As shown in Figure 1, methotrexate (MTX) injection significantly increases MDA levels in liver tissue when compared to the control group ($P < 0.05$). BEM 30 mg/kg therapy dramatically decreased MDA expression by 54% as compared to the MTX group. These findings suggest that bempedoic acid may protect against MTX-induced hepatotoxicity by reducing the MDA signal.

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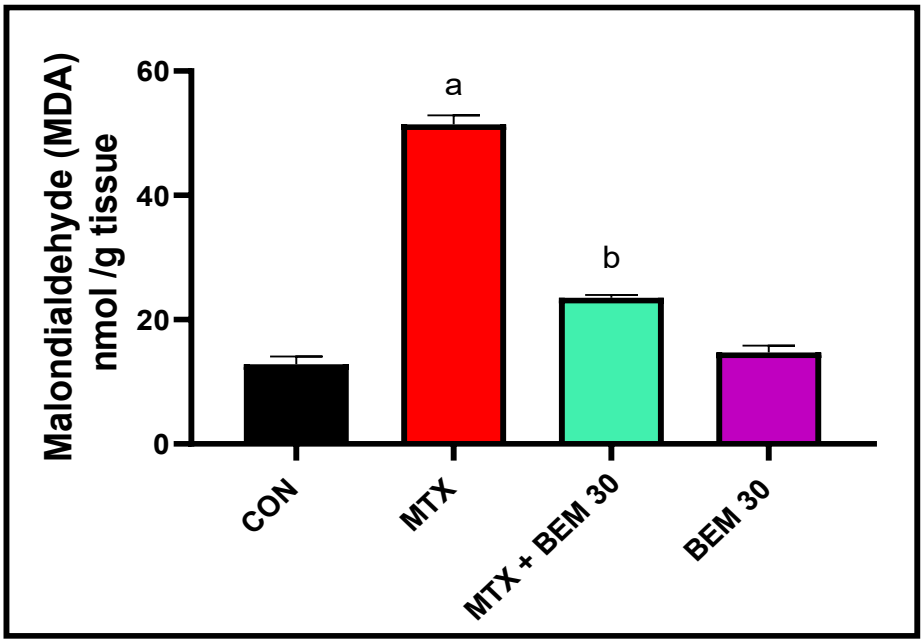


Figure 1: Effect of Bempedoic acid on MDA Activation in MTX-Induced hepatotoxicity in rats. a vs. control group, b vs. MTX group. Values are expressed as mean ± SD of 10 rats per group. (one-way ANOVA followed by Tukey multiple comparisons test; p <0.05). BEM; Bempedoic acid, CON; Control, MTX; Methotrexate.

3.1.2 EFFECT OF BEMPEDOIC ACID ON GSH IN METHOTREXATE-INDUCED HEPATOTOXICITY

As shown in Figure 2, methotrexate (MTX) injection significantly reduces GSH levels in liver tissue when compared to the control group ($P < 0.05$). Treatment with BEM 30 mg/kg markedly raised GSH expression by 196% in comparison to the MTX group. These results imply that BEM enhances the GSH signal and has a protective function against the hepatotoxicity caused by MTX.

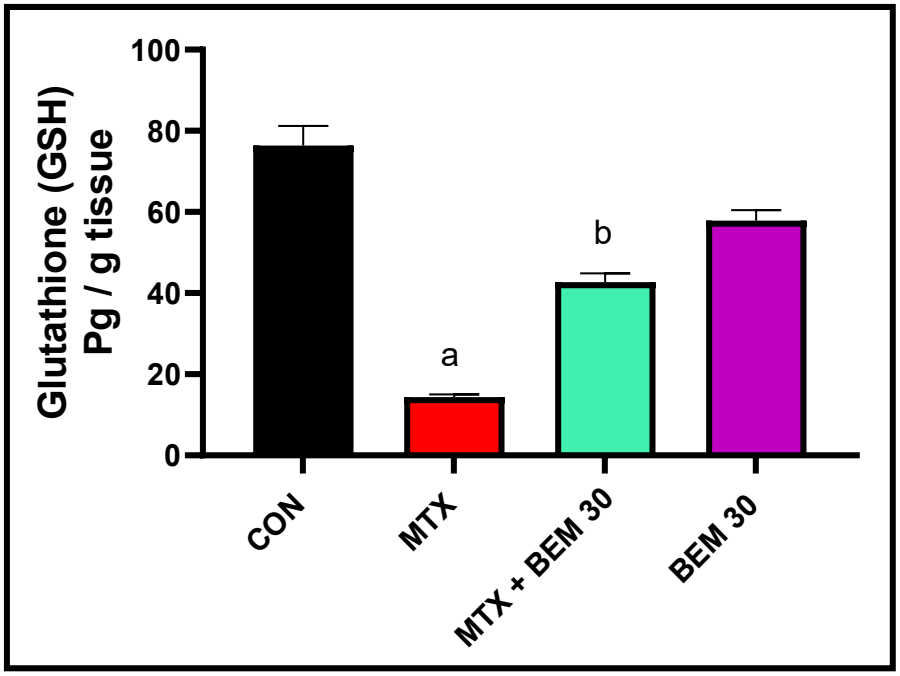


Figure 2: Effect of Bempedoic acid on GSH Activation in MTX-Induced hepatotoxicity in rats. a vs. control group, b vs. MTX group. Values are expressed as mean ± SD of 10 rats per group. (one-way ANOVA followed by Tukey multiple comparisons test; p <0.05). BEM; Bempedoic acid, CON; Control, MTX; Methotrexate.

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3.1.3 EFFECT OF BEMPEDOIC ACID ON CAT IN METHOTREXATE-INDUCED HEPATOTOXICITY

Methotrexate (MTX) injection dramatically reduces CAT levels in liver tissue as shown in Figure 3, in comparison to the control group ($P < 0.05$). In contrast to the MTX group, treatment with BEM 30 mg/kg significantly increased BEM expression by 194%. These results imply that BEM enhances the GSH signal and has a protective function against the hepatotoxicity caused by MTX.

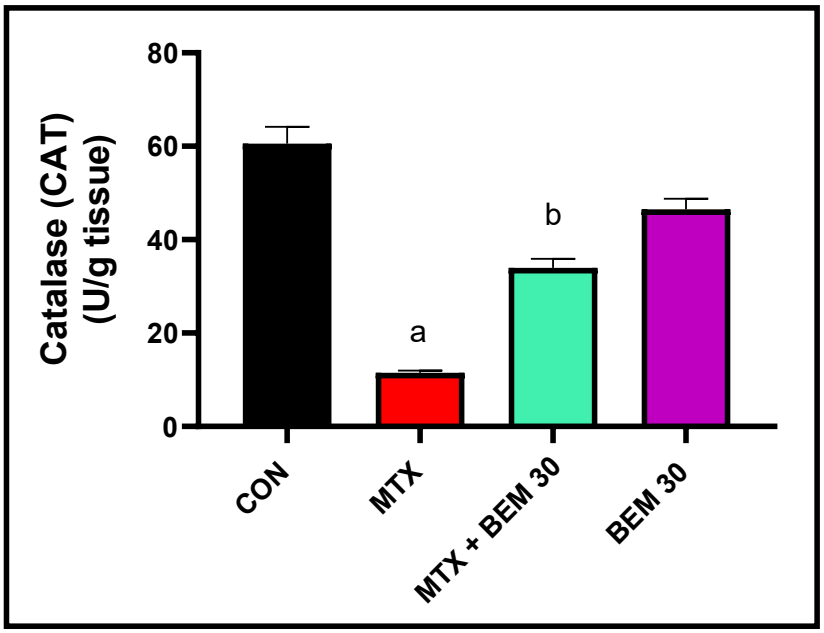


Figure 3: Effect of Bempedoic acid on CAT Activation in MTX-Induced hepatotoxicity in rats. a vs. control group, b vs. MTX group. Values are expressed as mean ± SD of 10 rats per group. (one-way ANOVA followed by Tukey multiple comparisons test; $p < 0.05$). BEM; Bempedoic acid, CON; control, MTX; methotrexate.

3.1.4 EFFECT OF BEMPEDOIC ACID ON SOD IN METHOTREXATE-INDUCED HEPATOTOXICITY

As shown in Figure 4, methotrexate (MTX) injection significantly lowers SOD levels in liver tissue as compared to the control group ($P < 0.05$). As opposed to the MTX group, treatment with BEM 30 mg/kg significantly increased SOD expression by 252%. These results imply that BEM enhances the SOD signal and has a protective function against the hepatotoxicity caused by MTX.

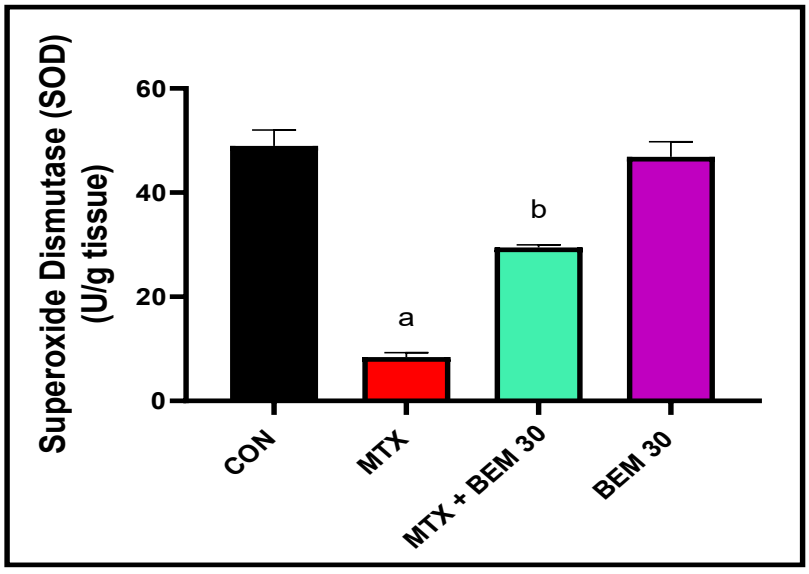


Figure 4. Effect of Bempedoic acid on SOD Activation in MTX-Induced hepatotoxicity in rats. a vs. control group, b vs. MTX group. Values are expressed as mean ± SD of 10 rats per group. (one-way ANOVA followed by Tukey multiple comparisons test; $p < 0.05$). BEM; Bempedoic acid, CON; control, MTX; methotrexate.

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3.2 EFFECT OF BEMPEDOIC ACID ON AMPK ACTIVATION IN METHOTREXATE-INDUCED HEPATOTOXICITY

As shown in Figure 5, methotrexate (MTX) injection significantly reduced AMPK levels in liver tissue when compared to the control group ($P < 0.05$), indicating impaired cellular energy management. As opposed to the MTX group, treatment with BEM 30 mg/kg significantly elevated AMPK expression by 83%. Although BEM showed the highest level of AMPK activation, These results imply that BEM protects against MTX-induced hepatotoxicity by enhancing AMPK signaling.

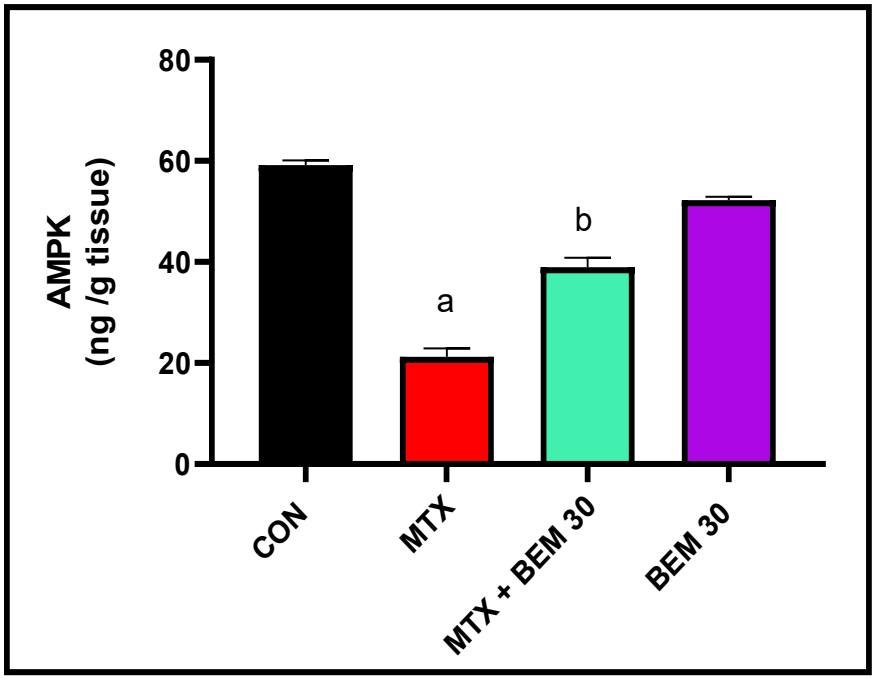


Figure 5: Effect of Bempedoic acid on AMPK Activation in MTX-Induced hepatotoxicity in rats. a vs. control group, b vs. MTX group. Values are expressed as mean ± SD of 10 rats per group. (one-way ANOVA followed by Tukey multiple comparisons test; $p < 0.05$). BEM; Bempedoic acid, CON; control, MTX; methotrexate.

3.3 EFFECT OF BEMPEDOIC ACID ON LIVER FUNCTIONAL TEST IN METHOTREXATE-INDUCED HEPATOTOXICITY

Table 1: Effect of Bempedoic acid on liver functional test markers (ALT and AST) in methotrexate induced-hepatotoxicity in rats.

Table 1 illustrates that following MTX treatment, ALT and AST levels in liver tissue were significantly higher than in the control group ($P < 0.05$). This implies a disruption in the regulation of cellular energy. Treatment with BEM 30 mg/kg significantly decreased ALT and AST expression by 51.29% and 48.14%, respectively, as compared to the MTX group. These results imply that BEM increases ALT and AST signaling, which contributes to its protective function against MTX-induced hepatotoxicity, even though BEM showed the greatest activation of ALT and AST.

Group	ALT (U/L)	AST (U/L)
Control	30.50 ± 1.04	49.50 ± 0.54
Methotrexate (14 mg/kg)	77.00 ± 2.36 a	121.5 ± 5.61 a
Methotrexate + Bempedoic acid (30 mg/kg)	37.50 ± 1.87 b	63.00 ± 2.82 b
Bempedoic acid (30 mg/kg)	27.50 ± 1.04	47.00 ± 2.28

Values are expressed as mean ± SD of 10 rats per group. a vs. control group, b vs. MTX group (one-way ANOVA followed by Tukey multiple comparisons test; $p < 0.05$). ALT; alanine aminotransferase, AST; aspartate aminotransferase, BEM; Bempedoic acid, CON; control, MTX; methotrexate.

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3.4 EFFECT OF BEMPEDOIC ACID ON HISTOPATHOLOGICAL HEMATOXYLIN AND EOSIN (H&E) STAIN IN METHOTREXATE-INDUCED HEPATOTOXICITY

Histological analysis was performed on liver tissue sections stained with haematoxylin and eosin (H&E), and the results showed that the experimental groups had different pathological changes (Figure 6). A near-normal histological structure or a good protective/therapeutic response preserving structural integrity is suggested by the control group (A). MTX group (B) significant toxic or metabolic stress resulting in diffuse hepatocellular degeneration, MTX + BEM 30 group (C) Highlights prominent circulatory disturbances and congestion-induced parenchymal stress, BEM 30 group (D) Reflects optimal recovery or a robust protective outcome with minimal residual tissue damage.

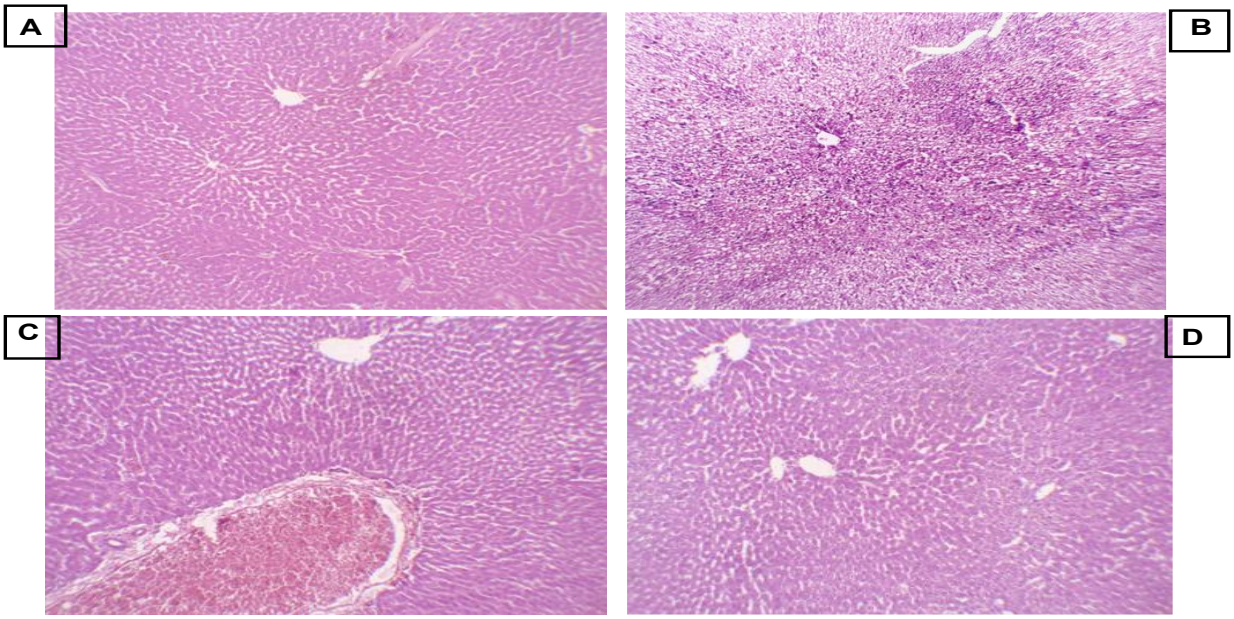


Figure 6: Photomicrographs of liver sections (H&E; 10×) showing the effect of Dulaglutide on methotrexate (MTX)–induced hepatotoxicity. A: control group demonstrates a well-preserved hepatic parenchyma with defined cellular boundaries and regular hepatocyte cords that radiate orderly around the central vein, B: MTX group displaying significant cellular changes, including a central vein, modest sinusoidal dilatation, and widespread diffuse cytoplasmic vacuolation (hydropic degeneration/steatosis) impacting hepatocytes throughout the lobule, C: MTX + BEM 30 group showing Characterized by marked vascular congestion with a severely dilated central vein/portal tract engorged with red blood cells, accompanied by surrounding pressure atrophy and focal degenerative changes in adjacent hepatocytes, D: BEM 30 group showing Shows close restoration of normal hepatic architecture, featuring well-organized hepatocyte cords separated by thin, clear blood sinusoids and prominent central veins with minimal pathological changes.

4. DISCUSSION

The results of the study unequivocally show that MTX causes hepatotoxicity in rats, as shown by increased oxidative, protein kinase, and liver functional test levels in liver tissue. The study's main conclusions include that MTX causes oxidative damage, protein kinase, and liver functional tests in liver tissue via several biomolecular mechanisms. According to the current study, Rats given MTX (14 mg/kg/week) showed markedly lower levels of SOD, CAT, and GSH in their lungs than the control group, but their MDA levels were significantly greater. These results suggest that an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defence mechanisms led to oxidative stress and MTX-induced lung damage (Mammadov et al., 2019). MTX may inhibit a number of antioxidant enzymes, which could result in increased formation of reactive oxygen species (ROS), oxidative stress-mediated lipid peroxidation, and decreased activity of antioxidant enzymes due to increased lipid peroxidation (Uzar et al., 2006). Superoxide radicals are converted to hydrogen peroxide ($H2O_2$) by the metal-containing enzyme SOD, which is mostly found in the mitochondria. While glutathione peroxidase (GPx) further converts $H2O_2$ into water by oxidising GSH, catalase breaks down $H2O_2$ into water and oxygen to prevent it from turning into very dangerous hydroxyl radicals (Aziz et al., 2020). By upregulating important enzymes like GSH, SOD, and CAT, therapy restored antioxidant defence in contrast to MTX-induced oxidative damage. The alveolar lining fluid of patients with pulmonary oxidative damage frequently lacks glutathione, an essential ROS scavenger (Wei et al., 2025).

BEM medication significantly reduced MDA levels at 30 mg/kg/day while increasing GSH, CAT, and SOD levels, in contrast to the MTX group. BEM can activate AMPK, according to a number of previous reports. Here, we observe that our findings corroborate the theory that reestablishing AMPK activation lessens the harm caused by oxidative stress (Ford et al., 2015). Reactive oxygen species (ROS) levels are significantly reduced when the AMPK protein is activated, according to a prior study. (Guo et al., 2014).

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In this study, compared to the control, MTX treatment (14 mg/kg/week) dramatically decreased the level of AMPK in lung tissue. However, prior research in liver tissue revealed that methotrexate metabolites reduce AMPK activity and limit DNA synthesis, which is consistent with the reduction in AMPK brought on by MTX (Atia et al., 2025). In contrast to the MTX group, BEM significantly raised AMPK expression levels in a dosage-dependent manner when administered at a dose of 30 mg/kg/day, p.o. Proinflammatory cytokines and chemokines were discovered to be produced less frequently when AMP-activated protein kinase (AMPK) levels were elevated (Filippov et al., 2013).

It has been demonstrated that liver damage is also possible, especially when acute high dosages or long-term MTX use are administered (Sener et al., 2006). According to reports, hydrogen peroxides and oxygen radicals are connected to the emergence of several pathological processes linked to the negative effects of MTX in the liver (Moghadam et al., 2015). Following MTX treatment, In comparison to the control group, both ALT and AST levels—known indicators of liver damage—were higher in our study. Our findings are consistent with earlier studies. According to reports, MTX-induced hepatotoxicity in this instance was preceded by a notable increase in blood AST and ALT levels (Vardi et al., 2009). However, therapy with bempedoic acid also significantly reduced plasma ALT and AST levels and total cholesterol (Sanjay et al., 2021). Additionally, it was discovered that AMPK activation increased enzymatic antioxidant activity, decreased oxidative stress, and inhibited the production of ROS (Wu et al., 2014).

Rats treated with MTX had much more liver damage than control rats, based on a histopathological examination. Haematoxylin and eosin (H&E) staining revealed that treatment with BEM at a dose of 30 mg/kg/day greatly reduced the liver damage.

5. CONCLUSION

The present study concluded that MTX significantly increases oxidative stress, preotein kinase, and liver functional tests in liver cells. Significant liver damage, including fibrosis, necrosis, and tissue degradation, resulted from MTX treatment. Bempedoic acid treatment significantly reduced these pathological effects by restoring antioxidant capacity, lowering oxidative stress markers MDA while raising GSH, CAT, and SOD, increasing protein kinase markers (AMPK), improving liver histology, and increasing liver functional tests (ALT and AST). In particular, our results demonstrate a robust correlation between the cytoprotective effects of bempedoic acid and the elevation of total AMPK levels.

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