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ORIGINAL RESEARCH

The Protective Effect of Roflumilast Against Acute Hepatotoxicity Caused by Methotrexate in Wistar Rats: In vivo Evaluation

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Introduction: Methotrexate (MTX) is one of the most widely used drugs in cancer chemotherapy and treating rheumatoid arthritis. The hepatotoxicity of MTX is one of its major side effects. Roflumilast (ROF) has been recognized to have antioxidant and anti-inflammatory activity in in-vivo and in-vitro models. The present study aimed to explore the potential protective effects of roflumilast against MTX-induced liver toxicity in male Wistar rats.

Methods: High dose of 5 mg/kg for 4 consecutive days subcutaneous (S.C) injection of methotrexate for induction of acute liver injury. A total of 24 Wistar rats, rats were used in four different groups. The NS injections were given S.C to the control group once a day for 4 consecutive days. SC injections of MTX (5 mg/kg) were given to the MTX group daily for four days. At 5 mg/kg once daily for four days, the roflumilast group was given daily oral roflumilast. An injection of MTX and oral roflumilast were given to the MTX + roflumilast group once daily for four consecutive days.

Results: Administration of high dose MTX (5 mg/kg) today 4 produced a significant decrease in hepatic glutathione (GSH) levels and a significant increase in ALT and AST liver enzymes, hepatic malondialdehyde (MDA), tumor suppressor protein (p53), interleukin 6, interleukin 1 levels compared to the control group. Treatment with roflumilast for 4 days significantly attenuated unfavorable changes in these parameters. According to histopathological findings, Roflumilast significantly reduced MTX-induced inflammation and degeneration in the liver. In conclusion, the findings indicate that roflumilast may have a potential therapeutic benefit in treating rats with MTX-induced liver toxicity by mitigating its effects.

Purpose: The aim of this study is to investigate the potential protective effects of roflumilast against MTX-induced liver toxicity in Wistar rats.

Keywords: roflumilast, methotrexate, hepatotoxicity, glutathione, malondialdehyde, tumor suppressor protein-53, interleukin 6, interleukin 1

Introduction

Drug-induced liver toxicity is a major limitation of pharmacotherapy, which can lead to fatal adverse drug reactions and incidence of morbidity and mortality. Anticancer chemotherapeutic agents, when taken for long-term therapy, have been reported to cause many side effects such as cardiotoxicity, lung toxicity, testicular toxicity, and hepatotoxicity. In addition, they cause persistent damage to DNA molecules.[1] Methotrexate (MTX) has anti-proliferative and anti-inflammatory properties and regulatory influence on the immune system.[2] Although known for numerous advantages, MTX use can be detrimental to several viscera, causing serious toxic consequences on respiratory tract, hepatic tissue, and bone marrow.[3] Its side effects have been classified into two groups: those that cause symptoms only, such as nausea, headaches, lethargy, mucositis, and alopecia, and those that could lead to fatal conditions, such as cytopenia, interstitial lung pathology, or MTX-induced pneumonitis, and hepatic conditions, such as fibrosis and cirrhosis.[4] The exact processes or mechanisms responsible for the hepatotoxic effects of MTX are yet to be clearly understood.

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The effectiveness of MTX as a pharmaceutical agent and its adverse events may be governed by identical or dissimilar metabolic pathways. The pathogenesis of liver toxicity induced by MTX necessitates further investigation. Various processes have been hypothesized to disrupt control mechanisms that safeguard against cellular antioxidants and induce injury to hepatocytes due to oxidative stress.[5] Thus, understanding the mechanisms responsible for both advantageous and detrimental effects of MTX is crucial for identifying new targets for treatment and achieving optimal therapeutic approaches for controlling immune-mediated and inflammatory diseases while reducing detrimental adverse events related to toxicity. This has significant implications for the innovation of new pharmaceutical agents and the development of additional targeted drugs that would improve effectiveness while ameliorating toxic side effects.

Roflumilast, a phosphodiesterase 4 (PDE4) inhibitor, has been endorsed as a therapeutic agent for inflammatory airway or dermal conditions. A succession of de novo PDE4 inhibiting agents has been designed to moderate inflammation; studies have indicated positive treatment outcomes.[2] Roflumilast has been shown to be an effective anti-inflammatory drug for treating inflammatory airway conditions.[6] In vitro experiments have shown that PDE4 activity is selectively inhibited by roflumilast in neutrophils derived from humans. Thus, it has been linked to several studies where promising anti-inflammatory activity has been reported via the induction of several anti-inflammatory mediators such as fMLP-induced leukotriene B4 (LTB4), generation of reactive oxygen species (ROS) in human neutrophils, and lipopolysaccharide (LPS)-induced TNF-α production in dendritic cells as well as monocytes.[7] Furthermore, inflammatory mediator production was notably inhibited by roflumilast in macrophages in vitro; this was facilitated by the induction of the expression of heme oxygenase (HO-1) and inhibition of NF-kB, p38 MAPK, and JNK.[8] Therefore, the aim of the current research is to examine the potentially protective or mitigative properties of roflumilast with respect to the hepatic tissue damage caused by MTX.

Materials and Methods

Material

Methotrexate Injection, USP Isotonic Liquid, Contains Preservative is available in 25 mg/mL, 2 mL (50 mg) vials. Manufactured by Hospira, Inc. Origin: Australia. The roflumilast powder was purchased from Hubei Yuncheng Technology Co. Ltd, with an origin in Wuhan, China. Dimethyl Sulfoxide (DMSO) was supplied by Zoad International Co., a medical supplier located in Jeddah, Saudi Arabia (KSA). Diethyl ether was also supplied by Zoad International Co. Formaldehyde 37% solution was supplied by Zoad International Co. for medical use.

Animal

All animal study protocols were approved by the Biomedical Research Ethics Committee at Umm Al Qura University in Saudi Arabia. 24 male Wistar rats, weighing between 170–190 grams and eight weeks old, were obtained from Mansour Scientific Foundation (MSF) in Jeddah, KSA. Prior to the experiment, rats were housed under controlled laboratory conditions with a 12-hour light/dark automated cycle, temperature between 26°C–25°C, and relative humidity of 35–75%, for at least one week. Six rats were kept per cage equipped with free access to food and water.

Study Design

To induce acute liver injury, a liver toxicity model was established by administering high doses of methotrexate (5 mg/kg) via subcutaneous (S.C) injections for four consecutive days. This model was used to evaluate the histopathological changes, biochemical, and inflammatory markers for hepatic toxicity. A total of 24 male Wistar rats were randomly divided into four groups with six rats in each group as follows:

- Group I (control group): received subcutaneous injections of 0.9% NaCl (NS) once daily for four consecutive days and oral DMSO as the vehicle.
- Group II (MTX group): received subcutaneous injections of MTX (5 mg/kg) once daily for four consecutive days and DMSO as the vehicle.[9]
- Group III (roflumilast group): received subcutaneous injections of NS once daily for four consecutive days and oral roflumilast at 5 mg/kg once daily for four consecutive days.

- Group IV (MTX + roflumilast group): received subcutaneous injections of MTX (5 mg/kg) and oral roflumilast (5 mg/kg) once daily for four consecutive days. On the fifth day, all rats were sacrificed through decapitation. Blood samples were collected, and tissue samples from the liver were also extracted for further analysis.[9,10]

Biochemical Analysis in Serum

Rats were sacrificed by decapitation while under anesthesia on the fifth day, and carotid artery blood samples were collected into serum separation gel tubes. Samples of rats' livers were then obtained.

Blood Samples Preparation

The tube was inverted gently five times to mix the blood with the clot activator. To obtain serum, the blood was then allowed to clot for 10 minutes at room temperature before being centrifuged for 15 minutes at 3500 rpm using a SIGMA SM7000 centrifuge (SIGMA, UK). The resulting serum was stored at −80°C for up to four weeks prior to biochemical analysis. Biochemical assessments were performed using a DSX Best sells 2000 automated ELISA machine.

Liver Samples Preparation

Rats were sacrificed, and livers were rapidly removed. The isolated liver was washed with cold normal saline solution and weighed. The left liver lobe was removed for histological study, while the right lobes were stored at −80°C for the assessment of p53, IL-6, IL-1, GSH, and MDA levels in tissue homogenate.

Serum ALT and AST Level

ALT and AST levels were assessed using ELISA kits (Catalog # MBS269614) supplied by My BioSource (San Diego, United States). ALT and AST levels were expressed as IU/L.

Liver Homogenate Assessment

Before homogenizing, 100 mg of liver tissue was rinsed with phosphate-buffered saline (PBS) to remove any blood. The tissue was then homogenized in 10 mL PBS for five cycles at 3000 rpm using a PT 3100 polytron homogenizer. After being kept at −20°C for 20 minutes, the tissue was centrifuged for 20 minutes at 3000 rpm. The supernatant was collected and stored in an Eppendorf tube at −80°C until analysis.

Assessment of Glutathione (GSH)

The antioxidant status of liver homogenate was assessed by measuring reduced glutathione (GSH) levels. GSH levels were determined using ELISA kits (Catalog # MBS724319, My BioSource, San Diego, USA). GSH levels were expressed as nanograms per milliliter (ng/mL).

Assessment of Malondialdehyde (MDA)

To assess oxidative damage to the liver in rats, MDA levels were determined using ELISA kits (Catalog # MBS738685, My BioSource, San Diego, USA). MDA levels were expressed as micromoles per liter (μmol/L).

Assessment of p53

ELISA kits (Catalog # MBS723886, My BioSource, San Diego, USA) were used to determine the levels of p53 in rat liver tissue. p53 levels were expressed as micrograms per liter (μg/L).

Assessment of IL-6

ELISA kits (Catalog # MBS268653, My BioSource, San Diego, USA) were used to determine the levels of IL-6 in rat liver tissue. IL-6 levels were expressed as picograms per milliliter (pg/mL).

Assessment of IL-1

ELISA kits (Catalog # MBS268833, My BioSource, San Diego, USA) were used to determine the levels of IL-1 in rat liver tissue. IL-1 levels were expressed as picograms per milliliter (pg/mL).

Hepatic Histopathological Assessment

The rat liver tissues were fixed for a week in 10% neutral buffered formalin and then prepared for paraffin embedding. Slices that were 5 µm thick were cut and subjected to hematoxylin and eosin (H&E) staining using standard techniques that were documented previously.[11] Scoring of tissue changes in the organ was carried out according to the criteria set forth in previous literature.[12,13] The criteria included the following:

(A) Apoptosis or necrosis
(B) Degeneration of hepatocytes
(C) Hepatic sinusoids, central vein (dilatation or congestion)
(D) Inflammatory cells
(E) Parenchyma architecture

Scoring was as follows: = No changes watched; "+" = Mild changes; "++" = Moderate changes; "+++" = Extreme changes.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 26 (Windows®) (IBM SPSS Inc). The data were tested for normality and one-way analysis of variance (ANOVA) and post-hoc least significant difference (LSD) tests were performed to compare differences between the groups. Mean ± standard deviation (SD) were reported, and a p-value less than 0.05 was considered significant, while a p-value greater than 0.05 was considered non-significant. Graphs were made using the GraphPad Prism program version 9.3.0 (Graph Pad ® Inc., USA).

Results

Biochemical Measurements

Serum Levels of (ALT) and (AST)

The mean serum ALT level was significantly higher ($P<0.05$) in the MTX group (82.33± 6.80) than in the control group (17.00 ±1.00), indicating liver function deterioration caused by MTX with [nearly 5-fold rise]. However, the mean ALT levels in serum of the MTX + ROF group (26.67 ± 6.80) and ROF group (18.67 ± 2.88) were comparable to the control group, unlike the MTX group. As depicted in Figure 1, a similar pattern was observed with AST levels. After administering MTX, the level of serum AST in the MTX group rose from 22.00 ± 1.00 in the control group to 109.3± 10.69 IU/L [approximately 5-fold]. In addition, the administration of ROF+ MTX attenuated the MTX-induced rise in mean serum AST level. This indicates that roflumilast partially inhibits the increase in aminotransferases induced by MTX and offers protection against its hepatotoxic effects.

Liver Homogenate Studies

The data illustrate the ability of ROF to attenuate the MTX-induced reduced (GSH) and increased (MDA) levels Figure 2. There is a significant ($P<0.05$) decrease in the mean GSH level (2.73 ±1.49) in the rats that were given methotrexate compared to the control group (12.80 ± 0.8). GSH levels were almost normal in ROF alone. Interestingly, rats treated with MTX + ROF (7.96 ±1.41) showed a significant ($P<0.05$) increase in the mean GSH level compared to those treated with MTX alone. In the group given MTX + ROF, there is a significant difference in the GSH level compared to the control group, but the significant difference observed was small in magnitude.

The mean MDA level rose significantly ($P < 0.05$) from 0.47 ± 0.03 µmol/L in the control group to 2.11 ± 0.25 µmol/L in the MTX group. In contrast, the MDA level in the ROF+MTX group was similar to that of the control group. In liver tissue, the mean MDA level in the ROF + MTX group (0.86 ± 0.11) was comparable to that in the control group, but significantly higher. The ROF group had a mean MDA level (0.61 ± 0.06) that did not differ significantly ($P > 0.05$) from the control group.

The level of IL-6 in liver tissue was significantly higher ($P < 0.05$) in the MTX group at 19.97 ± 1.37 pg/mL than in the control group (5.63 ± 0.35). The ROF group had a mean IL-6 level in liver tissue (5.867 ± 1.02) almost identical to that of the control group. In contrast, ROF + MTX showed minimal changes (8.50 ± 0.75), but they were significantly

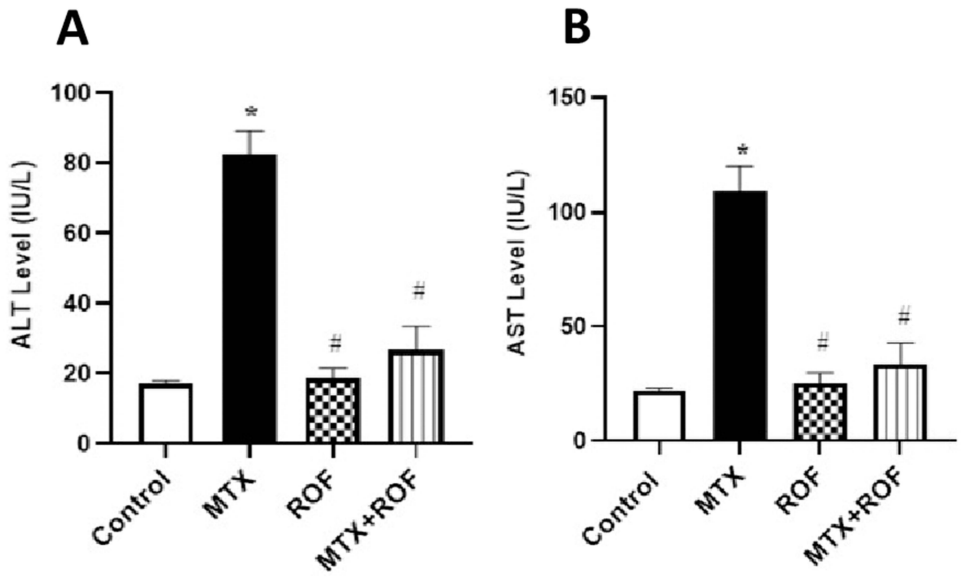

Figure 1 Effect of roflumilast (ROF) administration on methotrexate (MTX) induced increase in rat's serum alanine transaminase serum (ALT) level and serum aspartate transaminase (AST) level (**A**) Alanine aminotransferase (ALT) in IU/L, (**B**) Aspartate transaminase (AST) levels in IU/L. Values are mean ± standard deviation (SD), n=number of rats (6 rats/group). One-way ANOVA was followed by Turkey's multiple comparison test. *P <0.05 compared to normal control group. #P <0.05 compared to MTX group.

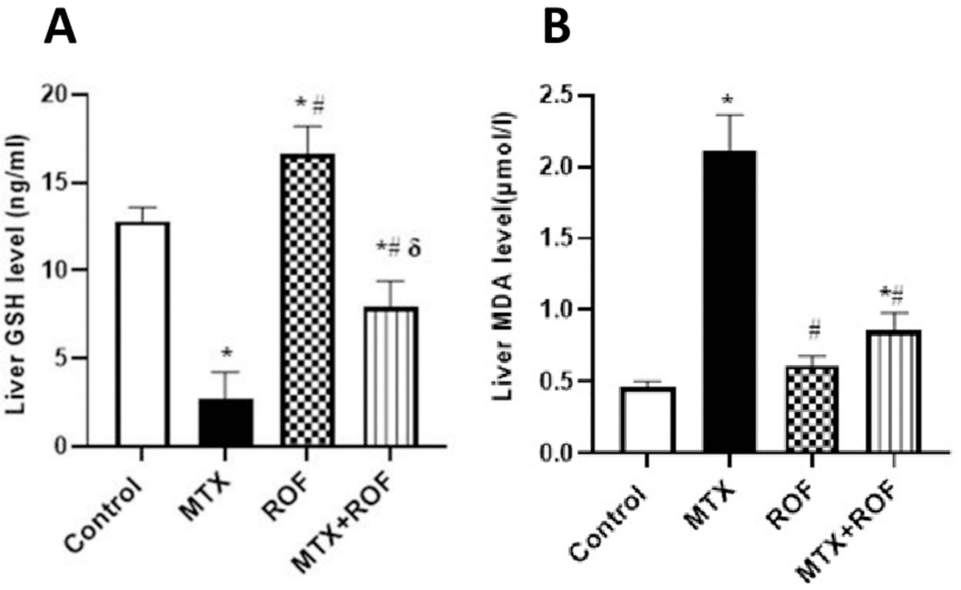

Figure 2 Effect of roflumilast (ROF) administration on methotrexate (MTX) induced change of rat's liver tissue content of glutathione (GSH) and (MDA). (**A**) Glutathione (GSH)) levels in ng/mL, (**B**) Malondialdehyde (MDA) levels in µmol/l. Values are mean ± standard deviation (SD), n=number of rats (6 rats/group). One-way ANOVA was followed by Turkey's multiple comparison test. *P <0.05 compared to normal control group. #P <0.05 compared to MTX group. δp <0.05 compared to MTX+ROF group.

higher ($P < 0.05$) than the control group. Similarly, the level of IL-1 in liver tissue was significantly higher ($P < 0.05$) in the MTX group at 47.33 ± 7.76 pg/mL than in the control group (12.43 ± 0.81). The ROF group exhibited a mean IL-1 level in liver tissue (12.93 ± 0.75) that was almost the same as that of the control group. Compared to the control group, ROF + MTX showed minimal change (20.67 ± 3.78) that was not significantly different ($P > 0.05$). Finally, the mean P53 level rose significantly ($P < 0.05$) from 0.16 ± 0.01 in the control group to 1.58 ± 0.52 µg/L in the MTX group. By contrast, the mean P53 levels seen in liver tissue in the ROF + MTX group (0.63 ± 0.33) and the ROF group (0.17 ± 0.02), shown in Figure 3, were comparable to the control group.

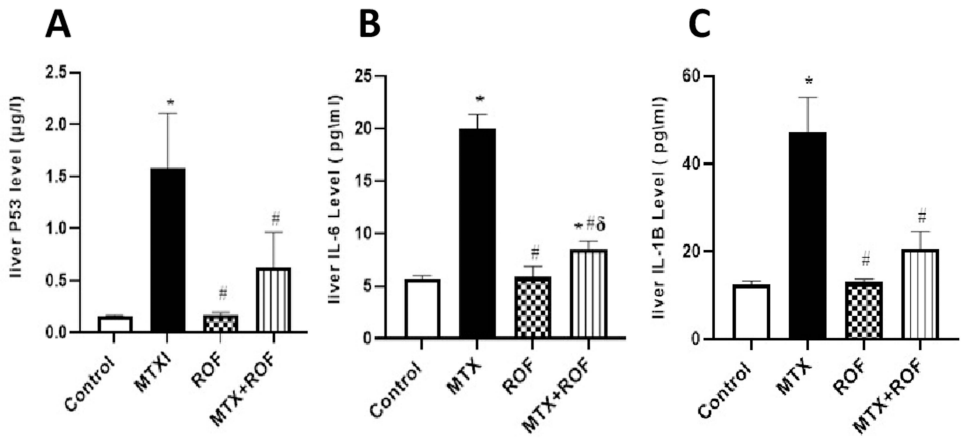

Figure 3 Effect of pre-treatment with Roflumilast (ROF) on methotrexate (MTX) induced change in hepatic tissue. (**A**) Tumor protein P53 (P53) level in (µgl/l), (**B**) Interleukin-6. (IL-6) levels in pg/mL (**C**) Interleukin-1 (IL-1) levels in pg/mL. Values are mean ± standard deviation (SD), n=number of rats (6 rats/group). One-way ANOVA was followed by Turkey's multiple comparison test. *P <0.05 compared to normal control group. #P <0.05 compared to MTX group. δp <0.05 compared to MTX+ROF group.

Histopathological Assessment of Liver Tissue

A histological study in this work was done to explain and confirm the biochemical data shown in this paper. In Figure 4 It was observed that, compared to the normal architecture and regular pattern of hepatocytes with active vesicular nuclei in G1 (Normal control), the liver of G2 (MTX) showed central vein dilation and congestion (a in Figure 4B), Focal cell

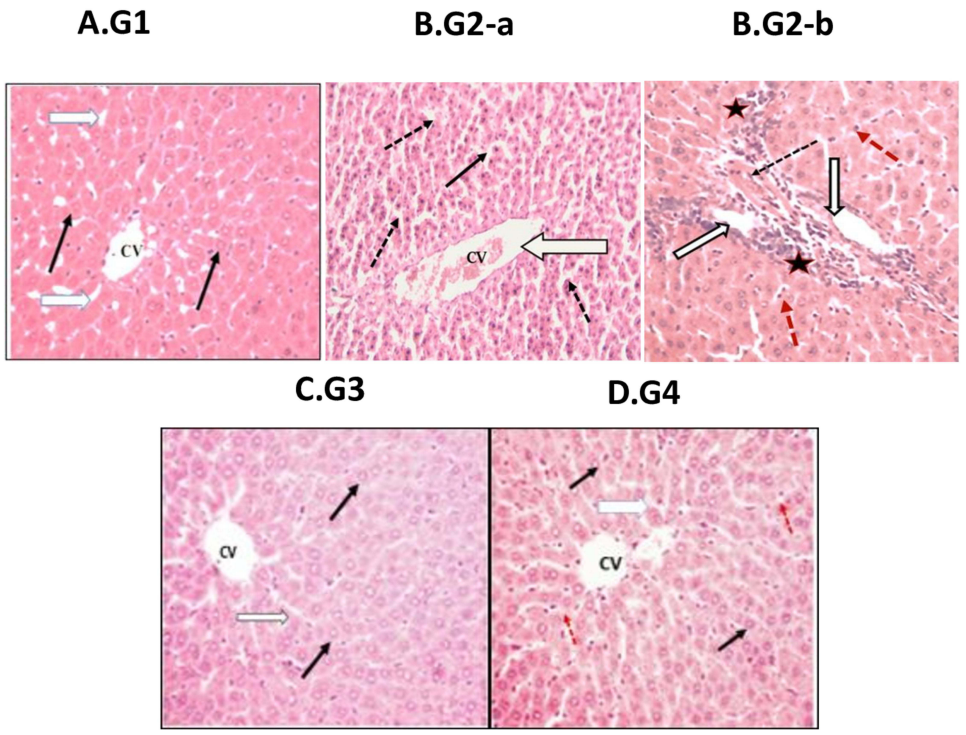

Figure 4 Sections from rat liver stained by H&E and photographed at ×400 scale bar = 50 µm to show: G1: Normal control; showing normal hepatocytes with active vesicular nuclei (thin black arrows) radiating from the central vein (CV) or blood sinusoids (white arrows) are thin and apparent. G2 (**A** and **B**): MTX; showing (**A**) Central vein dilation and congestion (CV), focal cell necrosis (black dotted arrows), hepatocyte nuclei of many cells (thin black arrow) are dark stained and small (apoptosis) with a dilated bile duct showing lining atrophy (white arrows) (**B**) Some sinusoids showed prominent Kupffer phagocytes (black dotted arrow) in the portal area, with a dilated bile duct showing lining atrophy (white arrows), marked inflammatory cell aggregations (black stars) and focal regions looked necrotic (red dotted arrows). G3 **(C)**: ROF; showing normal hepatic tissue, both hepatocyte nuclei (Black arrow) and blood sinusoids (white arrows) looked nearly normal. G4 **(D)**: MTX + ROF; showing marked preservation of hepatic tissue, both hepatocyte nuclei (black arrow) and blood sinusoids (white arrows) looked nearly normal, only the focal regions looked necrotic (red dotted arrows).

Table 1 Liver histopathological alterations scoring

Groups	Central Vein Dilatation / Congestion	Parenchyma Architecture Disorganization	Hepatocyte Nuclei Vesicular/ Dark Inactive	Focal Necrosis	Portal Area Inflammation
G1: NC	–	–	– / Vesicular	–	–
G: MTX	+++/ Congestion	++	++/Dark	+++	+++
G3: ROF	–	–	– / Vesicular	–	–
G4: MTX + ROF	+/ Mild Dilation	–	– / Vesicular	+	–

Notes: Scoring: – = No watched changes; + = Mild changes, ++ = Moderate changes; +++ = Extreme changes.

necrosis, hepatocyte nuclei of many cells are dark stained, and small apoptosis was also observed. In (b in Figure 4B), some sinusoids showed prominent Kupffer phagocytes, Portal area with dilated bile duct showing lining atrophy and marked inflammatory cell aggregations. In (Figure 4C) G3 receiving the ROF alone, no apparent changes were observed. On the other hand, in the group receiving MTX plus ROF, there was marked preservation of normal histological features of hepatic tissue. Table 1 shows the score evaluation of different hepatic components in different groups compared to the control.

Discussion

MTX is a potentially toxic agent for the liver,[2] due to its activation of various pathways involving oxidative stress, inflammation, and both intrinsic and extrinsic apoptotic pathways - including pathways such as MAPK and p53. These markers are being explored as potential indicators of MTX-induced liver damage, through various studies.[14] Researchers have investigated several ways to alleviate MTX-induced hepatotoxicity, including phosphodiesterase-4 (PDE4) inhibitors. The use of PDE4 inhibitors as a therapeutic approach for inflammatory disorders has been validated by treating conditions such as neuroinflammation, RA, lupus, inflammatory bowel disease, asthma, dermatitis, psoriasis, atopic conditions, and chronic obstructive pulmonary disease.[2] After extensive application of knowledge and resources, roflumilast, crisaborole, and apremilast have achieved serial endorsements for their effectiveness in treating respiratory or dermatological inflammation. Additionally, new PDE4 inhibitors have been developed to modify the inflammatory process and have demonstrated satisfactory clinical outcomes, highlighting the implication of novel therapeutic interventions in treating inflammatory-related disorders.[2,15]

Roflumilast, a potent and selective inhibitor of PDE4, has been approved for use in reducing the frequency of COPD exacerbations in patients with severe disease associated with chronic bronchitis and who have had previous exacerbations.[16,17] By specifically inhibiting PDE4, roflumilast suppresses the hydrolysis of cAMP within inflammatory cells.[18] Elevated intracellular levels of cAMP, in turn, lead to broad-spectrum anti-inflammatory effects, such as decreased liberation of inflammatory mediators derived from neutrophils, reduced cytokine secretion,[16] downregulation of cell surface markers in various types of cells, and decreased incidence of programmed cell death. These effects have been observed to be beneficial in patients with COPD exacerbations, particularly those with elevated inflammatory biomarkers compared to baseline values.[19] Roflumilast has been observed to decrease inflammation in response to allergens[20] and to help stabilize the systemic inflammatory response to inflammation caused by lipopolysaccharide.[21] Animal studies have also shown that roflumilast may have the potential to reduce liver inflammation and damage caused by other agents. When administered to rats with carbon tetrachloride-induced liver fibrosis, roflumilast increased liver function while reducing inflammation and fibrosis markers.[22] In rats with lipopolysaccharide-induced liver injury, roflumilast was found to reduce inflammation, oxidative stress markers, and cytokines associated with liver injury.[23] These favorable characteristics provided a rationale for testing the utility of high doses of roflumilast to prevent MTX-induced toxicity in critical organs. In the present study, the dose of roflumilast was derived from previous research that showed its anti-inflammatory effect in the animal model.[24] Consequently, the findings of the current research revealed that roflumilast was successful in safeguarding Wistar rat livers from methotrexate-induced acute hepatotoxicity.

The study showed a significant increase in ALT and AST levels induced by MTX, consistent with previous findings.[25–27] However, chronic treatment with ROF prevented the MTX-induced increase in these enzyme levels and confirmed its hepatoprotective effect. Other studies, such as Feng[28] and Essam,[29] have also found that a single dose of ROF improved liver damage and stimulated hepatic levels of GSH in C57BL/6 mice. In our study, MTX was shown to significantly elevate MDA levels while reducing GSH levels in liver tissues, contributing to oxidative stress and the formation of lipid peroxides as observed in other studies.[30,31] Previous research has suggested that the ROS pathway plays a significant role in MTX-induced hepatic toxicity.[31–34] MTX can create DNA adducts upon cell entry, resulting in the production of ROS and activation of p53,[35] which is a well-known tumor suppressor gene and has been linked to MTX-induced apoptosis.[36]

In the present study, MTX caused a significant elevation in p53, which is explained as an early sign of apoptosis in rat hepatic tissues. Furthermore, activated p53 caused cell cycle arrest and change in the mitochondrial membrane potential in cells.[37] These observations lead to the release of cytochrome c and activation of Caspase-9 and −3, ultimately leading to apoptosis.[38,39] The current data revealed that ROF inhibited the activation of p53 induced by MTX. In view of this finding, the protective effect of ROF against MTX-induced liver damage was mediated by its anti-apoptotic effects. The present finding confirms previously reported observations regarding the efficacy of ROF in suppressing the expression of the p53 level.[40]

The toxicity of MTX was associated with an increased expression of the proinflammatory cytokines, eg, IL-6, due to the increased production of ROS and lipid peroxidation. Increased expression of IL-6 induces liver cell damage and accelerates apoptotic changes.[40,41] The present study demonstrated that MTX caused a significantly increased levels of IL-6 and IL-1β. Similar findings have been reported in[42,43] documented that the expression of cytokines was significantly increased after a single dose of MTX. It was suggested that the expression of the pro-inflammatory cytokines and chemokines, including IL-6, IL-1β, and TNF-α, are due to activated NF-κB transfers to the nucleus of the liver cell and binds to their DNA.[44]

MTX in an animal model induced marked histological changes in the hepatic tissues. For example, the most prominent alteration in the liver of the MTX-treated rats' was congestion of the central vein, focal hepatocyte necrosis with loss of lobular pattern, nuclear lysis, and Blood sinusoids are dilated and full with mononuclear inflammatory cells.[45] These observations were explained by El Bana,[46] who reported dilatation of blood sinusoids due to the very thin cell plates that are widely separated. The thinning of cell plates is explained by the compression of the hepatocytes in the cords adjacent to the dilated and congested sinusoids where the initial toxic effects probably occurred. In the current study, histological analysis of hepatic tissues treated for four days with MTX (5 mg/kg) resulted in remarkable variations. The findings include apoptosis and necrosis of hepatocytes, abnormal activation of inflammatory cells in hepatocytes, congestion, central vein dilation, a noticeable increase in collagen around the dilated congested central vein and hepatic sinusoids. These alterations are the probable result of the lengthy cellular damage induced by MTX resulting in increased levels of ROS leading to DNA injury, oxidation, and proliferation inhibition.[47]

Limitations of the Study

Finally, it is crucial to acknowledge the limitations of this study, which include that it is an acute study, not a chronic study. The study also does not explore the effect of Roflumilast on Methotrexate (MTX) pharmacokinetics or on MTX's anticancer efficacy. These limitations present opportunities for further investigation to gain a more comprehensive understanding of the study's findings.

Conclusions

Experiments were conducted on rats. Administration of MTX (5 mg/kg) to day 4 produced a significant decrease in hepatic glutathione (GSH) levels and a significant increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) liver enzymes, hepatic malondialdehyde (MDA), tumor suppressor protein (p53), Il-6 and IL-1 levels compared to the control group. Administration of ROF for 4 days before administration of MTX significantly attenuated all these changes to normal values comparable to the control. Histopathological analysis revealed that administering ROF dramatically reduced MTX-induced degenerative and inflammatory alterations in the liver. The findings indicate that roflumilast may have a potential therapeutic benefit in treating rats with MTX-induced liver toxicity by mitigating its effects.

Disclosure

The author reports no conflicts of interest in this work.

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