Possible Hepatoprotective Role of Berberine versus Silymarin on Methotrexate Toxicity: Histological and Biochemical Study

Original Article

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ABSTRACT

Introduction: Methotrexate (MTX) is a commonly prescribed chemotherapeutic agent; however, it can alter liver cells, leading to liver damage and cirrhosis. Berberine and silymarin are herbal compounds with potent anti-inflammatory and antioxidant effects.

The aim of this work: Was to assess the effect of BBR versus silymarin on liver histological alterations induced by MTX.

Material and Methods: Twenty-four adult albino rats were divided into six groups. The control group was injected with 0.5ml saline intraperitoneally in the first day and administered 1ml carboxymethylcellulose (CMC 0.5%) by oral gavage daily for 7 days. The berberine treated group was given BBR (50mg/kg) dissolved in 0.5% CMC by oral gavage daily for 7 days. The silymarin treated group was given silymarin (50mg/kg) by oral gavage daily for 7 days. The methotrexate-treated group was intraperitoneally injected with a single dose of MTX (20 mg/kg). The MTX- berberine treated group was intraperitoneally injected with a single dose of MTX and BBR (50mg/kg) dissolved in 0.5% CMC by oral gavage daily for 7 days. The MTX-silymarin treated group was intraperitoneally injected with a single dose of MTX and silymarin (50mg/kg) by oral gavage daily for 7 days. Liver histological changes were assessed histologically (by light microscope and electron microscope) and biochemically.

Results: MTX administration significantly increased serum liver enzymes, malondialdehyde, catalase and superoxide dismutase while it decreased total protein level, glutathione and glutathione reductase levels. Microscopically, it induced histological damage. Both BBR and silymarin ameliorated the histological and biochemical parameters to nearly the control state. silymarin had a superior protective effect on hepatocyte ultrastructure.

Conclusion: BBR and silymarin proved a protective effect against MTX-induced histological impairment in the liver of albino rats.

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Key Words: BBR, electron microscope, liver, MTX, silymarin.

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INTRODUCTION

The liver, which is the body's largest metabolic organ, is responsible for altering and eliminating synthetic materials, which can cause its malfunction. Overdoses of certain medications, or even the approved therapeutic doses, are responsible for this devastation[1]. Several studies have linked oxidative stress to the pathogenesis of liver disorders involving chronic hepatitis, fibrosis, fatty liver, hepatocellular carcinoma and cirrhosis[2].

Methotrexate (MTX) is a popular treatment for malignancies like leukemia. It can inhibit dihydrofolate folate reductase enzyme. As an immunosuppressive medication, it also treats systemic lupus erythematosus and rheumatoid arthritis. It is possible for MTX to have several side effects, like fever, nausea, vomiting, and diarrhea because it changes the metabolism of folate and delays synthesis of DNA. Toxicity of MTX has been demonstrated to have an impact on the heart, kidneys, and liver. A notable side effect of MTX is drug-induced liver failure, which restricts its therapeutic use[3].

Hepatic damage in MTX-treated rats was attributed to hepatic oxidative stress and a decline in antioxidants. Studies have found a connection between mitochondrial dysfunction, and reactive oxygen species (ROS). ROS makes nuclear factor-kappa B (NF-kB) to become active, that causes the release of inflammatory mediators. MTX induced hepatic damage was found to occur through liver inflammation and death of hepatocytes as well as oxidative stress[4]. MTX is known as a profibrotic drug which stimulates the hepatic stellate cells. Smooth muscle actin (SMA), a marker of hepatic stellate cell activation and fibrosis, is a result of increased extracellular matrix and collagen deposition, which accelerate developing hepatic stellate cells and ultimately result in cirrhosis[5].

Berberine (BBR) is an isoquinoline alkaloid produced from Rhizoma coptidis, a Chinese medicinal herb that has been used for many years in Chinese medical world. BBR is well-known for its function in metabolic disorders and other inflammatory ailments[6]. BBR possesses a diverse set of pharmacological activities; anti-inflammatory, antioxidant,

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hepatoprotective, antidiabetic, cardioprotective[7], and neuroprotective effects[8].

Silymarin is a polyphenolic compound found in the Silybum marianum plant that has been proven to have antioxidant, anti-fibrotic, and anti-inflammatory characteristics. It exhibits significant hepato and neuroprotective properties[2]. The antioxidant, membrane stabilizing, and increasing hepatocyte protein synthesis capabilities of silymarin contribute to its anti-hepatotoxic action[9]. This work aimed to assess the impact of BBR versus silymarin on histological liver alterations experimentally induced by MTX in male albino rats.

MATERIALS AND METHODS

Animals and Treatments

Twenty-four adult male albino rats (Sprague-Dawley strain) weighing (200-250 g) and aging 10-12 weeks were kept in an animal house at a constant temperature of 22±2°C with a 12-hour light-dark cycle in this study. They have unlimited access to water and food. All tests were performed in line with the Animal Care and Use Committee's recommendations at Beni-Suef University (BSU-IACUC) (Approval No. 021-155).

Chemicals

MTX was purchased from (RAMCO Company, Cairo, Egypt). BBR was obtained from (Sigma Company, St. Louis, MO, USA). Silymarin was purchased from Medical Union Pharmaceutical Company, Pharma, Cairo, Egypt as Hepaticum. BBR was dissolved in dissolved in 0.5 percent CMC for use.

The experimental groups

Randomly, the rats were subdivided into six groups of four animals for every group, as summarized in (Table 1). Control group (Group I), rats injected 0.5 saline as a single IPI on the first day and 1ml carboxymethylcellulose (CMC-0.5%) using oral gavage daily for seven days[10]. BBR-treated group (group II) given 1ml BBR (50 mg/kg body weight) through oral gavage daily[10]. Silymarin group (group III) given 1 ml Silymarin (50 mg/kg body weight) using oral gavage daily[11]. MTX-treated group (group IV) given 0.5 ml MTX (20 mg/kg body weight) single IPI[10]. MTX + BBR-treated group (group V) given MTX as a single IPI dose and BBR orally daily. MTX+ Silymarin-treated group (group VI) given 0.5 ml MTX as a single IPI and Silymarin orally daily. After 7 days of therapy, blood was collected for serological assessment then the animals were sedated with urethane (40mg/ml), sacrificed and their livers were obtained. Every liver was splitted into two parts; a part stored in -20°C for biochemical assessments and the other part processed for histological and ultrastructural examinations.

Biochemical study

Diamond assay kits were used to assess various parameters in the serum or in the liver tissue homogenates of the studied rat groups. The techniques were performed in the Department of Biochemistry, Faculty of Veterinary Medicine, Beni Suef University as per the respective manufacturer guidelines. Serological assessment of liver function tests; serum ALT, AST, ALP, and total protein (Biodiagnostic, Cairo, Egypt)[12]. Frozen liver tissues were homogenized (0.5 g of the liver in 5 ml of normal saline) by homogenizer (Ortoalresa, Spain). The homogenates were centrifuged for 15 minutes at 1000 X g. The supernatant was collected in Eppendorf tubes and stored in the deep freezer at - 20°C for biochemical analysis. Glutathione reductase (GR), glutathione (GSH), catalase, malondialdehyde (MDA), and superoxide dismutase (SOD) were estimated in tissue homogenates by bio diagnostic colorimetric kits (Biodiagnostic, Cairo, Egypt)[12].

Histological study

For histological analysis, the liver was perfused and immersed in a fixative solution (10 percent neutral-buffered formalin) for 24 hours, whereby the samples were processed for paraffin embedding. Blocks were sectioned into 5 µm thick serial sections and stained with H&E and Masson's trichrome[13, 14]. This study was done in the Department of medical histology and cell biology, Faculty of Medicine, Beni Suef University.

Ultrastructural study

Small sections of rat liver were fixed for 3 hours at 4Co in 3% glutaraldehyde in sodium phosphate buffer (200 mM, pH 7.2), then postfixed for 1 hour in 1 percent osmium tetroxide (cold). After being dehydrated in ascending sequences of ethanol solutions (70%, 80%, 90%, 95% and 100%) the tissue specimens were treated with acetone for 1 hour and then embedded with Araldite. Then, the blocks were sectioned and cut by the Leica EM UC6 ultramicrotome. Ultrathin sections (80–100 nm) were picked onto 200-mesh Cu grids and double-stained with 4% uranyl acetate (15 min) and 1 percent lead citrate (15 min) (2 min). Stained grids were investigated utilizing a transmission electron microscope (Jeol JEM 1011) at 80 kV[15]. This study was done in the Faculty of Agriculture, Mansoura University.

Morphometrical study

The area percent of stained collagen fibers in the liver sections of the studied groups was estimated by the Leica Qwin 500 image analyzer computer system at Beni-Suef University's Faculty of Veterinary Medicine (Leica Imaging Systems, Cambridge, England). The image analyzer involved a colored display, a Panasonic WV. GP 210 color video camera, and a Leica IBM personal computer hard drive coupled to an Olympus BX41 microscope (Tokyo, Japan) and controlled using Lecia Qwin 500 software. The measurements were performed in binary mode in 10 high power fields (HPF) of the studied groups.

Statistical analysis

We used SPSS software, version 16 to perform statistical analysis. Data were represented as a mean ±standard deviation. The one-way analysis of variance (ANOVA) was utilized to compare the rat groups. Significance was considered for p-values less than 0.05[16].

RESULTS

Histological Results:

Haematoxylin and Eosin

Microscopic analysis of liver sections from group I (Control group) revealed normal liver architecture, normal morphology of the central vein, and polygonal hepatocytes arranged in radiating cords (Figure 1A). There were no histopathological alterations in groups II (BBR group) (Figure 1B) or III (Silymarin group) (Figure 1C). Histopathological abnormalities in the liver tissues of group IV (MTX group) included Dilatation and congestion of blood sinusoids with mononuclear cell infiltration. Most of hepatocytes showed cytoplasmic rarefaction. Some hepatocytes showed dense eosinophilic cytoplasm and pyknotic nuclei and others showed karyolysis. Some areas showed complete resolution of hepatocytes. (Figures 2A, 2B & 2C). Concomitant injection of BBR with MTX medication in group V and silymarin with MTX medication in group VI showed remarkable restoration of the hepatic architecture with normal radiating cords of polygonal hepatocytes (Figures 3A & 3B).

Masson's trichrome

In the Masson's trichrome-stained liver sections of groups I (control group), II (BBR group), and III (silymarin group), the quantity of collagen fiber in the portal tracts was negligible (Figures 4A, 4B & 4C). MTX therapy resulted in an apparent increase in collagen deposition surrounding the portal tract in group IV liver sections (Figure 4D). On the contrary, collagenous fibers deposition surrounding the portal tract was reduced when BBR was given concurrently with MTX medication in group V, and silymarin was given concurrently with MTX medication in group VI (Figures 4E & 4F) (Table 4), (Histogram 1).

Transmission electron microscopic Findings:

According to results of the sections of Groups I (Control group), II (BBR group), and III (Silymarin group), hepatocytes were normal with euchromatic nuclei and prominent nucleoli. Distinct cytoplasmic organelles like rough endoplasmic reticulum, and mitochondria were seen (Figure 5). Hepatocytes of group IV (MTX group) displayed dark pyknotic irregular nucleus, cytoplasmic vacuolations and dilatation of rough endoplasmic reticulum cisternae (Figure 6). Interstitial fibroblast immersed in an abundant bundles of collagen fibers also noticed. Von Kupffer cell showed an irregular heterochromatic nucleus and cytoplasm with marked vacuolation (Figure 7). Ultrathin sections of Group V (MTX + BBR group) demonstrated a reduction in the histological alterations caused by MTX, with hepatocytes resembling the control, while others indicated some mitochondria with loss of cristae, and little affection of rough endoplasmic reticulum and presence of few cytoplasmic vacuoles (Figure 8A). Ultrathin sections of Group VI (MTX + Silymarin group) revealed normal ultrastructure of hepatocyte with normal mitochondria and normal rough endoplasmic reticulum but with few cytoplasmic vacuoles (Figure 8B).

Biochemical results:

Examination of liver enzymes (AST, ALT, and ALP) and protein content in the serum of rats from the six groups revealed that the MTX administered group had a significantly greater level of liver enzymes (*P-value* less than 0.05) than the control, MTX+BBR, and MTX + Silymarin administered groups. In contrast, the MTX-treated group had a significant drop in total proteins (*P-value* greater than 0.001) when compared to the other three groups. No significant differences in biochemical features were found between the MTX+BBR, MTX + Silymarin, and control groups (Table 2). The concentration of lipid peroxidation "MDA" and antioxidant parameters "GSH, catalase, GR, and SOD" in liver tissue homogenates revealed a significant reduction in GSH and GR action (*P-value* less than 0.05) in the MTX administered group contrasted to the other three groups. In contrast, the MTX-treated group had a substantial rise in MDA, catalase, and SOD (*P-value* less than 0.05) as contrasted to the other three groups (Table 3).

Morphometric results:

Morphometric assessment of collagen deposition demonstrated a significant ($P<0.05$) improve in MTX-treated group compared to control group). Interestingly, MTX + BBR-treated and MTX + Silymarin-treated groups reported a significantly ($P<0.05$) reduction in collagen content by comparison to the MTX-treated group. There was no significant difference between MTX + BBR-treated and MTX + Silymarin-treated groups. Moreover, there was no significant difference between groups BBR-treated, Silymarin-treated groups and the control group. Data were summarized in (Table 4).

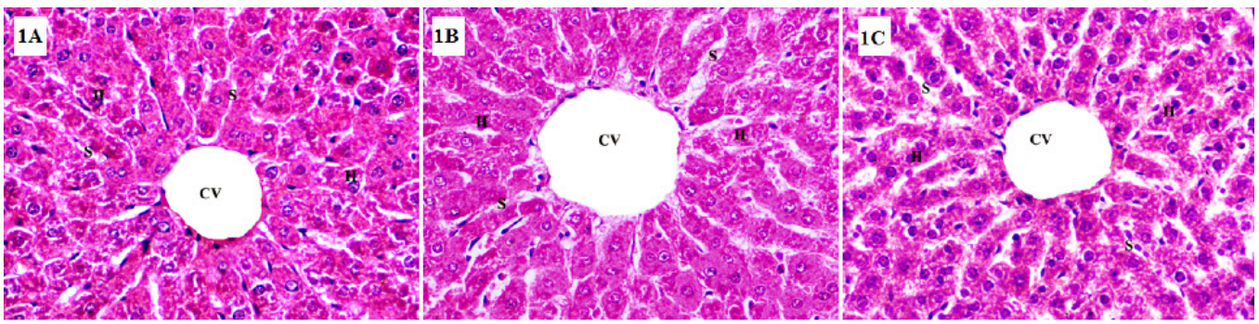


Fig. (1A): photomicrograph of the liver of Group I (control group) displaying the normal histological structure of the liver. Hepatocytes (H) are radiating from the central vein (CV). Blood sinusoids are present between the cords of hepatocytes. (HE x400).
Fig. (1B): photomicrograph of the liver of Group II (BBR group) displaying the normal architecture of the liver. Hepatocytes (H) are radiating from the central vein (CV). Blood sinusoids are present between the cords of hepatocytes. (HE x400).
Fig. (1C): photomicrograph of the liver of group III (silymarin group) displaying the normal architecture of the liver. Hepatocytes (H) are radiating from the central vein (CV). Blood sinusoids are present between the cords of hepatocytes. (HE x400).

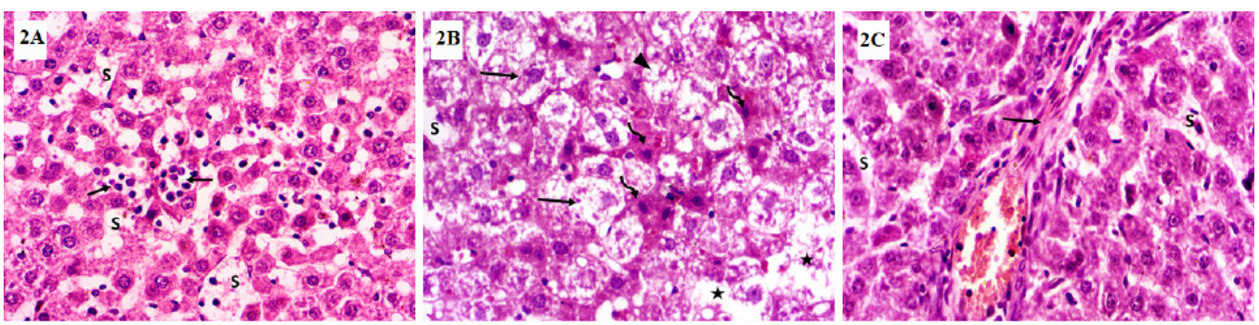


Fig. (2A): photomicrograph of the liver of group IV (MTX group) showing dilated hyperemic blood sinusoids (S) with mononuclear cell infiltration (arrow). (HE x400).
Fig. (2B): photomicrograph of the liver of group IV (MTX group) showing Hepatocytes with cytoplasmic rarefaction (arrow), Some hepatocytes showing dense eosinophilic cytoplasm with pyknotic nuclei (curved arrows) while others showing karyolysis (arrowhead). Dissolution of hepatocytes can be seen (asterisk). (HE x400).
Fig. (2C): photomicrograph of the liver of group IV (MTX group) showing thickened connective tissue septa (arrow). (HE x400).

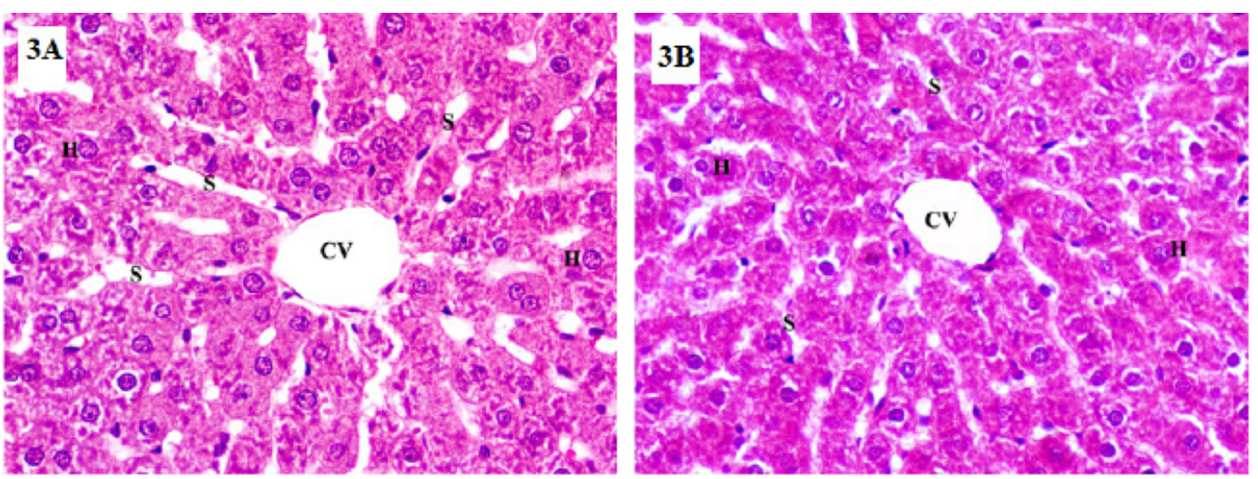
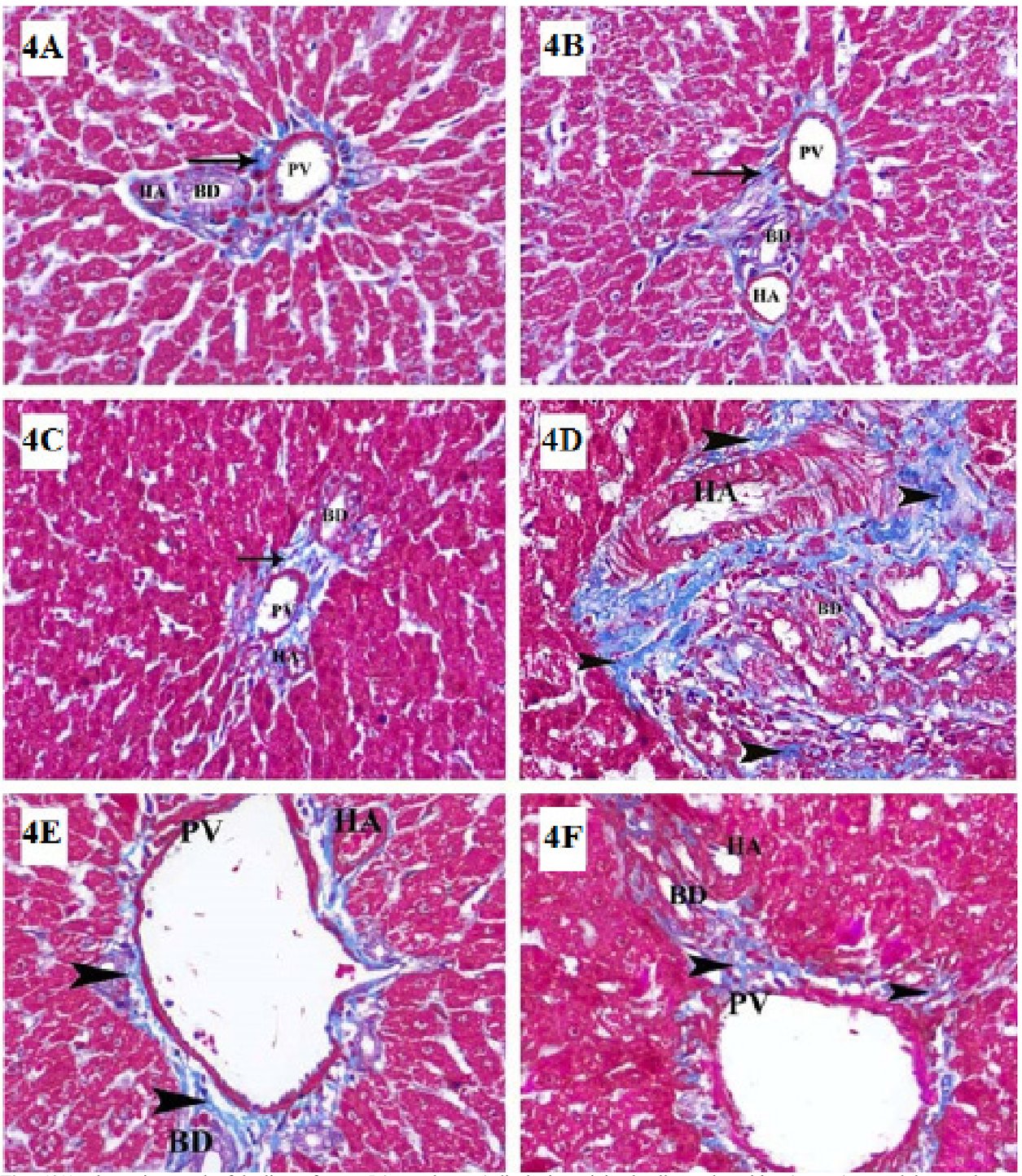


Fig. (3A): photomicrograph of the liver of group V (BBR + MTX group) displaying normal hepatocytes (H) radiating from the central vein (CV). Blood sinusoids (S) are present between the cords of hepatocytes. (HE x400).
Fig. (3B): photomicrograph of the liver of group VI (Silymarin + MTX group) displaying normal hepatocytes (H) are radiating from the central vein (CV). Blood sinusoids (S) are present between the cords of hepatocytes. (HE x400).



Figs. (4A): photomicrograph of the liver of group I (control group) displaying minimal collagen deposition (arrows) around the portal area. (Note: portal vein (PV), hepatic artery (HA) & bile duct (BD). (Masson's Trichrome x400).
Figs. (4B): photomicrograph of the liver of group II (BBR group) displaying minimal collagen deposition (arrows) around the portal area. (Note: portal vein (PV), hepatic artery (HA) & bile duct (BD). (Masson's Trichrome x400).
Figs. (4C): photomicrograph of the liver of group III (silymarin group) displaying minimal collagen deposition (arrows) around the portal area. (Note: portal vein (PV), hepatic artery (HA) & bile duct (BD). (Masson's Trichrome x400).
Figs. (4D): photomicrograph of the liver of group IV (MTX group) displaying massive collagen deposition (arrowheads) around the portal area. (Note: portal vein (PV), hepatic artery (HA) & bile duct (BD). (Masson's Trichrome x400).
Figs. (4E): photomicrograph of the liver of group V (BBR + MTX group) displaying moderate collagen deposition (arrowheads) around the portal area. (Note: portal vein (PV), hepatic artery (HA) & bile duct (BD). (Masson's Trichrome x400).
Figs. (4F): photomicrograph of the liver of group VI (Silymarin + MTX group) displaying moderate collagen deposition (arrowheads) around the portal area. (Note: portal vein (PV), hepatic artery (HA) & bile duct (BD). (Masson's Trichrome x400).

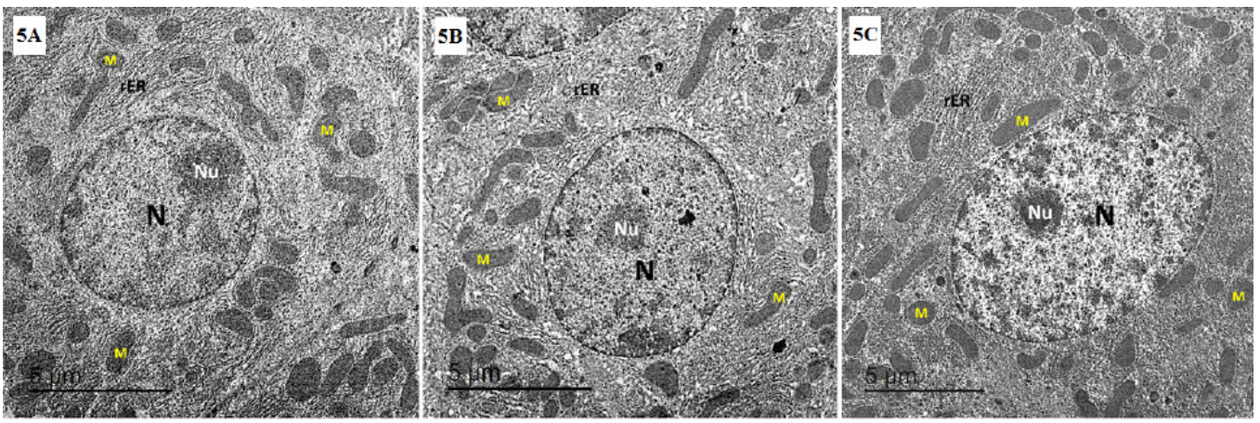


Fig. (5A): Transmission electron micrographs of rat liver of group I (control group) showing hepatocyte with a nucleus (N) has circular smooth outline and normal distribution of euchromatin and prominent nucleolus (NU), The cytoplasm contains numerous mitochondria (M). Notice the presence of well-developed parallel cisternae of the rough endoplasmic reticulum (rER). (TEM Scale Bar: 5 µm.).
Fig. (5B): Transmission electron micrographs of rat liver of group II (BBR group) showing hepatocyte with a nucleus (N) has circular smooth outline and normal distribution of euchromatin and prominent nucleolus (NU), The cytoplasm contains numerous mitochondria (M). Notice the presence of well-developed parallel cisternae of the rough endoplasmic reticulum (rER). (TEM Scale Bar: 5 µm.).
Fig. (5C): Transmission electron micrographs of rat liver of group III (silymarin group) showing hepatocyte with a nucleus (N) has circular smooth outline and normal distribution of euchromatin and prominent nucleolus (NU), The cytoplasm contains numerous mitochondria (M). Notice the presence of well-developed parallel cisternae of the rough endoplasmic reticulum (rER). (TEM Scale Bar: 5 µm.).

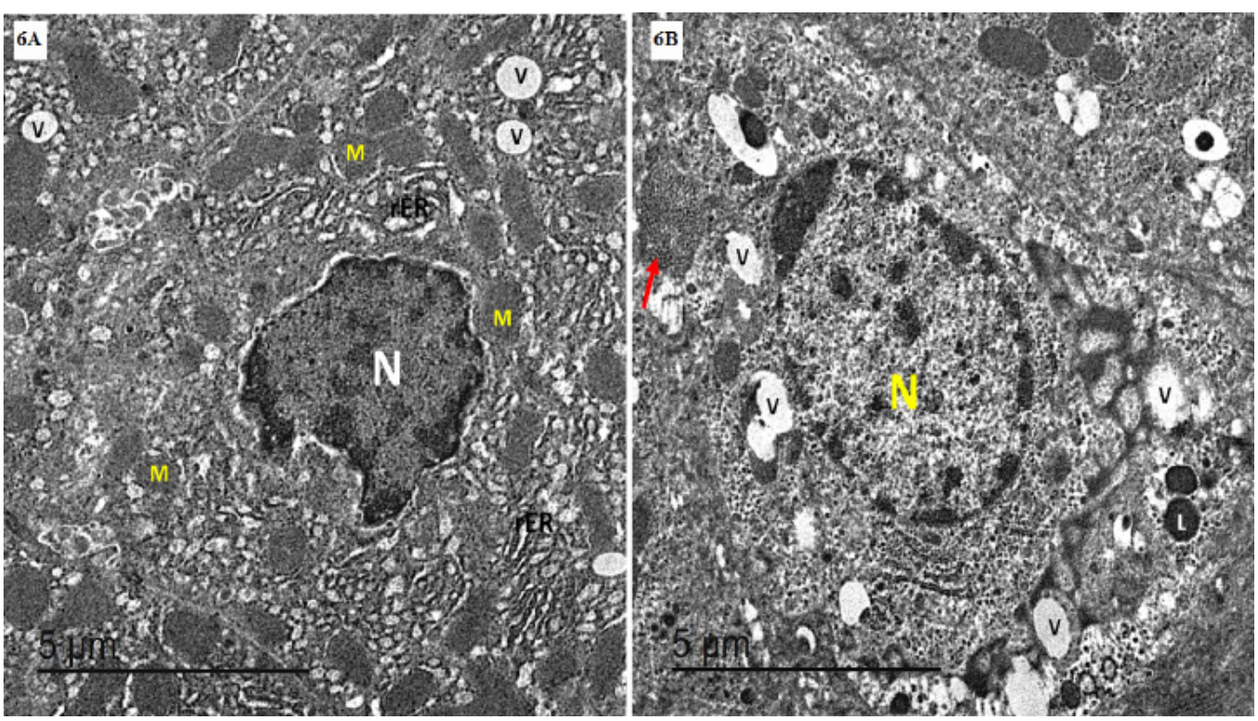


Fig. (6A): Transmission electron micrographs in rat liver of group IV (MTX group) showing hepatocyte with normal mitochondria (M) dark pyknotic irregular nucleus (N), cytoplasmic vacuolations (V) and dilatation of rough endoplasmic reticulum cisternae (rER). (TEM Scale Bar: 5 µm.).
Fig. (6B): Transmission electron micrographs in rat liver of group IV (MTX group) showing Hepatocyte with many cytoplasmic vacuolations (V). an extensive bundle of collagen fibrils (red arrow) can also be noticed. (TEM Scale Bar: 5 µm.).

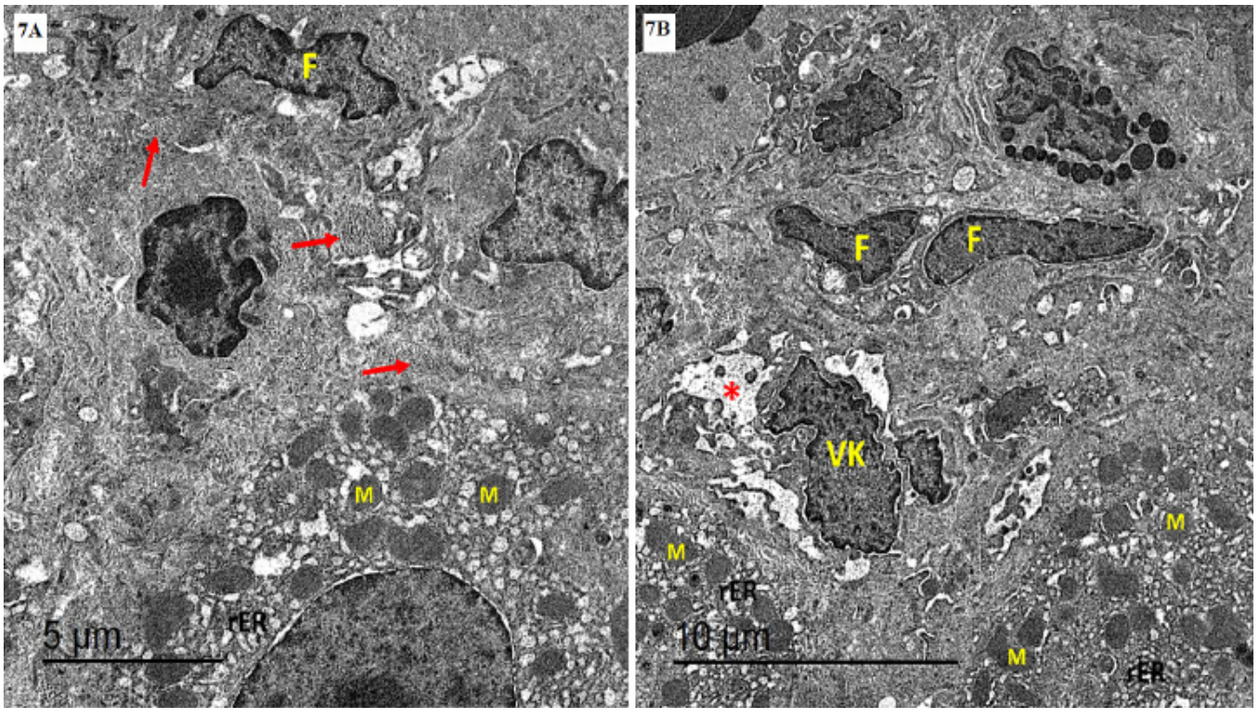


Fig. (7A): Transmission electron micrographs in rat liver of group IV (MTX group) showing hepatocyte with normal mitochondria (M) and dilated rough endoplasmic reticulum cisternae (rER). Interstitial fibroblast (F) immersed in an abundant bundles of collagen fibers (red arrow). (TEM Scale Bar: 5 µm.).

Fig. (7B): Transmission electron micrographs in rat liver of group IV (MTX group) showing Von Kupffer cell (VK) contains an irregular heterochromatic nucleus and cytoplasm with marked vacuolation (asterisk). (TEM Scale Bar: 10 µm.).

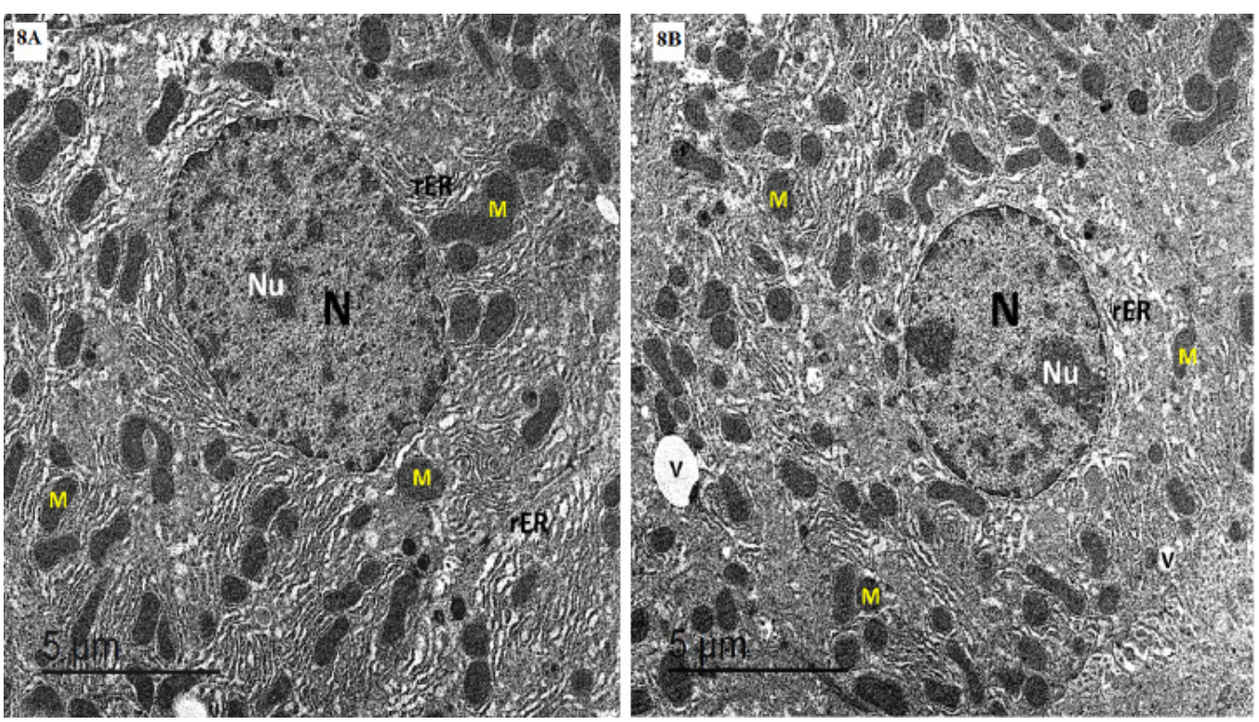


Fig. (8A): Transmission ele¬ctron micrographs in rat liver of group V (BBR + MTX group) showing nearly normal hepatocyte with nucleus (N) irregular outline and prominent nucleus (Nu). Mild dilation of rough endoplasmic reticulum (rER) and normal mitochondria (M) can be noticed. (TEM Scale Bar: 5 µm.).

Fig. (8B): Transmission ele¬ctron micrographs in rat liver of group VI (Silymarin + MTX group) showing nearly normal hepatocyte with normal mitochondria (M) and circular euchromatic nucleus (N) with prominent nucleus (Nu), and slightly dilated rough endoplasmic reticulum (RER). Few cytoplasmic vacuoles are still present(V). (TEM Scale Bar: 5 µm.).

Table 1: Schematic diagram of the study protocol

Groups	No. of rats	Drugs	Route	Dose
I	4	Saline	IPI	Single dose in the 1st day
		0.5% CMC	oral gavage	7 days
II	4	BBR	oral gavage	50 mg / kg body weight) dissolved in 0.5% CMC for 7 days
III	4	Silymarin	oral gavage	50 mg / kg body weight) for 7 days
IV	4	MTX	IPI	20 mg / kg body weight Single dose in the 1st day
V	4	BBR	oral gavage	50 mg / kg body weight) dissolved in 0.5% CMC for 7 days
		MTX	IPI	20 mg / kg body weight Single dose in the 1st day
VI	4	Silymarin	oral gavage	50 mg / kg body weight) for 7 days
		MTX	IPI	20 mg / kg body weight Single dose in the 1st day

Table 2: Changes in serum ALT, AST, ALP activities and total proteins concentration in response to MTX, BBR and Silymarin administration

Groups	ALT (U//L)	AST (IU/L)	ALP (IU/L)	TP (g/dl)
I (Control)	36.364 ± 2.73	31.3 ± 3.7	161.2 ± 0.57	6.64 ± 0.55
II (BBR)	37.43 ± 0.18	32.3 ± 2.8	187.43 ± 8.54	6.81 ± 0.59
III (Silymarin)	38.9 ± 0.45	34.52 ± 1.78	185.99 ± 8.62	6.15 ± 0.57
IV (MTX)	70.33 ± 3.5[a]	93.6 ± 7.1[a]	277.61 ± 10.56[a]	3.28 ± 0.41[a]
V (MTX + BBR)	41.5 ± 3.37[b]	48.26 ± 0.36[b]	209.42 ± 8.7[b]	6.01 ± 0.54[b]
VI (MTX + Silymarin)	42.1 ± 3.66[b]	42.3 ± 5.6[b]	201.32 ± 5.2[b]	6.05 ±0.69[b]

Values are shown as mean ± S.D.
BBR Berberine, MTX, Methotrexate, ALT Alanine Aminotransferase, AST Aspartate Aminotransferase, ALP Alkaline Phosphatase, TP Total Proteins. aSignificant difference with control group at *P<0.05*. bSignificant difference with MTX at *P<0.05*

Table 3: Changes in liver reduced glutathione (GSH), catalase (CAT) and malonaldehyde (MDA) in response to MTX, BBR and Silymarin administration

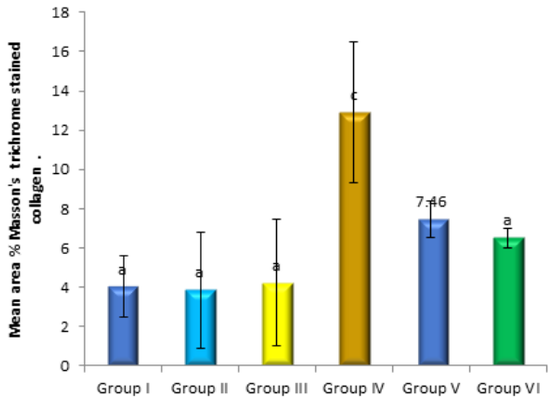
Groups	GSH-Px (U/mg protein	CAT (U/mg protein)	MDA (nmol/mg protein)	SOD (U/mg protein)	GR (U/gm tissue)
I (Control)	3.9 ± 0.39	2.21 ± 0.24	51.4 ± 3.2	3.34 ± 0.32	1.64 ± 0.11
II (BBR)	4.22 ± 0.72	2.54 ± 0.51	50.8 ± 3.5	3.77 ± 0.39	1.73 ± 0.16
III (Silymarin)	4.67 ± 0.58	3.33 ± 0.65[a]	54.4 ±1.39	4.43 ± 0.53	1.61 ± 0.09
IV (MTX)	2.12 ± 0.23[a]	1.03 ± 0.27[a]	98.5 ± 6.0[a]	1.76 ± 0.25[a]	0.68 ± 0.11[a]
V (MTX + BBR)	3.6 ± 0.48	2.11 ± 0.19[b]	63.87 ± 6.1[b]	3.12 ± 0.19[b]	1.48 ± 0.26[b]
VI (MTX + Silymarin)	3.23 ± 0.31[b]	2.84 ±0.23[b]	60.2 ± 3.9[b]	3.08 ± 0.36[b]	1.24 ± 0.12[b]

Values of SOD, GSH-Px and CAT and MDA are shown as mean ± S.D.
BBR Berberine, MTX Methotrexate, SOD superoxide dismutase, GSH-Px Glutathione peroxidase, CAT Catalase. aSignificant difference with control group at *P < 0.05*. bSignificant difference with MTX at *P < 0.05*

Table (4): Mean ± SD of the Area % of Masson's trichrome-stained collagen of the liver of the six experimental groups

Morphometrical parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Area % of Masson's trichrome stained collagen	4.04± 1.55	3.87± 2.96	4.24±3.2	12.91 ± 3.56	7.46±0.93[*]	6.52 ± 2.2[#]

• There was a significant increase in the area percentage of Masson's trichrome-stained collagen in group IV, in comparison with group I. *P value <0.5*
• There was a significant decrease in the area percentage of Masson's trichrome-stained collagen in group V in comparison with group IV. *P value <0.5*
• There was a significant decrease in the area percentage of Masson's trichrome-stained collagen in group VI in comparison with group IV. *P value <0.5*



Histogram 1: Mean area % of Masson's trichrome stained collagen

DISCUSSION

Methotrexate (MTX) is a folic acid inhibitor utilized to antagonize some tumors as an anti-inflammatory and chemotherapeutic drug. Several adverse effects have been discovered in experimental animal investigations based on the long and short-term use of MTX. The incidence of oxidative stress, which produces free radicals, has been linked to liver impairment induced by some medications and toxic chemicals[17]. The defense system of cells is strengthened by exogenous antioxidant intervention, which enables it to counteract these adverse effects at the cellular level[18]. As a result, this study assesses the possible hepatoprotective role of silymarin and berberine (BBR) in reducing the hepatotoxic effect of MTX in experimental rats. We assessed the histological, biochemical, and ultrastructural alterations for this reason.

In the current study, 20 mg/kg of MTX intraperitoneally was enough to establish MTX-induced hepatotoxicity model, in agreement with previous studies[10,19,20].

Microscopic examination of the liver of the MTX group showed hepatocyte degeneration, cellular infiltration, blood sinusoidal dilatation and congestion. Collagen in the portal tract was increased. The markers of oxidative stress, and serum levels of liver enzymes also markedly increased. On the other hand, adding BBR and silymarin to MTX improved the histological architecture of the liver and biochemical abnormalities. Serum AST and ALT values are frequently employed as hepatic damage markers because they reflect cellular leakage of intracellular enzymes and lack of liver cell membrane stability[17]. The destruction of hepatocytes produced by MTX resulted in a significant increase in AST and ALT ($P < 0.05$) in MTX group. This agrees with previous studies that denoted an increase in ALT and AST in rats given 50 mg/kg of MTX[21,22].

Peroxidation of membrane-bound lipids is promoted by cellular oxidative injury, and the toxic products cause macromolecule damage. Decreased SOD and GSH were utilized as indicators of hepatoprotection for cells in this study, while the quantity of MDA was used as a lipid peroxidation indicator. The higher concentration of MDA in rat liver tissues, as well as lower amounts of both GSH and SOD, indicate that lipid peroxidation is accelerated, culminating in tissue dysfunction and a lack of the body's antioxidant defense mechanisms to avoid excessive free radical production. MTX hepatotoxicity has been implicated in suppression of folic acid conversion to tetrahydrofolate[23]. MTX toxicity is caused by several mechanisms[24]. One of the underlying mechanisms in MTX-induced damage in the liver and other organs is oxidative stress[21]. Furthermore, MTX induces disbalance between oxidant-antioxidants levels, it reduces antioxidants such as GSH and SOD, and increases oxidants like MDA and myeloperoxidase (MPO) in hepatic and renal tissues[25]. Furthermore, MTX induces residual oxygen radicals which cause oxidative DNA damage with subsequent cell damage[26]. Therefore, there are several evidence indicating the MTX-induced toxicity could be antagonized by exogenous antioxidant supplement.

According to our findings, BBR and silymarin treatment restored all alterations in liver enzymes, oxidative enzymes, and antioxidants. The capacity of BBR to alleviate the detrimental effects of MTX is suggested by the considerable reduction in enzyme activities of rats treated with it. Reduced transaminase levels indicate that the plasma membrane integrity has been restored and that hepatocytes have been protected from hepatotoxin injury. This is consistent with the belief that serum transaminase levels return to normal as hepatocytes regenerate and the hepatic parenchyma heals[27].

Additionally, BBR significantly hindered the MTX-induced decrease in the protective enzyme SOD's level. It has been extensively demonstrated that SOD can prevent tissue peroxidation of lipids. This drop in the level of antioxidants leads to increased DNA oxidation in the cells, which enhances mutagenic damage development and cell proliferation in the target organs[28].

In our study, histological results obtained from MTX group including dilatation, congestion in the hepatic sinusoids, inflammation, hepatocyte degeneration, vacuolization, and fibrosis were supported by biochemical results. Our light microscopic findings were validated by electron microscopic results. Hepatocytes had vacuolated cytoplasm, swollen mitochondria, and dilated rough endoplasmic reticulum. The nuclei were irregular and indented. Interstitial fibroblasts and collagen fibers were in abundant extracellular matrix. These results agree with previous studies which declared that MTX causes focal necrosis, dilatation in the central vein, accompanied by inflammatory cells, many bodies of apoptosis with dense cytoplasm and peripheralized pyknotic nuclei[29,30].

Based on Dimkpa *et al*. 2013 's research, many stress factors, such as ROS and toxins, affect the ultrastructure of mitochondria and lysosomes, disrupting their functions. The proteolytic enzymes of the lysosomes and the apoptotic enzymes of the mitochondrial matrix are released into the

cytoplasm as a result of this damage, triggering cell death and the appearance of most of the observed microscopic alterations[31].

The fact that the BBR-treated group experienced less MTX-induced liver damage suggests that BBR maintained a respectable level of hepatic integrity. The observed outcomes may be related to the growing significance of BBR as an antioxidant in the fight against MTX free radical damage mechanism. Ghareeb *et al*. 2015 reported similar results where BBR succeeded to regain serum enzymes and liver structure brought about by hepatic oxidative damage prompted by CCL4 and ascribed that impact to its its antioxidant properties[32].

In the current study inflammatory cell infiltration was seen in the liver of the MTX-treated group. As per this research, it was accounted that inflammatory cells were attracted by the cytokines secreted by the stimulated hepatocyte stem cells, and this potentially explains this result[33,34]. In contrast, liver inflammatory cell infiltration was minimal following BBR treatment. This could be due to the antioxidant effect of BBR, which causes hepatic stellate cells to secrete a small number of cytokines. These cytokines may increase the number of mononuclear and polymorphonuclear leukocytes infiltrating the tissues[33].

In Masson trichrome stained sections, there was massive deposition of the fibers of collagen within the portal area. This was attributed to the inflammatory cellular infiltration shown in MTX-treated group. This could be caused by lipid peroxidation products and reactive oxygen species, which can cause programmed cell death and necrosis, as well as the initiation of a pathway that contributes to collagen deposition and fibrosis[35].

In MTX-BBR and MTX-Silymarin-treated groups deposition of collagen fibers was significantly decreased. This was attributed to the antioxidant effect of BBR and silymarin which inhibited HSCs activity[36].

CONCLUSION

It was demonstrated that BBR and silymarin improved MTX-induced impairment in hepatic architecture, as well as both recovered biochemical parameters to nearly the control state in adult albino rats, yet silymarin had a superior protective effect. These results can be applied clinically after further studies to affirm their potency against MTX-induced injury of hepatic structure. These findings, also, could encourage further studies on other natural agents that possess both anti-inflammatory and antioxidant activities against MTX-induced hepatotoxicity.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

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مقدمة: الميثوتريكسات (MTX) هو عامل علاج كيميائي موصوف بشكل شائع. ومع ذلك ، يمكن أن يغير خلايا الكبد، مما يؤدي لتلف الكبد وتليف الكبد. البربرين والسيليمارين مركبان عشبيان مع مضادات الالتهاب والأكسدة القوية تأثيرات.

الهدف من هذا العمل: تقييم تأثير BBR مقابل سيليمارين على التغيرات النسيجية للكبد التي يسببها MTX.

المواد والطرق: تم تقسيم أربعة وعشرين فأرًا بالغًا من الجرذان البيضاء إلى ست مجموعات. تم حقن مجموعة التحكم بـ ٠,٥ ملي محلول ملحي داخل الصفاق في اليوم الأول وإعطاء ١ مل كاربوكسي ميثيل سلولوز (CMC ٠,٥٪) بالتزقيم عن طريق الفم يوميًا من أجل ٧ أيام. أعطيت المجموعة المعالجة بربارين BBR (٥٠ ملجم / كجم) مذابة في CMC ٠,٥٪ بالتزقيم الفموي يومياً لمدة ٧ أيام. المجموعة المعالجة بالسيليمارين أعطيت سيليمارين (٥٠ مجم / كجم) بالتطعيم عن طريق الفم يومياً لمدة ٧ أيام. كانت المجموعة المعالجة بالميثوتريكسات يحقن داخل الصفاق بجرعة وحيدة من MTX (٢٠ ملجم / كجم). كانت المجموعة المعالجة بـ MTX- بربارين داخل الصفاق يتم حقنها بجرعة واحدة من MTX و BBR (٥٠ مجم / كجم) مذابة في CMC ٠,٥٪ عن طريق الحقن الفموي يومياً لمدة ٧ أيام. تم حقن المجموعة المعالجة بـ MTX- silymarin داخل الصفاق بجرعة وحيدة من MTX وسيليمارين (٥٠ مجم / كجم) عن طريق الحقن الفموي. يوميا لمدة ٧ أيام. تم تقييم التغيرات النسيجية في الكبد من الناحية النسيجية (بواسطة المجهر الضوئي والمجهر الإلكتروني) وكيميائيا.

النتائج: أدى إعطاء MTX إلى زيادة ملحوظة في إنزيمات الكبد في الدم، والمالونديالديهيد، والكتلاز، والأكسيد الفائق. ديسموتاز بينما قلل من مستوى البروتين الكلي ومستويات الجلوتاثيون واختزال الجلوتاثيون. مجهريا ، يسببها الضرر النسيجي. خفف كل من BBR و silymarin المعلمات النسيجية والكيميائية الحيوية إلى ما يقرب من السيطرة ولاية. سيليمارين كان له تأثير وقائي متفوق على البنية التحتية لخلايا الكبد.

الخلاصة: أثبت BBR والسيليمارين تأثير وقائي ضد التلف النسيجي الناجم عن MTX في كبد الألبينو. الفئران.