ejpmr, 2019,7(1), 43-52

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF THE POSSIBLE PROTECTIVE EFFECTS OF CAPTOPRIL AND VALSARTAN ON METHOTREXATE-INDUCED HEPATOTOXICITY IN ALBINO RATS

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Article Received on 29/10/2019 **Article Revised on 19/11/2019** **Article Accepted on 09/12/2019**

ABSTRACT

The aim of this study was to evaluate the ameliorating effect of both captopril and valsartan on methotrexate (MTX)-induced hepatotoxicity in rats. Forty two adult male albino rats weighing 180-250 g were divided into 6 groups; n= 7. Groups 1, 2, and 3 were orally received saline (control), captopril (20 mg/kg/day) and valsartan (30 mg/kg/day) respectively for 3 weeks. Groups 4, 5, and 6 were orally pretreated with saline (toxicity control), captopril and valsartan respectively in the same doses and duration as three previous groups. At the end of treatment, groups 4, 5, and 6 were subjected to single dose of MTX 5 mg/kg I.P. The impacts of MTX on the hepatic injury and its prevention by captopril and valsartan were assessed by estimation of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T.Bil), total protein and albumin. In addition, superoxide dismutase (SOD) activity, nitric oxide (NO), malondialdehyde (MDA) levels were measured in serum and liver homogenates. MTX resulted in significant increases ($P < 0.01$) in AST, ALT T.Bil, NO and MDA levels and decrease in serum albumin and protein levels and SOD activity; as well as severe congestion and inflammation in liver tissues compared to control group. On the other hand, pre-treatment with captopril or valsartan decreased MTX-induced alterations in liver function, NO, and MDA levels and SOD activity; also, ameliorated the induced changes in liver tissues. The oxidative stress plays an essential role in MTX-induced hepatotoxicity in rats. Captopril and valsartan have a protective effect on MTX-induced hepatotoxicity; which may be created by their antioxidant effect.

KEYWORDS: Captopril, Valsartan, Methotrexate, Hepatotoxicity, SOD and NO.

INTRODUCTION

Methotrexate (MTX) is 4-amino-4- deoxy-10- Methyl-Pteroyl-glutamic acid is an inhibitor of dihydrofolate reductase.[1] It is one of the most widely used drugs in the treatment of many types of cancer as osteosarcoma, Hodgkin's, non-Hodgkin's lymphomas, choriocarcinoma, head and neck cancer, breast cancer and lung cancer.[2] In addition, MTX is used in the treatment of chronic inflammatory diseases such as dermatomyositis, sarcoidosis, psoriasis and rheumatoid arthritis.[3]

There are many studies concluding that methotrexate toxicity arises from different mechanisms.[4] Some studies showed the importance of oxidative stress in the mechanism of MTX toxicity on the liver and other organs.[5] Some authors have found the suppression of antioxidant levels such as superoxide dismutase (SOD), glutathione (GSH), and the elevation of oxidant levels, such as myeloperoxidase (MPO) and malondialdehyde (MDA) in hepatic, renal and intestinal tissues.[6] Furthermore, residual oxygen radicals were shown to cause oxidative injury of DNA by reacting with it.[7][8] On the other hand, inflammation plays a role in MTX-induced hepatotoxicity where it activates the proinflammatory cytokines.[9]

Angiotensin II (Ang II), a peptide hormone, plays critical roles in the modulation of cardiovascular functions.[10] Ang II is the main effector of the renin-angiotensin system for maintaining homeostasis, as well as a powerful stimulator of NAD (P) H oxidase, which is the most important source and the main trigger for reactive oxygen species (ROS) generation in various tissues.[11]

Captopril is an angiotensin converting enzyme (ACE) inhibitor which is derivative of amino acid proline.[12] On the same point of view, captopril also has a sulfhydryl (SH) group in its molecule; where the antioxidant effect of it is related to its (SH) structural moiety that directly scavenges ROS by either hydrogen donation or electron-transferring.[13] Valsartan is a clinically used Ang II type 1 receptor subtype blocker. Valsartan has antioxidant and free radical scavenging abilities because it reverses the reduction of GSH and the decrease in GPx and SOD antioxidant activities.[14]

These findings suggest that the antioxidant and antiinflammatory agents which in the same time inhibit the Ang II predisposing effect of ROS generation may be helpful in prevention of methotrexate induced toxicity.

MATERIALS AND METHODS

Materials

Methotrexate, captopril and valsartan were purchased from the Sigma Aldrich Company, England. Kits used for measuring liver functions were obtained from Egyptian Company for Biotechnology, Cairo, Egypt. Kits for determination of (SOD, NO and MDA) were purchased from Bio-diagnostic Company Pharmaceutical Industries, Egypt.

Animals

Forty two male adult albino rats weighing 150-250 gm have been used. Animals were obtained from the animal house, Faculty of Science, Sohag University, Egypt. They were housed in animal place with room temperature being maintained at 22-24 ºC. Animals were fed on a commercial pellet diet and kept under normal light/dark cycle. Animals were given a free access to food and water up to their use. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Faculty of Medicine, Sohag University.

Experimental design

Rats were randomly divided into 6 groups, 7 animals each. Group1, 2 and 3 were treated orally with 2 ml/kg /day saline (normal control group), 20 mg/kg/day captopril[15] and 30 mg/kg/day valsartan[16] dissolved in saline for 3 successive weeks respectively. Group 4, 5 and 6 were pretreated with saline (hepatic toxicity control), captopril 20 mg/kg/day and valsartan 30 mg/kg/day; respectively. At the end of treatment period; after 24 hours of starvation, the animals of groups 4, 5, 6 were subjected to single I.P injection of 5 mg/kg MTX to induce hepatotoxicity.[17]

Blood Sampling and Serum Preparation

At the end of the treatment period, the investigated animals of all groups were fasted for 24 hours and sacrificed by decapitation. Blood samples were collected in centrifuge tubes. The serum was separated following centrifugation of blood at 3500 RPM for15 minutes using Heraeus Sepatech centrifuge (Labofuge 200, DJB Labcare Company), serum was stored quickly on -20˚c until the time of analysis for analysis of liver function, SOD, NO and MDA levels.

Tissues Sampling

Livers were quickly removed from the sacrificed rats, placed in an ice cold saline solution and blotted on filter papers and weighted, then homogenized using Glas-Col, LLC, USA motor-driven homogenizer in 10 ml ice-cold buffer (50 mM potassium phosphate pH 7.4) per gram tissue (v/w) for assay the hepatic SOD, NO and MDA levels. All homogenates were centrifuged at 4000 RPM for 15 min at 4ºC. The supernatant was removed and kept on -20ºC until the time of analysis.

Calculation of liver index

The liver index was calculated according to the formula: (rat liver weight / rat' weight) 100%.[18]

BIOCHEMICAL ANALYSIS

Determination of liver function

AST, ALT, T.Bil, total proteins and albumin levels in serum were assayed by using a commercial kit according to the method of **Bergmeyer et al.**[19]

Determination of serum and hepatic SOD enzyme activities

The activity of SOD in serum and tissue homogenate was determined by a calorimetric method using commercially available kits. The method was described by Nishikimi et al.[20] This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium (NBT) dye. The change in the absorbance was measured at 560 nm for control and sample at 25 ºC. A SOD enzyme activity was expressed in the serum and tissues in U/ml and U/g respectively.

Determination of serum and hepatic NO level

The level of NO was determined by a calorimetric method using commercially available kits according to the method of **Montgomery and Dymock.**[21] This assay depends on that, in an acid medium and in the presence of nitrite the formed nitrous acid diazotize sulphanilamide the product is coupled with N-(1-naphthyl) ethylenediamine. The resulting azo dye has a bright reddish- purple color which was measured at 540 nm. The level of NO was expressed in serum and liver by nmol/ml and nmol/g tissue respectively.

Determination of lipid peroxidation level

MDA level is an indicator of lipid peroxidation. MDA in serum and the liver tissue homogenate was determined by a calorimetric method using commercially available kits, as described by **Ohkawa** et al.[22] The principle of the method is based on spectrophotometric measurement of the color formed during the reaction of a thiobarbituric acid with MDA. The absorbance of the resultant pink product was measured at 534nm. MDA level and expressed in nmol/ml in serum and nmol/g in liver tissues.

Histopathological examination

Livers were fixed in formalin, and embedded in paraffin block. The samples were cut into 5 µm sections and were stained with hematoxylin and eosin and examined by light microscope.[23]

Statistical analysis

The results were presented as mean values ±SEM. The SPSS version 22.0 was used in statistical analysis. Biochemical markers among different groups were compared using the one-way ANOVA test. When a

significant difference was observed, post hoc Tukey test analysis was carried on for multiple comparisons. A difference was considered significant when the $p < 0.05$.

RESULTS

Changes of liver index in rats

The liver index revealed a significant increase ($p<0.01$) in MTX-treated group compared to the normal control group. On the other hand, pretreatment of rats with captopril or valsartan for 3 weeks prior to MTX, the liver index decreased significantly ($p< 0.01$) compared to MTX-treated group (Fig. 1).

Changes in liver function tests

Table (1) showed that administration of MTX in a single I.P dose of 5 mg/kg B.W. caused hepatotoxicity in rats as indicated by a significant increase ($p< 0.01$) in serum AST, ALT and T.Bil levels and a significant decrease ($p< 0.05$) in total protein and albumin, all compared to the control group. Whereas, animals pre-treated with captopril in a dose of 20 mg /kg/ day or valsartan in a dose of 30 mg /kg/ day for 3 weeks before MTX exhibited a significant decrease ($p< 0.01$) in the levels of these tests and significant elevation ($p< 0.05$) in total protein and albumin compared to MTX group.

Changes in serum and hepatic SOD activity

A significant decrease ($p< 0.01$) in serum and hepatic SOD activity showed in groups treated with MTX alone compared to the normal control group. However, groups pre-treated with 20 mg/ kg/ day captopril or 30 mg/ kg/ day valsartan for 3 weeks plus MTX 5 mg /kg single dose showed a significant promotion ($p< 0.01$) towards the control level (Fig. 2 and 3).

Changes in serum and hepatic NO levels

A significant elevation ($p< 0.01$) in serum and hepatic NO levels occurred in animals treated with MTX alone compared to the normal control group. On the other hand, animals pre-treated with 20 mg/ kg/ day captopril or 30 mg/ kg/day valsartan for 3 weeks plus MTX 5 mg /kg single dose showed a significant decrease ($p< 0.01$) towards the control level (Fig. 4 and 5).

Changes in serum and hepatic lipid peroxidation levels

Figure (6) and (7) revealed a significant increase ($p< 0.01$) in serum and hepatic MDA were showed in groups treated with MTX alone compared to the normal control group. However, groups pre-treated with 20 mg/ kg day captopril or 30 mg/ kg/ day valsartan for 3 weeks before MTX 5 mg /kg single dose showed a significant cessation ($p< 0.05$) towards the control level.

Histological Analysis of liver tissues

Light Microscopic Results

Group treated with saline, captopril or valsartan

Liver tissue histopathology was assessed in these groups revealed a normal liver parenchymal architecture (Fig 8 a, b & c).

MTX-treated group

In contrast, liver sections of rats treated with single dose MTX (5 mg/kg i.p) showed marked dilatation and congestion of central vein (CV) and portal vein (PV), multiple patchy necrotic foci of hepatocyte within the liver lobules, multiple foci of mononuclear cell infiltrate within liver lobules and portal areas and feathery degeneration of hepatic cells (Fig 9 a, b & c).

Group pretreated with captopril and MTX

The liver sections of rats pretreated with captopril 20 mg/kg orally for 3 weeks before I.P injection of a single dose of MTX (5 mg/ kg) revealed mild to moderate dilatation and congestion of central vein (CV) and portal vein (PV). The histopathological changes developed in the liver after single dose administration of MTX were ameliorated by pretreatment with captopril (Fig 10 a & b).

Group pretreated with valsartan and MTX

The liver sections of rats pretreated with valsartan 30 mg/ kg orally for 3 weeks before i.p injection of single dose MTX 5mg/kg illustrated mild dilatation of CV and PV. The histopathological changes developed in rat liver after single dose administration of MTX were almost disappeared by pretreatment with valsartan (Fig 11 a & b).

RESULTS

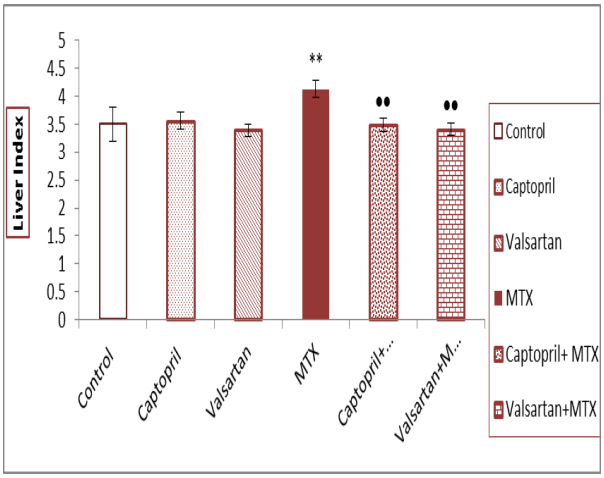

Figure (1): Effect of oral administration of captopril (20 mg/kg/day) or valsartan (30 mg/kg/day) for 3 weeks on liver index in I.P methotrexate-induced liver toxicity in albino rats. Each value represents the mean ±SEM.

** Significant at ($p<0.01$) vs. control group.

•• Significant at ($p<0.01$) vs. MTX-treated group.

Data were analyzed by one way ANOVA followed by post hoc Tukey's test.

Table (1): Effect of oral administration of captopril (20 mg/kg/day) and valsartan (30 mg/kg/day) for 3 weeks on liver function tests in I.P methotrexate-induced liver toxicity in albino rats.

Groups	Control	Captopril	Valsartan	MTX	Captopril + MTX	Valsartan + MTX
AST (U/L)	16.50 ± 3.83	15.50 ± 2.74	17.00 ± 3.46	103.15 ± 15.06 **	24.50 ± 4.93 ••	18.00 ± 4.38 ••
ALT (U/L)	27.50 ± 3.83	27.17 ± 8.50	23.67 ± 6.71	105.83 ± 18.06 **	32.67 ± 11.88 ••	30.17 ± 9.50 ••
T.Bil (mg/dl)	0.70 ± 0.09	0 .70 ± 0.11	0.75 ± 0.06	1.30 ± 0.11**	0. 85 ± 0.06 *••	0.75 ± 0.06 ••
T. P (g/dl)	7.43 ± 0.33	7 .08 ± 0.38	7.65 ± 0.24	5.48 ± 0.74 **	6. 70 ± 0.21 *•	7.17 ± 0.21 •
Alb (g/dl)	3.40 ± 0.23	3 .38 ± 0.32	3.00 ± 0.32	2.25 ± 0.33 **	2. 80 ± 0.16 *•	2.95 ± 0.43 •

AST: aspartate aminotransferase, AST: alanine aminotransferase (ALT), T. Bil: total bilirubin, T.P: total protein and Alb:albumin. MTX: methotrexate.

Each value represents the mean ±SEM.

* Significant at ($p<0.05$) vs. control group.

** Significant at ($p<0.01$) vs. control group.

• Significant at ($p<0.05$) vs. MTX-treated group.

•• Significant at ($p<0.01$) vs. MTX-treated group.

Data were analyzed by one way ANOVA followed by post hoc Tukey's test.

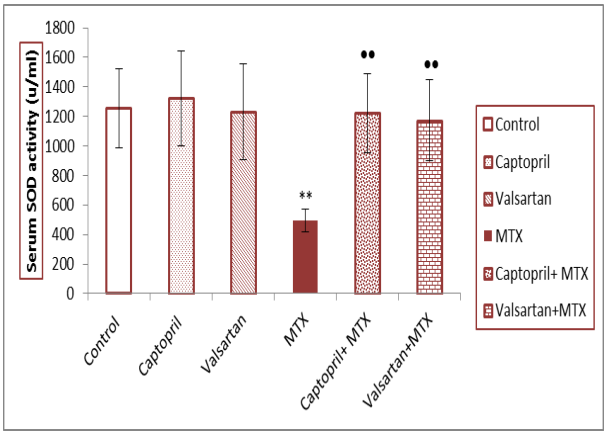

Figure (2): Effect of oral administration of captopril (20 mg/kg/day) and valsartan (30 mg/kg/day) for 3 weeks on serum SOD activity in I.P methotrexate-induced liver toxicity in albino rats.

SOD: superoxide dismutase. MTX: methotrexate.

Each value represents the mean ±SEM.

** Significant at ($p<0.01$) vs. control group.

•• Significant at ($p<0.01$) vs. MTX-treated group.

Data were analyzed by one way ANOVA followed by post hoc Tukey's test.

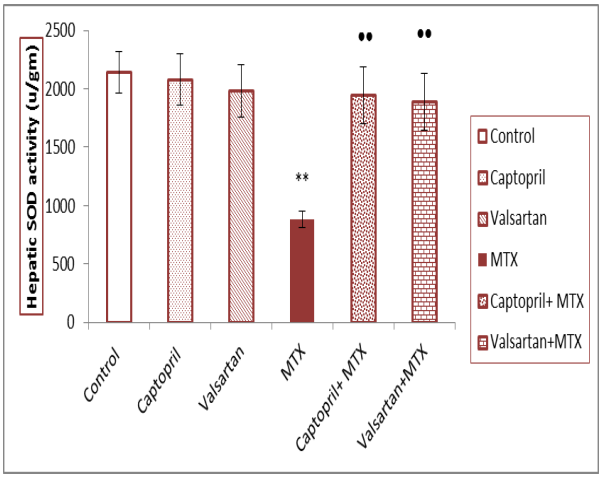

Figure (3): Effect of oral administration of captopril (20 mg/kg/day) and valsartan (30 mg/kg/day) for 3 weeks on hepatic SOD activity in I.P methotrexate-induced liver toxicity in albino rats.

SOD: superoxide dismutase. MTX: methotrexate.

Each value represents the mean ±SEM.

** Significant at ($p<0.01$) vs. control group.

•• Significant at ($p<0.01$) vs. MTX-treated group.

Data were analyzed by one way ANOVA followed by post hoc Tukey's test.

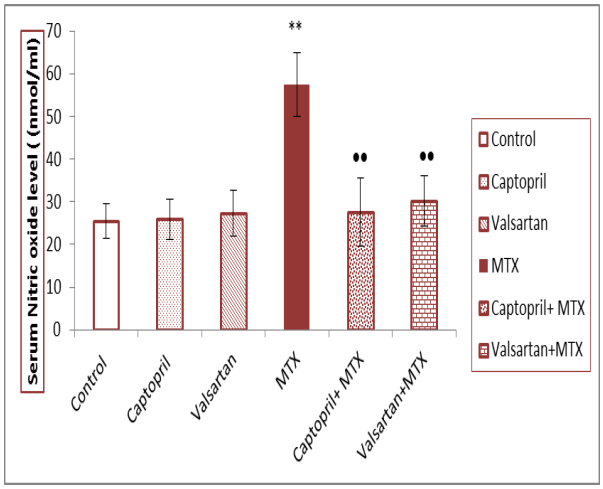

Figure (4): Effect of oral administration of captopril (20 mg/kg/day) and valsartan (30 mg/kg/day) for 3 weeks on serum NO levels in I.P methotrexate-induced liver toxicity in rats. MTX: methotrexate.

Each value represents the mean ±SEM.

** Significant at ($p<0.01$) vs. control group.

•• Significant at ($p<0.01$) vs. MTX-treated group.

Data were analyzed by one way ANOVA followed by post hoc Tukey's test.

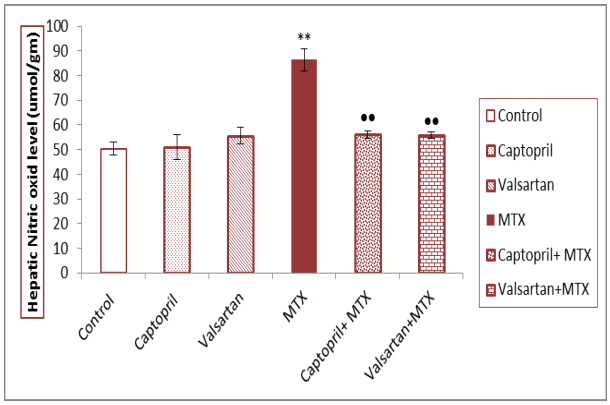

Figure (5): Effect of oral administration of captopril (20 mg/kg/day) and valsartan (30 mg/kg/day) for 3 weeks on hepatic NO levels in I.P methotrexate-induced liver toxicity in rats.

MTX: methotrexate.
Each value represents the mean ±SEM.
** Significant at ($p<0.01$) vs. control group.
•• Significant at ($p<0.01$) vs. MTX-treated group.
Data were analyzed by one way ANOVA followed by post hoc Tukey's test.

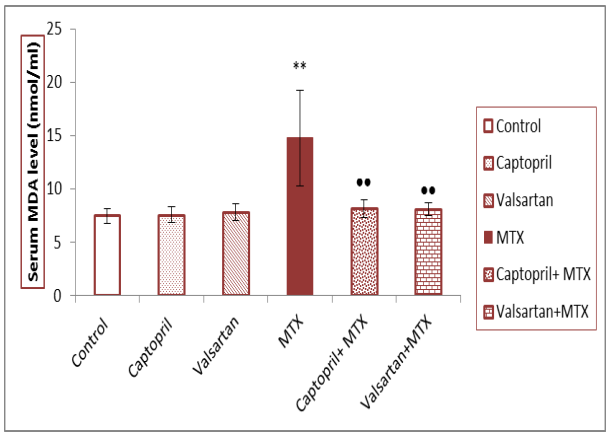

Figure (6): Effect of oral administration of captopril (20 mg/kg/day) and valsartan (30 mg/kg/day) for 3 weeks on serum MDA levels in I.P methotrexate-induced liver toxicity in rats. MDA: malondaldhyd. MTX: methotrexate.

Each value represents the mean ±SEM.
** Significant at ($p<0.01$) vs. control group.
•• Significant at ($p<0.01$) vs. MTX-treated group.
Data were analyzed by one way ANOVA followed by post hoc Tukey's test.

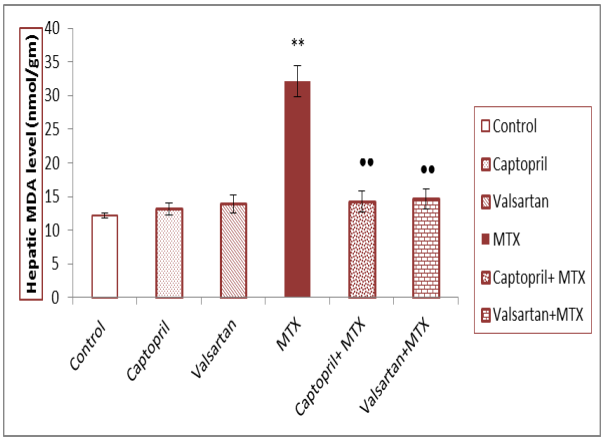

Figure (7): Effect of oral administration of captopril (20 mg/kg/day) and valsartan (30 mg/kg/day) for 3 weeks on hepatic MDA level in I.P methotrexate induced liver toxicity in rats.

Each value represents the mean ±SEM.
MDA: malondaldahyde. MTX: methotrexate.
** Significant at ($p<0.01$) vs. control group.
•• Significant at ($p<0.01$) vs. MTX-treated group.
Data were analyzed by one way ANOVA followed by post hoc Tukey's test.

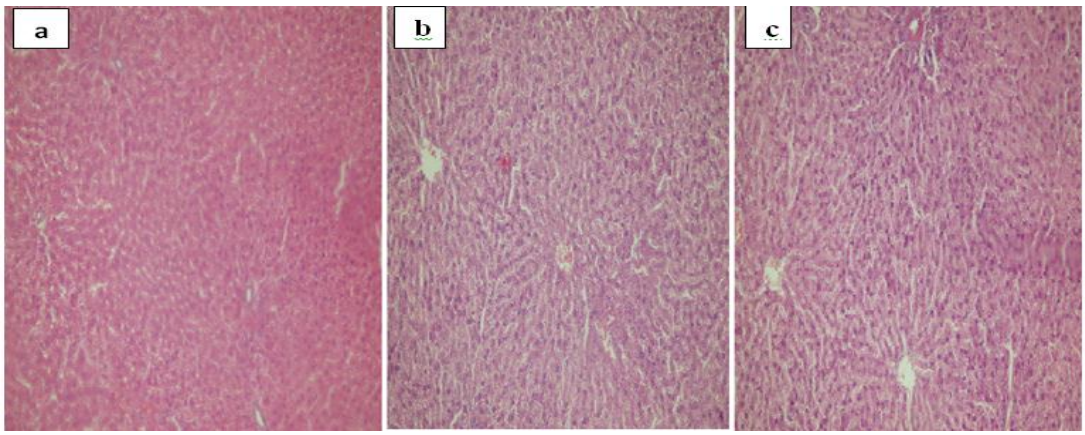

Figure (8): A photomicrographs of liver section from rats treated with saline (a), captopril 20 mg/kg orally for 10 days (b) and valsartan 30 mg/kg orally for 21 days (c) showed no pathological changes with normal liver architecture (H&E X 100).

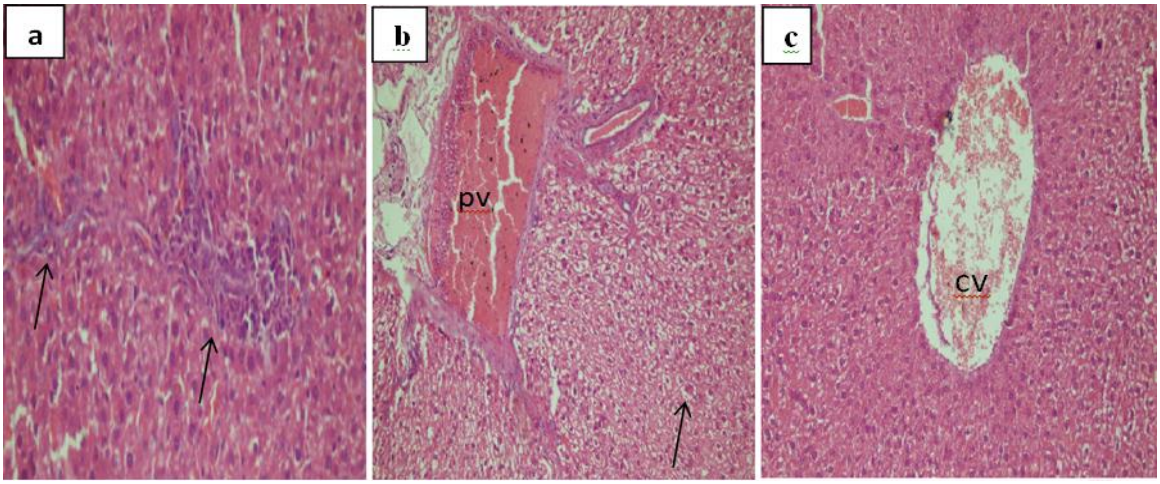

Figure (9): Photomicrographs of liver sections from rats treated with single dose MTX (5 mg/kg I.P) showed multiple foci of mononuclear cell infiltrate within liver lobules (arrows) (a), marked dilatation and congestion of portal vein (PV), multiple patchy necrotic foci hepatocyte within liver lobules and focal hydropic degeneration of hepatic cells arrow (b) and marked dilatation and congestion of central vein (CV) (c). (H&E X100).

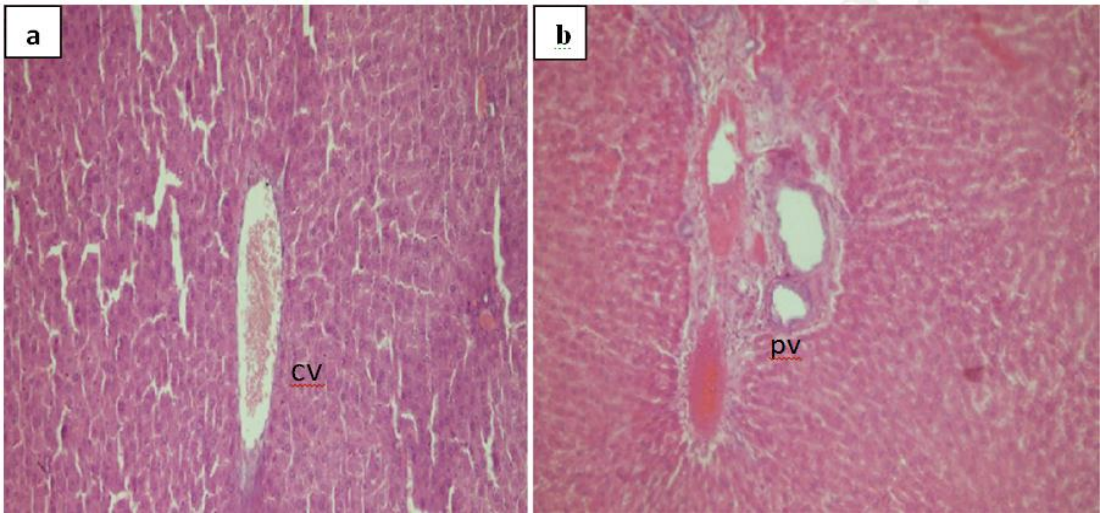

Figure (10): Photomicrograph of liver sections of rats pretreated with captopril 20 mg/kg orally for 10 successive days before I.P injection of a single dose of MTX (5 mg/ kg) showed mild to moderate dilatation and congestion of central vein (CV) (a) and portal vein (PV) (b) (H&E X100).

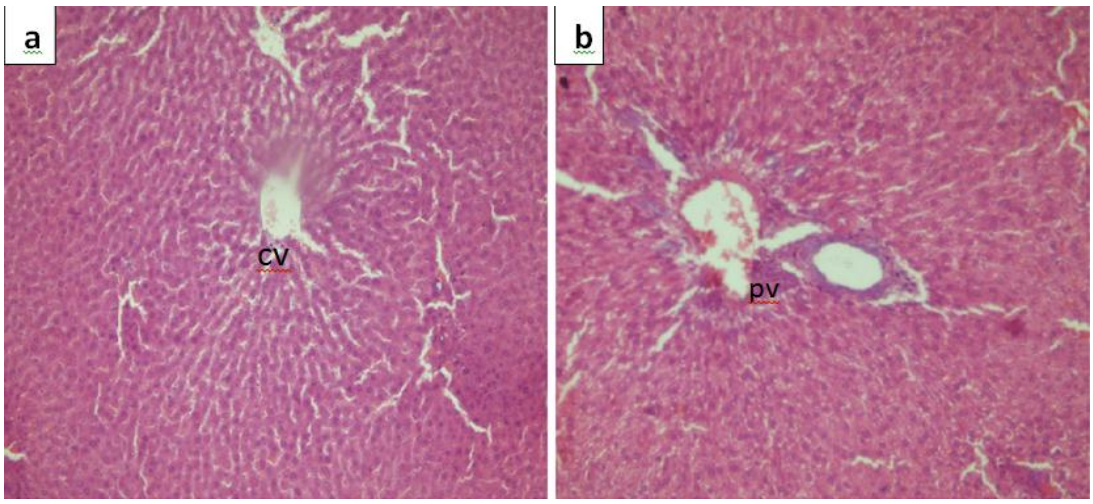

Figure (11): Photomicrograph of liver sections of rats pretreated with valsartan 30 mg/ kg orally for 21 days before I.P injection of a single dose MTX 5mg/kg showed mild dilatation of central vein (CV) (a) and portal vein (PV) (b).

DISCUSSION

It was reported that many mechanisms are involved in MTX hepatotoxicity showing that the toxicity is mediated mostly by the generation of ROS and lipid peroxidation. So, the use of antioxidant agents associated with MTX treatment could be a useful tool in attempting to reduce MTX induced side effects.[6] Our results showed that the serum levels of AST, ALT and T.Bil were highly significantly increased in MTX treated group compared with the control group and these results were confirmed by histopathological findings. This result was in agreement with[24][25][26][27][28][29] who mentioned that, I.P injection of MTX in a dose of 100 µg/kg for successive 40 days in rats induced hepatotoxicity manifested biochemically by increased serum AST, ALT, total bilirubin and alkaline phosphatase. The rise of levels of these parameters has been attributed to the damaged in the structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular damage.[30][31]

Many mechanisms for MTX induced toxicity have been postulated, where inflammation, apoptosis, activation of the NF-κB pathway and oxidative stress have all been suggested as contributing factors.[32] In addition, a reduction in hepatic folate stores by MTX is one of its possible toxic mechanisms.[33] Also, MTX indirectly affects the synthesis of thymidilate, thereby suppressing DNA synthesis.[6] Additionally, it was demonstrated that the cytosolic nicotinamide adenosine diphosphate $[NAD(P)]^2$ dependent dehydrogenases and NADP malic enzyme are inhibited by MTX, suggesting that the drug could decrease the availability of NADPH in cells.[34]

Also, the present study demonstrated that the disruption in the levels of the hepatic enzymes in MTX intoxicated rats was prevented by pretreatment with captopril or valsartan before MTX, as there was highly significant decrease in the levels of these parameters. These results coincide with, the results of.[35][36], who reported that oral administration of mice with captopril 10, 25, 50 mg/kg for three consecutive days has protective effect against Ccl_4 induced hepatotoxicity and it improved the AST and ALT levels. Also, Georgescu et al. and Rizwan et al. reveled that valsartan produced a significant decrease in AST, ALT and total bilirubin levels in hypertensive patients with nonalcoholic steatohepatitis and in gentamycin-induced nephrotoxicity in the rat model respectively.[37][38] These results mean that these parameters returned to near normal level by possibly preserving the functional integrity of the hepatocytes. As captopril or valsartan protect cell components from ROS-mediated damage and induce membrane stabilizing effect leading to decrease the levels of transaminases in serum indicating its hepatoprotective effect against MTX induced hepatotoxicity.[39]

Regarding other liver functions measured in this study, there was a significant decrease in serum level of total protein and albumin in MTX treated rats compared with the control group. Our results agree with the results of[40][41] who studied the protective effect of turmeric against MTX hepatotoxicity. The suggested mechanism may be attributed to impaired protein synthesis by damaged liver tissue and increased albumin turnover caused by its consumption at the site of inflammation[42], also due to increased renal loss of albumin due to the nephrotoxicity caused by MTX.[43] On the other hand, our results revealed that the administration of captopril or valsartan before MTX ameliorated these changes. This result is coincided with the result of.[44][45] The explanation of this effect may be due to captopril attenuates the occurrence of glomerulosclerosis and reduction of interstitial matrix content.[46] Also, valsartan inhibits the progression of renal injury by decreased albuminuria. On the other hands, it has anti-inflammatory effect that lowered urinary monocyte chemoattractant protein-1 (MCP-1); renal inflammatory marker; excretion to very low levels.[47] The renal protective effects of ARBs are mostly related to the improvement of hemodynamic status and direct blockade of prosclerotic effects stimulated by an Ang II.[48]

Antioxidant enzymes are important cellular defense system against free radical overproduction and decrease their cellular concentration.[49],[50] Our study revealed that the treatment of rats with MTX produced a highly significant decrease of SOD and highly significant increase in NO and MDA in serum and liver homogenates levels compared to control group. These results are in agreement with many studies as.[51],[52],[53],[54],[55] where MTX increase ROS and MDA production that are hallmarks of oxidative stress. Other studies revealed that lipid peroxidation and increase NO level is another important mechanism of MTX induced hepatotoxicity.[41],[56] Other reports have demonstrated the role of ROS/RNS in the pathogenesis of MTX induced hepatotoxicity. These highly reactive species react with biological macromolecules producing lipid peroxides, inactivating proteins and mutating DNA.[6],[52]

However, concurrent treatment of captopril or valsartan before MTX led to a highly significant elevation in the serum and liver homogenate levels of SOD and reduction of NO and MDA compared to MTX treated group. Also, these results were confirmed by histopathological findings. These results are in agreement with the results of[36],[57], which revealed that captopril has a hepatoprotective role on paraquat induced hepatotoxicity in isolated rat liver due to its antioxidant, free radical scavenging activities and anti-inflammatory properties. This protective effect was confirmed by[58], who studied the antioxidant effect of other members of ACE inhibitors (captopril and enalapril) on hepatotoxicity. This protective effect of captopril can be explained by may mechanisms; where it possesses antioxidative potential which might be mediated, by the limitation of free radicals and amelioration of oxidative stress through scavenging free radical because of its terminal-SH group where it might easily cross the cell membrane and cope

with the intracellular ROS formation,[59],[60] In addition, in case of treatment with valsartan the results are coincide1d with[39], who reported that damage of the liver by Ccl4 was partially abrogated by the concomitant administration of valsartan. Also the study of[16], revealed that valsartan has anti-inflammatory effect where it attenuates TNF-α and CRP expression in non-alcoholic fatty liver disease in transgenic rats. This effect can be explained by the important role of Ang II in increase the activity and expression of NADPH oxidase, which generates ROS and leads to hepatic stellate cells activation and liver fibrosis; where treatment with valsartan significantly reduces hepatic NADPH oxidase activity and expression in rats.[61],[62]

CONCLUSION

To conclude; the oxidative stress plays an essential role in MTX-induced hepatotoxicity in rats. Captopril and valsartan induces potent hepatoprotective mechanisms against MTX by reducing the production oxidant products and enhancing the activity of the antioxidant system. In addition, inhibition of RAS by either ACEI or ARBs has a protective effect on the liver by inhibiting action of Ang II, which is a powerful pro-oxidant agent in the liver Further researches will be necessary to understand the exact mechanisms by which captopril and valsartan is able to prevent hepatic damage.

ACKNOWLEDGEMENTS

We are thankful to Faculty of medicine pharmacology and pathology departments Sohag University for providing the facilities for carrying out this work.

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