DOI: https://doi.org/10.31351/vol31iss1pp87-94

The Ameliorative Effect of Fimasartan against Methotrexate-Induced Nephrotoxicity in Rats

Maryam Rasheed Abd*,1 and Ali Faris Hassan*

*Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

Drug-induced acute kidney injury is a serious disorder. Oxidative stress has a key role in its initiation and progression. In this study, the possible ameliorative effect of fimasartan against methotrexate-induced nephrotoxicity was investigated in comparison with α-tocopherol in rats. Wistar rats were allocated into six groups and treated as follows: **group I** received water on a daily basis for 8 successive days; **group II** received methotrexate (20 mg/kg) on day 1, followed by water for 7 successive days; **group III** received fimasartan (3 mg/kg/day) for 7 successive days; **group IV** received α-tocopherol (1 g/kg/day) for 7 successive days; **group V** received methotrexate (20 mg/kg) on day 1, followed by fimasartan (3 mg/kg/day) for 7 successive days; and **group VI** received methotrexate (20 mg/kg) on day 1, followed by α-tocopherol (1 g/kg/day) for 7 successive days. Finally, after euthanization of each animal by diethyl ether, the samples were collected for analysis. Administration of fimasartan and α-tocopherol resulted in a significant decline in serum creatinine and urea, a significant reduction of renal malondialdehyde, and a significant elevation of renal superoxide dismutase-1 compared to the methotrexate-treated rats. In conclusion, fimasartan has ameliorative effects, comparable to those of α-tocopherol, on methotrexate-induced nephrotoxicity in rats.

Keywords: Nephrotoxicity, Methotrexate, Oxidative stress, Fimasartan, α-Tocopherol.

التأثيرالمحسّن للفيماسارتان ضد السمية الكلوية المستحثة بواسطة الميثوتريكسيت في الجرذان

مريم رشيد عبد *،١ و علي فارس حسن *

* فرع الادوية والسموم، كلية الصيدلة ، جامعة بغداد، بغداد، العراق.

الخلاصة

الاعتلال الكلوي الحاد الناتج عن استخدام الأدوية هو اضطراب صحي خطير و للإجهاد التأكسدي دور رئيسي في حدوثه وتفاقمه. في هذه الدراسة، تم فحص التأثير المحسن المحتمل للفيماسارتان ضد السمية الكلوية المستحثة بواسطة الميثوتريكسيت مقارنة بالألفا توكوفيرول في الجرذان. تم تقسيم جرذان ويستار الى ست مجاميع وتم اعطاؤها على النحو الآتي: المجموعة الاولى تلقت الماء يوميا لمدة ٨ ايام متتالية، المجموعة الثانية تلقت جرعة واحدة من الميثوتريكسيت (٢٠ ملغم/ كغم) في اليوم الأول تلاها تلقي الماء يوميا لمدة ٧ ايام متتالية، المجموعة الثالثة تلقت الفيماسارتان (٣ ملغم/ كغم/ اليوم) لمدة ٧ ايام متتالية، المجموعة الرابعة تلقت الألفا توكوفيرول (١ غم/ كغم/ اليوم) لمدة ٧ ايام متتالية، المجموعة الخامسة تلقت جرعة واحدة من الميثوتريكسيت (٢٠ ملغم/ كغم) في اليوم الأول تلاها الفيماسارتان (٣ ملغم/ كغم/ اليوم) لمدة ٧ ايام متتالية، والمجموعة السادسة تلقت جرعة واحدة من الميثوتريكسيت (٢٠ ملغم/ كغم) في اليوم الأول تلاها تلقي الألفا توكوفيرول (١ غم/ كغم/ اليوم) لمدة ٧ ايام متتالية. في الختام، تم اجراء القتل الرحيم للحيوانات بواسطة ثنائي إيثيل الإيثر وجمع العينات منها لفحصها. نتج عن إعطاء الفيماسارتان والألفا توكوفيرول تناقص كبير في مستويات اليوريا والكرياتينين في المصل وانخفاض كبير في المالون داي الديهايد وارتفاع كبيرفي انزيم ديسموتاز الفائق-١ في نسيج الكلى مقارنة بالجرذان المعاملة بالميثوتريكسيت. كإستنتاج، لدى الفيماسارتان تأثيرات محسنة، مقاربة لتأثيرات الألفا توكوفيرول، ضد التسمم الكلوي الناتج عن الميثوتريكسيت في الجرذان.

الكلمات المفتاحية: التسمم الكلوي، ميثوتريكسيت، الإجهاد التأكسدي ، فيماسارتان، ألفا توكوفيرول.

Introduction

Acute kidney injury (AKI) is a serious medical problem characterized by a rapid and reversible decline in renal function [(1)]. It is associated with a high risk of irreversible renal injury, poor prognosis, and high healthcare costs [(2)]. Many important medications have been reported to cause AKI, which limits their clinical usefulness [(3)]. Among these agents, methotrexate (MTX), which is a widely used antimetabolite, has been reported to cause AKI in about 12% of patients receiving high dose-MTX (HDMTX) [(4)]. HDMTX therapy, defined as the administration of MTX in doses exceeding (500 mg/m^2), is generally used in chemotherapy against various malignancies [(5)]. MTX-induced AKI is a complex process that arises from tubular obstruction via precipitation of MTX and its metabolites within the renal tubules [(5,6)], as well as direct tubular toxicity linked to inflammation, mitochondrial dysfunction, and increased production of reactive oxygen species (ROS) in renal tissue [(4-8)].

[1]Corresponding author E-mail: maryamrasheed.a.r@gmail.com
Received:12 /6/2021
Accepted: 14/8 /2021

Given that oxidative stress (OS) has a key role in the development and progression of MTX-induced renal injury, many studies have been directed to identify interventions that promote the antioxidant defences of the cells in order to circumvent MTX-induced AKI development or its complications, and the results are encouraging [7, 9-11]. Previous researches have proved that α-tocopherol (α-Toc), which is the predominant and most biologically active form of vitamin E, can mitigate renal injury and inhibit renal fibrosis due to its potent antioxidant and anti-inflammatory properties [12-14]. Furthermore, high doses of α-tocopherol were shown to protect the renal and hepatic tissues against oxidative damage induced by various drugs [14].

Fimasartan (FMS) is an efficacious and potent angiotensin II receptor blocker (ARB) that was recently developed and approved in Korea as an antihypertensive medication [15]. It is metabolically stable and chiefly excreted via the bile, and its use exhibited a good safety profile [15-17]. Experimental data proposed that fimasartan exert organ-protecting effects beyond its hypotensive action and a previous study revealed that it has a protective role against renal inflammation and fibrosis through the induction of the antioxidant pathway [15,18,19]. Besides, Cho *et al*. (2018) found that fimasartan preserves kidney structure and function in a murine model of ischemia-reperfusion injury through its anti-inflammatory and antiapoptotic properties [20]. All these factors make fimasartan an attractive candidate to be examined as a renoprotective adjuvant to the standard MTX chemotherapy.

In view of the above considerations, this study was conducted to examine the possible ameliorative activity of fimasartan, in comparison to α-tocopherol, against MTX-induced renal injury in rats, pointing to its ability to suppress OS.

Materials and Methods

Chemicals, drugs, and kits

(+)-α-Tocopherol was obtained from Santa Cruz Biotechnology Inc. (Texas, USA). Fimasartan potassium trihydrate was purchased from Novachemistry (Loughborough, UK). Methotrexate (50mg/2ml) solution for injection was supplied from Mylan S.A.S. (Saint-Priest, France). Diethyl Ether (ROMIL LTD, Cambridge, UK) and phosphate-buffered saline (EuroClone, S.p.A., Milan, Italy) were also used in the study. All enzyme-linked immunosorbent assay (ELISA) kits utilized in the study were obtained from MyBioSource, Inc. (California, USA) and include: rat blood urea nitrogen (BUN) ELISA kit, rat creatinine (Cr) ELISA kit, rat malondialdehyde (MDA) ELISA kit, and rat superoxide dismutase [Cu-Zn] (SOD-1) ELISA kit.

Animal selection

Thirty-six adult Wistar rats (8 weeks old) of both sexes, weighing 150-240 g, were used in the present study. They were obtained from and maintained in the Animal House at the College of Pharmacy/University of Baghdad under conditions of controlled temperature, humidity and light periodicity (12-hour light/dark cycle). They were fed commercial pellets and tap water *ad libitum* throughout the experimental period. To get adapted, these rats were routinely handled and acclimatized for 7 days in the above-stated conditions before drug administration.

Experimental protocol

This study was approved by the Scientific and Ethical Committees of the College of Pharmacy/University of Baghdad. The rats employed in this study were randomly divided into six groups of six rats each, as follows:

Group I (negative control group): Rats received sterile water for injection in a volume of (6 ml/kg) intraperitoneally [21] for 8 days starting from day 1.

Group II (MTX group): Rats received a single dose of MTX (20 mg/kg) intraperitoneally on day 1, followed by daily intraperitoneal (IP) administration of sterile water for injection (6 ml/kg) for 7 days starting from day 2 [22].

Group III (FMS group): Rats received a daily IP injection of fimasartan (3 mg/kg/day) for 7 successive days. A solution of (0.05% w/v) FMS was prepared by dissolving fimasartan potassium trihydrate in water on the day of administration [18,23,24].

Group IV (α-Toc group): Rats received α-tocopherol (1 g/kg/day) orally for 7 successive days [14].

Group V (MTX plus FMS group): Rats received a single dose of MTX (20 mg/kg) intraperitoneally on day 1, followed by daily IP injection of fimasartan (3 mg/kg/day) for 7 successive days starting from day 2.

Group VI (MTX plus α-Toc group): Rats received a single dose of MTX (20 mg/kg) intraperitoneally on day 1, followed by daily administration of α-tocopherol (1 g/kg/day) orally for 7 successive days starting from day 2.

Samples collection and preparation of kidney tissue homogenate

After twenty-four hours from the final drug administration, blood samples were withdrawn from the carotid artery (at the neck) and collected in gel activated tubes and allowed to stand for 30 minutes to clot. Then, it was centrifuged at 3000 rpm for 15 minutes using a centrifuge (EBA 20, Andreas Hettich GmbH & Co. KG, Germany) to obtain serum [25]. The obtained sera were utilized for the estimation of urea and creatinine levels.

Next, all rats were sacrificed by cervical dislocation under diethyl ether anaesthesia and kidney tissues were isolated and processed for analysis [25]. Briefly, the kidneys were rapidly excised, cleaned from fatty tissues, and washed with a pre-cooled PBS (pH=7.4, 4˚C) to rinse away any residual blood. Then, each kidney was blotted on filter paper, weighed, and chopped into fine pieces. For each rat, the left kidney was used to prepare the kidney tissue homogenate by adding 0.4 g of the minced tissue and 3.6 ml of PBS (pH=7.4, 4˚C) into a tube [26]. Homogenization was then accomplished using a tissue homogenizer (Dyna-Passion® WT130, Success Technic Industries, Selangor, Malaysia) at set 3 for 1 minute at 4˚C. Samples were kept on ice throughout all the above-mentioned steps. The resultant suspension was then subjected to a freeze-thaw cycle and centrifuged in a refrigerated centrifuge (HERMLE Labortechnik GmbH, Germany) at 10,000 rpm for 10 minutes at 4˚C. The resultant supernatant was immediately collected and stored at −20˚C until the day of analysis when it was used for the estimation of MDA and SOD-1 levels [26,27].

Biochemical analysis

Serum levels of kidney function biomarkers, blood urea nitrogen (BUN) and creatinine (sCr), were measured using ELISA kits according to the manufacturers' instructions. Moreover, to assess the oxidant/antioxidant status in the tissue, the concentrations of MDA and SOD-1 were quantified in the renal tissue homogenate by sandwich ELISA method according to the kit manufacturers' instructions [11, 22].

Measurements of the relative kidney weight (kidney index)

On the morning of sacrifice day, the bodyweight of each rat was measured. Then, the rats were euthanized and the weights of right and left kidneys were measured immediately after harvesting the kidneys from the rat carcasses. Then, the kidney-to-body weight ratio, also called the relative kidney weight or kidney index (KI), had been calculated for each rat by dividing the total right and left kidney weights by the total body weight of the rat, then multiplying the result by 100 [9].

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Analysis was carried out using Statistical Package for Social Sciences (SPSS, version 25) software. The differences between the groups were evaluated by a two-way analysis of variance (ANOVA). The differences among the groups were considered statistically significant at a *P* value of less than 0.05 (*P*<0.05).

Results

Effects on serum markers of kidney function

(Table 1) and (Figures 1 and 2) revealed that administration of MTX in group II resulted in a significant increase of BUN and sCr levels compared to the negative control group (group I) (*P*<0.05). At the same time, there were no significant differences in the FMS and α-Toc groups when compared to group I (*P*>0.05). Besides, comparing the FMS group and α-Toc group with the MTX group revealed significant differences between them (*P*<0.05).

Interestingly, rats in the MTX plus FMS group showed a significant decrease in BUN and sCr levels compared to the MTX group (*P*<0.05). Similarly, rats in MTX plus α-Toc group showed significantly lower levels compared to the MTX group (*P*<0.05), as shown in (Table 1) and (Figures 1 and 2).

By comparing the levels of BUN and sCr in rats treated with MTX alone (group II), FMS alone (group III), and MTX followed by FMS (group V), the obtained results showed that there were significant differences between the three groups (*P*<0.05). Likewise, when we compare BUN and sCr levels among the MTX group, α-Toc group, and MTX plus α-Toc group, statistically significant differences between them can be noted (*P*<0.05), as shown in (Table 1) and (Figures 1 and 2).

The same table also showed a significant difference in BUN levels when we compare the MTX plus FMS group to the MTX plus α-Toc group (*P*<0.05), which has significantly lower BUN levels compared to the MTX plus FMS group. However, there is a nonsignificant difference in sCr levels when we compare the MTX plus FMS group to the MTX plus α-Toc group (*P*>0.05).

Table 1. Effects of fimasartan on the serum levels of BUN and creatinine.

Groups	Serum BUN (mmol/L)	Serum Cr (mmol/L)
I. Negative control group	1.79±0.46	1.03±0.48
II. MTX group	$2.86 \pm 0.79^{*aA}$	$2.73 \pm 0.61^{*aA}$
III. FMS group	$1.78 \pm 0.62^{\psi b}$	$1.201 \pm 0.69^{\psi b}$
IV. α-Toc group	$1.74 \pm 1.27^{\psi B}$	$1.42 \pm 0.32^{\psi B}$
V. MTX + FMS group	$2.05 \pm 0.34^{\delta c}$	$1.79 \pm 0.92^{\delta c}$
VI. MTX + α-Toc group	$1.69 \pm 0.24^{\beta C \clubsuit}$	$1.83 \pm 0.48^{\beta C}$

- The data are expressed as mean ± SD, number of rats in each group = 6
- Superscript (*) indicates significant differences when groups II, III and IV are compared to the negative control group (*P*<0.05)
- Superscript (ψ) indicates significant differences when group III and IV are compared to the MTX group (*P*<0.05)
- Superscript (δ) indicates a significant difference when group V is compared to group II (*P*<0.05)
- Superscript (β) indicate a significant difference when group VI is compared to group II (*P*<0.05)

- Small letter superscripts (a, b, c) indicate significant differences among the groups (II, III, V) (P<0.05)
- Capital letter superscripts (A, B, C) indicate significant differences among the groups (II, IV, VI) (P<0.05)
- Superscript (♣) indicates a significant difference when group V is compared to group VI (P<0.05)

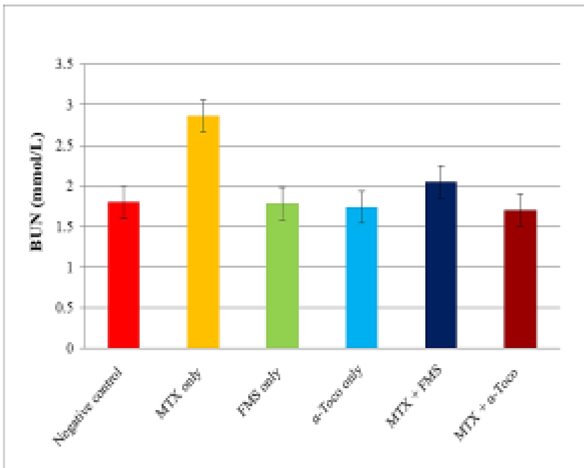

Figure 1.Effects of fimasartan on the serum levels of BUN.

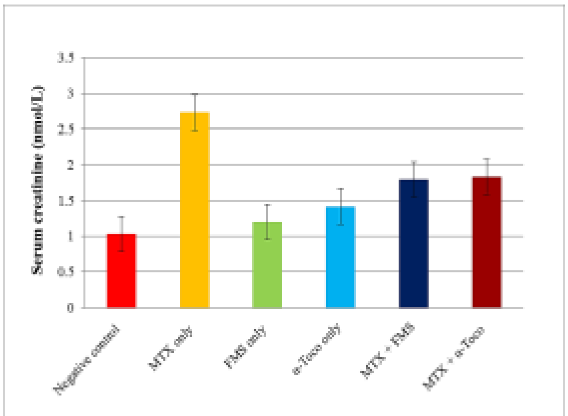

Figure 2. Effects of fimasartan on the serum levels of creatinine.

Effects on renal lipid peroxidation and antioxidant parameters

Table 2 and Figures 3 and 4 revealed that administration of MTX in group II resulted in a significant increase in renal MDA levels, coupled with a significant decrease in renal SOD-1 contents, as compared to the negative control group (group I) (P<0.05). At the same time, there were no significant differences in the levels of MDA and SOD-1 in the FMS and α-Toc groups as compared to group I (P>0.05). Besides, comparing the FMS group and α-Toc group with the MTX group revealed significant differences between them (P<0.05).

Notably, the rats in MTX plus FMS group showed a significant decrease in MDA along with a significant increase in SOD-1 levels as compared to the MTX group (P<0.05). Similarly, rats in MTX plus α-Toc group showed significantly decreased MDA and increased SOD-1 levels as compared to the MTX group (P<0.05), as shown in (Table 2) and (Figures 3 and 4).

By comparing the levels of MDA and SOD-1 in the renal tissue homogenate of rats in the MTX group, FMS group, and MTX plus FMS group, the obtained results showed that there were significant differences between the three groups (P<0.05). Likewise, when we compare the renal MDA and SOD-1 levels among the MTX group, α-Toc group, and MTX plus α-Toc group, statistically significant differences between them can be noted (P<0.05), as shown in (Table 2) and (Figures 3 and 4).

The same table also showed a nonsignificant difference in renal MDA levels when we compare the MTX plus FMS group to the MTX plus α-Toc group (P=0.05). However, MTX plus FMS group has significantly higher renal SOD-1 levels compared to MTX plus α-Toc group (P<0.05).

Table 2.Effects of fimasartan on the renal MDA and SOD-1 levels.

Groups	**Renal MDA (nmol/ml)**	**Renal SOD-1 (ng/ml)**
I. Negative control group	8.44±1.22	149.11±11.35
II. MTX group	16.58±1.03^{*aA}	109.66±19.21^{*aA}
III. FMS group	9.07±0.94$^{\psi b}$	151.87±23.87$^{\psi b}$
IV. α-Toc group	8.32±1.265$^{\psi B}$	148.51±18.82$^{\psi B}$
V. MTX + FMS group	11.69±0.99$^{\delta c}$	158.36±16.86$^{\delta c♣}$
VI. MTX + α-Toc group	10.82±0.59$^{\beta C}$	135.23±20.83$^{\beta C}$

- The data are expressed as mean ± SD, number of rats in each group = 6
- Superscript (*) indicates significant differences when groups II, III and IV are compared to the negative control group (P<0.05)
- Superscript (ψ) indicates significant differences when group III and IV are compared to the MTX group (P<0.05)
- Superscript (δ) indicates a significant difference when group V is compared to group II (P<0.05)
- Superscript (β) indicate a significant difference when group VI is compared to group II (P<0.05)

- Small letter superscripts (a, b, c) indicate significant differences among the groups (II, III, V) (P<0.05)
- Capital letter superscripts (A, B, C) indicate significant differences among the groups (II, IV, VI) (P<0.05)
- Superscript (♣) indicates a significant difference when group V is compared to group VI (P<0.05)

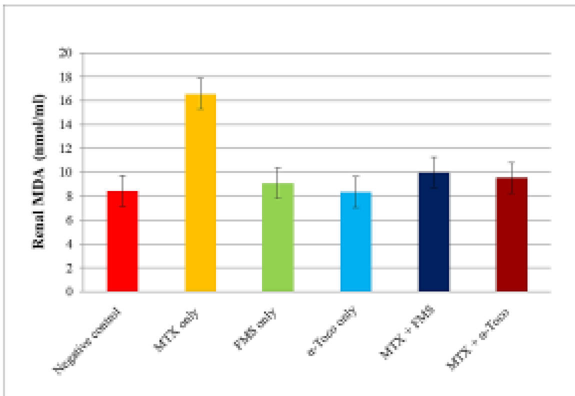

Figure 3.Effects of fimasartan on the renal MDA levels.

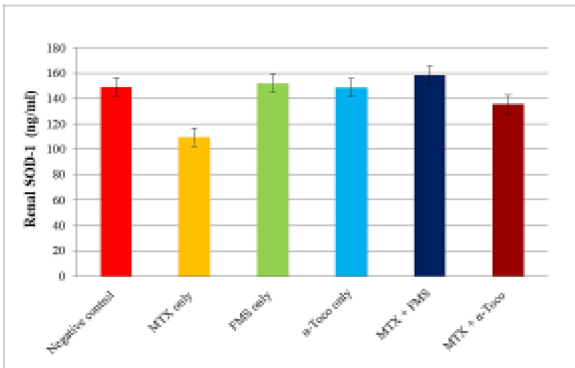

Figure 4. Effects of fimasartan on the renal SOD-1 levels.

Effects on the relative kidney weight (kidney index)

As shown in (Table 3) and (Figure 5), MTX administration in group II resulted in a significant increase in KI as compared to the untreated rats (group I) (P<0.05). At the same time, there were no significant differences in the FMS and α-Toc groups rats as compared to the untreated rats in group I (P>0.05). Besides, comparing the FMS group and α-Toc group with the MTX group revealed significant differences between them (P<0.05).

Remarkably, the rats in the MTX plus FMS group showed a significant decrease in KI as compared to the MTX group (P<0.05). Likewise, rats in MTX plus α-Toc group showed a significantly decreased KI as compared to the MTX group (P<0.05), as shown in (Table 3) and (Figure 5).

By comparing the KI of rats in the MTX group, FMS group, and MTX plus FMS group, the obtained results showed that there were significant differences between the three groups (P<0.05). Likewise, when we compare among MTX group, α-Toc group, and MTX plus α-Toc group, statistically significant differences between them can be noticed (P<0.05), as shown in (Table 3) and (Figure 5).

The same table also showed a nonsignificant difference in KI when we compare the MTX plus FMS group to the MTX plus α-Toc group (P>0.05).

Table 3.Effects of fimasartan on the kidney index (KI).

Groups	KI
I. Negative control group	0.69±0.074
II. MTX group	$1.12 \pm 0.29^{*aA}$
III. FMS group	$0.696 \pm 0.04^{\psi b}$
IV. α-Toc group	$0.74 \pm 0.03^{\psi B}$
V. MTX + FMS group	$0.71 \pm 0.12^{\delta c}$
VI. MTX + α-Toc group	$0.725 \pm 0.11^{\beta C}$

- The data are expressed as mean ± SD, number of rats in each group = 6
- Superscript (*) indicates significant differences when groups II, III and IV are compared to the negative control group (P<0.05)
- Superscript (ψ) indicates significant differences when group III and IV are compared to the MTX group (P<0.05)
- Superscript (δ) indicates a significant difference when group V is compared to group II (P<0.05)
- Superscript (β) indicate a significant difference when group VI is compared to group II (P<0.05)
- Small letter superscripts (a, b, c) indicate significant differences among the groups (II, III, V) (P<0.05)
- Capital letter superscripts (A, B, C) indicate significant differences among the groups (II, IV, VI) (P<0.05)
- Superscript (♣) indicates a significant difference when group V is compared to group VI (P<0.05)

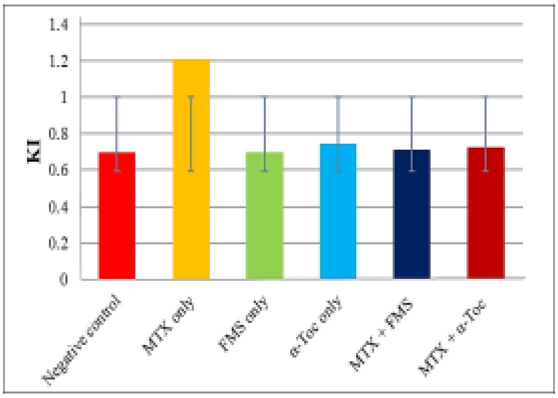

Figure 5.Effects of fimasartan on the kidney index (KI).

Discussion

AKI induced by HDMTX represents a serious challenge, especially among hospitalized patients, with a high risk of progression to irreversible renal impairment. Moreover, the nephrotoxicity induced by MTX is of special importance because MTX is eliminated primarily by the kidneys [(4,7)]. Hence, if MTX-induced AKI developed, excess amounts of the drug and its metabolites will accumulate in the body, leading to enhancement of other MTX toxicities including hepatotoxicity, myelosuppression, and neurotoxicity [(5,28)]. Oxidative stress has been well-established as a key player in the pathogenesis of MTX-induced renal injury [(9,29)]. Previous investigations reported that MTX can promote ROS production by inducing mitochondrial dysfunction and activating NADPH oxidases, which generate excessive amounts of ROS, especially superoxide anion, as their main products [(22)]. The resultant oxidative burst, coupled with impairment of the antioxidant defences of the body; can result in oxidative damage of important cellular components including lipids, proteins, and nucleic acids; contributing to lipid peroxidation and cell death [(22,30)].

In the current study, the administration of MTX to the rats resulted in renal dysfunction evidenced by the significant increase in serum creatinine and BUN levels; which have been widely utilized to assess renal impairement in numerous preclinical studies [(31-33)]; combined with a significant increase in renal MDA levels (a lipid peroxidation marker), and a significant decrease in renal contents of the antioxidant enzyme, SOD-1. These results were similar to previous studies, indicating the important role of oxidative stress in the development of peroxidative damage and renal injury upon exposure to MTX [(10,12,22)]. MTX-induced nephrotoxicity was further confirmed by a significant increase in KI in the MTX group compared to the untreated rats, confirming the harmful effects of MTX on the kidneys, since any change in KI from normal is an indicator of renal toxicity [(9,34,35)].

Importantly, the present study showed that fimasartan ameliorated the renal injury induced by MTX, evidenced by the significant decrease in the serum levels of renal function parameters and KI, coupled with a significant decrease in renal MDA and a significant increase in renal SOD-1 contents, reflecting a restoration of the cellular redox balance in the kidney. These results are consistent with previous researches that reported the renoprotective effects of fimasartan and other ARBs in various drug-induced renal injury models [(18,20,35,36)]. A study by Kim *et al.* (2015) in a mice model of unilateral ureteral obstruction showed that the ameliorating effect of fimasartan against renal oxidative stress, inflammation, and fibrosis was mediated through upregulation of the antioxidant enzymes (including SOD-1) along with counteracting the effects of angiotensin II (Ang II), which is a major component of the renin-angiotensin-aldosterone-system [(18)]. They found that the locally expressed Ang II in the kidneys contributed to the oxidative stress by enhancing NADPH oxidases, and blockade of Ang II/AngII type 1 receptor signalling by fimasartan reduced the oxidative stress and inflammation induced by Ang II [(18)]. Similarly, in another preclinical study, fimasartan was found to preserve renal structure and function in an ischemia/reperfusion injury model by preventing apoptosis induced by the inflammatory pathway [(20)]. Notably, the mechanisms offered by fimasartan and other renoprotective ARBs are the same, where it was reported that irbesartan displayed a protective role in gentamicin-induced nephrotoxicity via its antioxidant effect [(36)]. Similarly, losartan showed a renoprotective effect through counteracting oxidative stress in an ischemic renal injury model [(37)]. Consequently, the beneficial effects of fimasartan against renal injury can be attributed to its antioxidant activity mediated by the enhancement of the endogenous antioxidants, with the resultant attenuation of lipid peroxidation in renal tissue.

On the other hand, α-tocopherol appears to have antioxidant effects which contribute to its renoprotective effects. In agreement, many investigators verified that α-tocopherol can mitigate the nephrotoxicity induced by several agents, and the protective effect was credited to its antioxidant and anti-inflammatory properties [(12,38,39)]. However, fimasartan appears to be a more powerful antioxidant than α-tocopherol, since it resulted in a more significant increase in the renal SOD-1 levels.

Conclusion

In conclusion, the present study revealed that treatment of rats with fimasartan have ameliorative effects, that are comparable to those of α-tocopherol, against MTX-induced nephrotoxicity through boosting the antioxidant defences in the kidneys; with fimasartan being more effective than α-tocopherol since it increased the renal SOD-1 levels to a greater extent.

Acknowledgement

The data of this article were abstracted from the M.Sc. thesis submitted to the Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad. The authors are extremely grateful to the College of Pharmacy/University of Baghdad for supporting this work.

References:

1. Soares S, C R Souza L, T Cronin M, M Waaga-Gasser A, F Grossi M, R Franco G, et al. Biomarkers and in vitro strategies for nephrotoxicity and renal disease assessment. Nephrol Ren Dis. 2020;5(1):1–14.
2. Darmon M, Joannidis M, Schetz M. Focus on critical care nephrology. Intensive Care Med. 2019;45(9):1288–91.
3. Soares S, C R Souza L, T Cronin M, M Waaga-Gasser A, F Grossi M, R Franco G, et al. Biomarkers and in vitro strategies for nephrotoxicity and renal disease assessment. Nephrol Ren Dis. 2020;5(1):1–14.
4. Howard SC, McCormick J, Pui C, Buddington RK, Harvey RD. Preventing and managing toxicities of high-dose methotrexate. Oncologist. 2016;21(12):1471–82.
5. Ramsey LB, Balis FM, O'Brien MM, Schmiegelow K, Pauley JL, Bleyer A, et al. Consensus Guideline for Use of Glucarpidase in Patients with High-Dose Methotrexate Induced Acute Kidney Injury and Delayed Methotrexate Clearance. Oncologist. 2018;23(1):52–61.
6. Ahmed W, Zaki A, Nabil T. Prevention of methotrexate-induced nephrotoxicity by concomitant administration of garlic aqueous extract in rat. Turkish J Med Sci. 2015;45(3):507–16.
7. Aladaileh SH, Hussein OE, Abukhalil MH, Saghir SAM, Bin-Jumah M, Alfwuaires MA, et al. Formononetin upregulates nrf2/ho-1 signaling and prevents oxidative stress, inflammation, and kidney injury in methotrexate-induced rats. Antioxidants. 2019;8(10):1–18.
8. Gai Z, Gui T, Kullak-Ublick GA, Li Y, Visentin M. The role of mitochondria in drug-induced kidney injury. Front Physiol. 2020;11:1–13.
9. Hassanein EHM, Shalkami AGS, Khalaf MM, Mohamed WR, Hemeida RAM. The impact of Keap1/Nrf2, P 38 MAPK/NF-κB and Bax/Bcl2/caspase-3 signaling pathways in the protective effects of berberine against methotrexate-induced nephrotoxicity. Biomed Pharmacother. 2019;109(October 2018):47–56.
10. Abdel-Daim MM, Khalifa HA, Abushouk AI, Dkhil MA, Al-Quraishy SA. diosmin attenuates methotrexate-induced hepatic, renal, and cardiac injury: A biochemical and histopathological study in mice. Oxid Med Cell Longev. 2017;2017:3281670.
11. Aldossary SA. protective Effect of hesperidin against methotrexate-induced nephrotoxicity in rats. Life Science Journal. 2019;16(2):18–22.
12. Abdel-Daim MM, Aleya L, El-Bialy BE, Abushouk AI, Alkahtani S, Alarifi S, et al. The ameliorative effects of ceftriaxone and vitamin E against cisplatin-induced nephrotoxicity. Environ Sci Pollut Res. 2019;15248–54.
13. Wu TK, Pan YR, Wang HF, Wei CW, Yu YL. Vitamin E (α-tocopherol) ameliorates aristolochic acid-induced renal tubular epithelial cell death by attenuating oxidative stress and caspase-3 activation. Mol Med Rep. 2018;17(1):31–6.
14. Jilanchi S, Nematbakhsh M, Bahadorani M, Talebi A, Eshraghi-Jazi F, Mansouri A, et al. Vitamin E Is a Nephroprotectant Agent in Male but Not in Female in a Model of Cisplatin-Induced Nephrotoxicity. ISRN Nephrol. 2013;2013:1–6.
15. Angeli F, Verdecchia P, Trapasso M, Pane M, Signorotti S, Reboldi G. PK/PD evaluation of fimasartan for the treatment of hypertension Current evidences and future perspectives. Expert Opin Drug Metab Toxicol. 2018;14(5):533–41.
16. Lee HY, Oh BH. Fimasartan: A new angiotensin receptor blocker. Drugs. 2016;76(10):1015–22.
17. Han SE, Jeong SH, Kang HJ, Hong MS, Paek E, Cho H, et al. Safety and efficacy of fimasartan with essential hypertension patients in real world clinical practice: data from a post marketing surveillance in Korea. Transl Clin Pharmacol. 2018;26(3):118–27.
18. Kim S, Kim SJ, Yoon HE, Chung S, Choi BS, Park CW, et al. Fimasartan, a novel angiotensin-receptor blocker, protects against renal inflammation and fibrosis in mice with unilateral ureteral obstruction: The possible role of Nrf2. Int J Med Sci. 2015;12(11):891–904.
19. Yang X, Sun J, Kim TJ, Kim YJ, Ko SB, Kim CK, et al. Pretreatment with low-dose fimasartan ameliorates NLRP3 inflammasome-mediated neuroinflammation and brain injury after intracerebral hemorrhage. Exp Neurol. 2018;310(August):22–32.
20. Cho JH, Choi SY, Ryu HM, Oh EJ, Yook JM, Ahn JS, et al. Fimasartan attenuates renal ischemia-reperfusion injury by modulating inflammation-related apoptosis. Korean J Physiol Pharmacol. 2018;22(6):661–70.
21. Machholz, E., Mulder, G., Ruiz, C., Corning, B.F., Pritchett-Corning, K.R. Manual restraint and common compound administration routes in mice and rats. J. Vis. Exp. (67), e2771:1-8.
22. Abd El-Twab SM, Hussein OE, Hozayen WG, Bin-Jumah M, Mahmoud AM. Chicoric acid

prevents methotrexate-induced kidney injury by suppressing NF-κB/NLRP3 inflammasome activation and up-regulating Nrf2/ARE/HO-1 signaling. Inflamm Res. 2019;68(6):511–23.

23. Han J, Park SJ, Thu VT, Lee SR, Long LT, Kim HK, et al. Effects of the novel angiotensin II receptor type I antagonist, fimasartan on myocardial ischemia/reperfusion injury. Int J Cardiol. 2013;168(3):2851–9.

24. Park H, Kim HS, Hong YJ, Min J-J, Kim HB, Kim MC, et al. Therapeutic effect of fimasartan in a rat model of myocardial infarction evaluated by cardiac positron emission tomography with [18 F]FPTP. Chonnam Med J. 2019;55(2):109–115.

25. Khudhai AR, Al-Shawi NN. Possible protective effects of high- versus low- dose of lutein in combination with irinotecan on liver of rats: Role of oxidative stress and apoptosis. Indian J Forensic Med Toxicol. 2021;15(1):2439–45.

26. Lampl T, Crum JA, Davis TA, Milligan C, Moore VDG. Isolation and functional analysis of mitochondria from cultured cells and mouse tissue. J Vis Exp. 2015;2015(97):1–9.

27. Gagne F. Biochemical Ecotoxicology. 1st Edition. Cambridge, Massachusetts, USA: Academic Press; 2014. Chapter 2, Tissue preparation and subcellular fractionation techniques; p.21-31.

28. Shirali AC, Perazella MA. Tubulointerstitial injury associated with chemotherapeutic agents. Adv Chronic Kidney Dis. 2014;21(1):56–63.

29. Perazella MA. Drug-induced acute kidney injury: Diverse mechanisms of tubular injury. Curr Opin Crit Care. 2019;25(6):550–7.

30. Saka S, Aouacheri O. The investigation of the oxidative stress-related parameters in high doses methotrexate-induced albino wistar rats. J Bioequiv Availab. 2017;09(02):372–376.

31. Faria J, Ahmed S, Gerritsen KGF, Mihaila SM, Masereeuw R. Kidney-based in vitro models for drug-induced toxicity testing. Arch Toxicol. 2019;93(12):3397–418.

32. Pajaro-Galvis N, Rico-Fontalvo J, Daza-Arnedo R, Cardona-Blanco MX, Abuabara-Franco E, Leal-Martinez V, et al. Biomarkers in acute kidney injury. J Clini Nephrol. 2020;4:027–035.

33. Abd ella A, El-Kotby H, Abd El-Lateef AE-L, Abd Elhai W. Study the potential nephro-protective effect of stem cells compared to perindopril on experimentally induced nephropathy. Al-Azhar Int Med J. 2020;1(1):36–45.

34. Perera T, Ranasinghe S, Alles N, Waduge R. Experimental rat model for acute tubular injury induced by high water hardness and high water fluoride: Efficacy of primary preventive intervention by distilled water administration. BMC Nephrol. 2020;21(1):1–16.

35. Al-joda AM, Zalzala MH. Inhibition of NF-κB pathway by guggulsterone in the protective effects of cyclophosphamide-induced renal toxicity. Iraqi J Pharm Sci. 2019;28(2):180–185.

36. Al-Kuraishy HM, Al-Gareeb AI, Al-Nami MS. Irbesartan attenuates gentamicin-induced nephrotoxicity in rats through modulation of oxidative stress and endogenous antioxidant capacity. Int J Prev Med. 2020;11(Ang II):16.

37. Miloradović Z, Ivanov M, Jovović Đ, Karanović D, Vajić UJ, Marković-Lipkovski J, et al. Angiotensin 2 type 1 receptor blockade different affects postishemic kidney injury in normotensive and hypertensive rats. Vol. 72, Journal of Physiology and Biochemistry. 2016. p. 813–20.

38. Kandeil MAM, Hassanin KMA, Mohammed ET, Safwat GM, Mohamed DS. Wheat germ and vitamin E decrease BAX/BCL-2 ratio in rat kidney treated with gentamicin. Beni-Suef Univ J Basic Appl Sci. 2018;7(3):257–62.

39. Stojiljkovic N, Ilic S, Veljkovic M, Todorovic J, Mladenovic M. α-Tocopherol reduces morphological changes and oxidative stress during gentamicin-induced acute renal failure. Bull Exp Biol Med. 2018;164(4):442–5.