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RESEARCH ARTICLE

Protective Effects of *Momordica charantia* (Bitter Melon) against Methotrexate-induced Kidney Damage

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Abstract: ***Background*:** Methotrexate is a cytotoxic chemotherapeutic agent that has severe side effects, such as nephrotoxicity. *Momordica charantia* is a bright yellow-orange fruity plant that has been shown to have antioxidant, antidiabetic, and anti-inflammatory properties.

***Objective*:** This study scrutinized the protective effects of *Momordica charantia* extract against methotrexate-induced nephrotoxicity.

***Methods*:** 24 Sprague Dawley male rats were divided into three experimental groups (8 rats in each): Control (C); Methotrexate (MTX); and Methotrexate plus *Momordica charantia* (MTX+MC). All rats were fed ad libitum and tap water. Methotrexate was administered at 20 mg/kg intraperitoneally as a single dose. In the MTX+MC group, MC was administered at a dose of 50mg/kg for 5 days orally. At the end of the 5th day, the rats were decapitated and kidney samples were taken to analyze glutathione (GSH), malondialdehyde (MDA), myeloperoxidase (MPO), 8-hydroxy-2'-deoxyguanosine (8-OHdG) and caspase-3 activity. Data was analyzed with GraphPad Prism 5.0.

***Results*:** Findings showed that while there was a significant increase in MDA, MPO, 8-OHdG levels, and an essential reduction in GSH levels in the MTX-treated group when compared with the control group, bitter melon treatment significantly reversed MDA, MPO, and 8-OHdG levels ($p< 0.001$). GSH level elevation was observed in the MTX-MC group when compared to the MTX-treated group ($p< 0.001$).

***Conclusion*:** This study showed that bitter melon is thought to have a protective effect against kidney damage caused by methotrexate. With future studies, we believe that the use of bitter melon extract as a protective agent in kidney damage caused by drug-induced oxidative damage will bring an innovative approach to treatment.

Keywords: *Momordica charantia*, protective effect, kidney, methotrexate, nephrotoxicity, oxidative damage.

1. INTRODUCTION

Methotrexate (MTX) is a toxic neoplastic drug that has an antimetabolite property, used in the treatment of such diverse illnesses as autoimmune, neurological, and neoplastic disorders [1, 2]. It antagonizes folate metabolism by binding to dihydrofolate reductase (DHFR), resulting in inhibiting the conversion of dihydrofolate to tetrahydrofolate [3, 4]. Therefore, methotrexate prevents the proliferation of quickly-dividing neoplastic cells. On the other hand, although MTX is a highly effective antineoplastic agent, it has also much toxicity on tissue cells. MTX is associated with the toxicity in liver, kidney, bone marrow, lungs, and gastrointestinal system. However, MTX is primarily eliminated from the kidney, nearly 90% without changing. MTX-induced kidney damage may be dangerous for our life because it delays MTX elimination and thus causes permanent and increased plasma MTX level and increasing of other side events [5, 6]. High doses of MTX cause functional injury resulting in nephrotoxicity [7]. If crystal nephropathy occurs by the use of high dose MTX and precipitation of MTX and its metabolites, it leads to nephrotoxicity. As MTX is an acidic compound, alkalization excessively increases MTX solubility and elimination. The first sign of crystal-induced kidney damage is increasing serum creatinine asymptomatically; however, after that, it causes severe kidney damage [4]. Administration of high doses of MTX causes increased creatinine levels; however, it has also been shown to cause gastrointestinal, kidney, liver, and bone marrow toxicity in many studies [8, 9]. *Momordica*

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charantia (MC) is a valuable medicinal plant which belongs to the *Cucurbitaceae* family; it is widely named as bitter melon or bitter gourd [10]. Complete parts of the bitter melon have a very bitter taste [11, 12]. This plant is commonly cultivated in the tropical and subtropical regions of the world [13, 14]. MC has very rich contents including essential oils, flavonoids, phenolic acids, fatty acids, amino acids, lectins and other constituents. These constituents are responsible for its biological activity [15]. From ancient times, it has been used in the healing of diverse diseases like toothache, diabetes [16], diarrhea. There are many pieces of literature indicating that this plant possesses different pharmacological functions; antiulcer [17, 18], antifungal [19], and wound healing [20]. In addition to these properties, *Mamordica charantia* also has an antioxidant effect due to its components like polysaccharides [21], peptides and proteins [22, 23], lipids [24], terpenoids [25, 26] and phenolics [27]. It shows its antioxidant effect by scavenging diverse free radicals, especially with phenolic contents [28]. In addition, bitter melon fruit extract increases caspase activity in neoplastic cells. Therefore, apoptosis induction and neoplastic cell growth inhibition occur [29]. In light of this information, this study scrutinized whether *Momordica charantia* (bitter melon) has an antioxidant effect in rats with MTX-induced nephropathy.

2. MATERIALS AND METHODS

2.1. Experimental Method

Twenty-four male Sprague Dawley rats (200–250 g, 3 months old) were purchased from Marmara University Experimental Animals Research and Implementation Centre. Animals were kept at a constant temperature (22 ± 1°C), relative humidity (50-60%), and 12 h light and dark cycles. The animals were fed with tap water and ad libitum. The experiment termination criteria were determined as animals losing more than 20% of their body weight and cannot get proper food and water. No adverse events were reported during the experiment. All experimental protocols were approved by Laboratory Animal Experiments Local Ethics Committee of Marmara University (54.2020.mar).

2.2. Preparation of the *Momordica charantia* Extract

Momordica charantia plants were collected from the rural district of Gemlik in Bursa, Turkey, in August 2019. Identification and preparation of plant extracts were conducted at the Departments of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmacy, Marmara University, respectively. The leaves of bitter gourd were washed with water and dried in the shade at room temperature. After that, the dried leaves were smashed and stored at 4°C in airtight containers until the analysis procedure. After that, 1000 mg of MC powder was boiled in 1 L distilled water for 1 hour. The obtained extract was filtered and dried by spray-dried method.

2.3. Experimental Groups

Twenty-four rats were randomly separated into three groups (n=8) as control, MTX, and MTX+MC. A single dose of MTX (20 mg/kg) was applied to MTX and MTX+MC groups by intaperitoneal injection. MTX+MC group was given *Momordica charantia* extract dissolved in saline (600 mg/kg) by oral gavage for 5 days [30]. The control and MTX groups were administered saline for 5 days. To finalize the experiment, at the 5th day, decapitation was done, followed by collecting kidney tissues.

2.4. Biochemical Assessment in Kidney Tissue

Beheading under anesthesia, kidney tissue samples were collected and put into the formaldehyde solution. Different methods were performed to determine antioxidant parameters -MDA and GSH- levels in all groups [31, 32]. As a determinant of lipid peroxidation, MPO activity analysis of kidney tissue was performed according to the method developed before [33]. 8-OHdG, indictor of DNA damage, was measured by kit (Oxi Select Oxidative Damage Elisa Kit (STA-320, Cell Biolabs)). Tissue samples were also performed by the purchased kit (Pure Link® Genomic DNA Mini Kit (K182001, Life Technology)). The most important apoptotic factor, Caspase-3 enzyme activity, was measured by a caspase colorimetric attempt kit (Abbkine Rat Caspase-3 Elisa Kit, Catalogue NO: KTE100992, China).

2.5. Statistical Analysis

Data were analysed with GraphPad Prism 5.0v. (GraphPad Software, San Diego, CA, USA) statistical program. All data were given as mean ± SD (Standard Deviation). One-way analysis of variance (ANOVA) followed by post hoc Tukey test was performed to compare the multiple groups with each other. $P \leq 0.05$ was considered statistically significant.

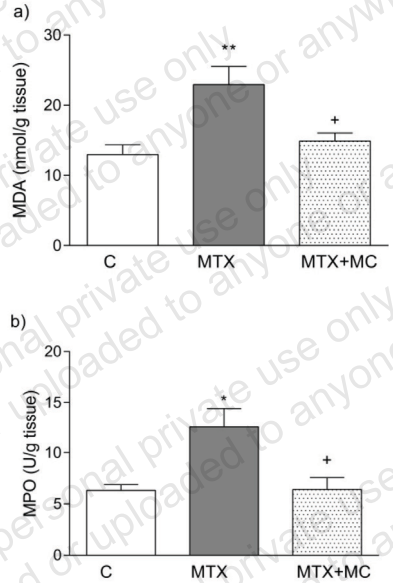

Fig. (1). Activity levels of **a**) MDA and **b**) MPO in kidney tissue of the experimental groups. Each group consists of eight animals. C: Saline-treated control group; MTX: Methotrexate-treated group; MTX+MC: Methotrexate plus *Momordica charantia*-treated group.*: $p< 0.05$ compared to control; **: $p< 0.01$ compared to control group; +: $p< 0.05$ compared to MTX group. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3. RESULTS

Fig. (**1**) shows that while MDA and MPO activities determinants of lipid peroxidation were essentially elevated in MTX group when compared to control group ($p< 0.01$ and $p< 0.05$), MDA and MPO were found to be reduced in MTX+MC group when compared to MTX group ($p< 0.05$).

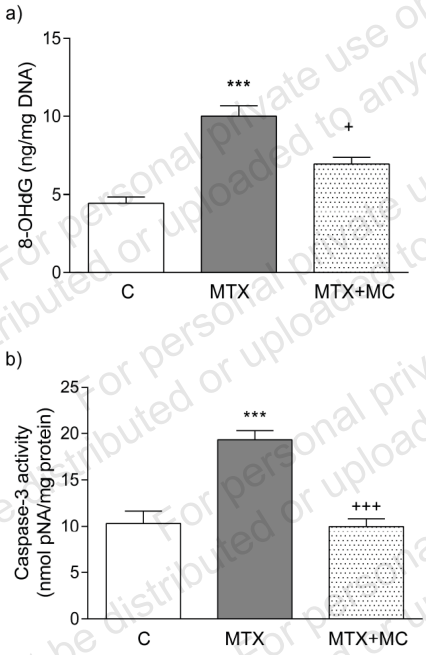

Fig. (2). a) 8-OHdG levels and **b**) Caspase-3 enzyme activity level in kidney tissues of experimental groups. Each group consists of eight animals. C: Saline-treated control group; MTX: Methotrexate-treated group; MTX+MC: Methotrexate plus *Momordica charantia*-treated group. ***: $p< 0.001$ compared to control group; +: $p< 0.05$ compared to MTX group; +++: $p< 0.001$ compared to MTX group. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

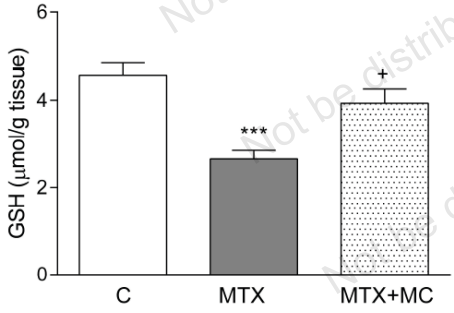

Fig. (3). GSH levels in experimental animals. Each group consists of eight animals. C: Saline-treated control group; MTX: Methotrexate-treated group; MTX+MC: Methotrexate plus *Momordica charantia*-treated group. +: $p< 0.05$ compared to MTX group; ***: $p< 0.001$ compared to control group. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Fig. (**2**) demonstrated that 8-OHdG level was significantly elevated in MTX-treated group when compared to the control group ($p< 0.001$). Essential decrease was demonstrated in the MC-treated group when compared to MTX group ($p< 0.05$) (Fig. **2a**). In addition, when compared with the control group, caspase-3 activity level was observed essentially raised in the MTX-treated group ($p< 0.001$). The treatment of the rats with MC reversed the caspase-3 elevation ($p< 0.001$), as shown in Fig. (**2b**).

As demonstrated in (Fig. **3**), GSH level meaningfully reduced in MTX-treated group more than the control group ($p< 0.001$). When comparing MTX-treated group with MTX-MC group, a significant elevation was found in the amount of GSH in MTX-MC group.

4. DISCUSSION

The aim of the current study was to show that single-dose methotrexate-induced renal damage can be healed with *Momordica charantia* treatment. In order to prove healing in a targeted way, DNA damage, lipid peroxidation parameters, and apoptotic caspase-3 marker were performed in the study. Our results show that MC fruit extract administration to experimental animals with MTX-induced kidney damage has reversed nephrotoxicity with its phenolic content. Chemotherapeutic agents like methotrexate are used to treat neoplastic illnesses [34]. However, they lead to the accumulation of free oxygen radicals in tissues. Free radical accumulation impairs the functional process of all tissues because DNA damage and/or lipid peroxidation occur in these tissues. Previous studies reported that bitter melon has many advantageous therapeutic properties like antiapoptotic [35] and antioxidant activities [30]. In a performed study, it was demonstrated that MDA and MPO levels increased in MTX-treated mice and oral MC administration significantly reversed and normalized these levels [36]. In parallel to this study, MDA level decreased with the bitter gourd treatment in another study [37]. Due to this effect, many researchers considered that bitter melon can be consumed as an alternative antioxidant treatment in order to prevent tissue damage if it is standardized. A different study investigated the impact of melatonin on liver and kidney tissues of MTX-treated rats. As might be expected, while MDA and MPO levels rose and GSH level reduced in MTX-toxicated group, melatonin treatment improved all parameters [38]. In a different study, effects of MTX were examined on stomach tissue of thirty male rats. Obtained results showed that MDA and MPO levels went up as a lipid peroxidation determinant and inflammatory response indicator, respectively [39]. In another study, the effect of bitter melon polysaccharide extract on endothelial dysfunction in case of myocardial infarction. MC was given during twenty-five days, and MDA, MPO, and caspase-3 levels reduced at the end of the experiments. Based on this, it has been shown that bitter gourd might be preferred as a protective agent against myocardial infarction [40]. Similar to findings of studies performed before, the current study showed similar findings that when MTX was given, MDA and MPO levels increased in kidney tissue; however, bitter melon treatment essentially reduced both MDA and MPO levels when compared to control group. Furthermore, regarding the mechanisms of kidney toxicity induced with MTX, various theories have been put forward; oxidative stress is likely among these. 8-OHdG is the determinant of oxidative DNA damaged in tissues [41]. In a study investigating the protective effects of castacin and myricetin on MTX-toxified liver tissues, 8-OHdG

was also observed beside different parameters. Findings showed that MTX plus castacin and MTX plus myricetin groups were found significantly different than MTX-treated group [42]. In another study performed whether thiamine and/or thiamine pyrophosphate has protective effect on MTX-generated oxidative stress, while thiamine pyrophosphate caused significant decrease in 8-OHdG level MTX-toxified hepatic tissue in rats, only thiamine administration did not cause a decrease when compared to control group [43]. Moreover, parallel to previous studies, an increase in 8-OHdG level was observed in rats with MTX-induced reproductive toxicity. Results of the study showed that administration of bitter melon fruit extract improved the 8-OHdG level in the testis and epididymis tissues [44]. Findings of our study showed bitter melon improved the 8-OHdG level in MTX-treated group in line with previous studies. Caspase-3 is the primary executor caspase in apoptosis. Increased caspase-3 expression shows apoptosis activation [44]. It is well known that the use of methotrexate disrupts the apoptosis mechanism in cells. In many studies investigating the MTX-induced toxicity of several tissues, it was stated that caspase-3 activity rose up in MTX-administrated group [45]. Another study investigated the impact of MTX in patients with psoriasis in terms of oxidative stress determinants and apoptosis parameters and they reported that caspase-3 expression increased [46]. In a study related to preventive pathways of thymoquinone on MTX-induced toxicity in intestines, it was indicated that while MTX administration caused increased expression of caspase-3, thymoquinone treatment significantly reversed caspase-3 marker when compared to MTX group [47]. Another study clarifying the liver protection effect of rhein on MTX-induced hepatotoxicity reported that rhein treatment reduced caspase-3 in MTX-treated rat liver [48]. Moreover, protective effect of propolis on MTX-induced nephrotoxicity rats was shown in a study [7]. Obtained findings revealed propolis was a potent antioxidant as well as reduced caspase-3 activity. Consistent with studies performed before, it was observed that bitter melon treatment differently reduced the caspase-3 activity in current study. Glutathione (GSH), an essential antioxidant enzyme defense system against ROS, plays an important restrictive role in the reactions of free radicals. If GSH level is not enough, extensive lipid peroxidation-mediated damages occur in the cell membrane of the tissue. Methotrexate exposure is believed to cause cell membrane damage with the same mechanism [49]. Several studies were performed on different tissues treated with MTX, and antioxidant parameters were examined like GSH. In a study, investigating whether resveratrol has a beneficial effect in rats with MTX-induced hepatic damage in rats, while MTX reduced GSH level in hepatic tissue, concomitant use of resveratrol with MTX significantly improved GSH level [50]. Another study examined the protective effect of apigenin, which is an important flavonoid with anti-inflammatory and antioxidant properties, on MTX-treated hepatotoxicity and nephrotoxicity. Findings demonstrated that GSH depletion was observed in MTX-treated rats. However, apigenin usage replenished the GSH levels in both kidney and liver tissue, resulting in reversing of toxicities in liver and kidney [51]. A different study on diabetic rats investigated the antioxidant effects of *Momordica charantia* was examined in cardiac tissue. It was observed that while GSH level was low in the cardiac tissue of diabetic control rats, bitter melon treatment differently changed the GSH level when compared to diabetic control rats [52]. Consistent with other studies, it was clearly showed that bitter melon had antioxidant property in STZ-induced diabetic rat model [53]. Obtained findings indicated that *M. charantia* reversed GSH level approximately to normal level. Similar to studies performed before, the obtained findings of the current study reported the possibly antioxidant effect of *M. charantia* that improved GSH level in animals with MTX-induced nephrotoxicity.

CONCLUSION

In conclusion, obtained data of current study support the hypothesis that *M. charantia* extract had potential antioxidant property on MTX-induced kidney damage in rats. Findings showed that while MTX treatment changed parameters negatively, administration of bitter melon extract to MTX-induced toxified group essentially improved and normalized. Obtained findings suggest that bitter gourd might be regarded as a supportive therapeutic agent in preventing nephrotoxicity in patients using toxic, neoplastic agents.

AUTHORS' CONTRIBUTIONS

Caglar Macit: Study Design, Analysis, Investigation, Writing. Dilek Ozbeyli: Data analysis, Investigation. Ozge Cevik: Study Design, Data analysis. Melisa Cetin: Data analysis, Investigation, Writing. Goksel Sener: Supervision, Study Design, Data Analysis, Writing.

LIST OF ABBREVIATIONS

8-OHdG	=	8-hydroxy-2-deoxyguanosine
C	=	Control
DHFR	=	Dihydrofolate Reductase
GSH	=	Gluthation
MC	=	*Momardica charantia*
MDA	=	Malondialdehyde
MPO	=	Myeloperoxidase
MTX	=	Methotrexate
STZ	=	Streptozotocin

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical Approval was obtained by the Marmara University School of Medicine Animal Care and Use Committee (54.2020.mar).

HUMAN AND ANIMAL RIGHTS

No humans were used. All animal procedures were performed according to the guideline of Laboratory Animal Experiments Local Ethics Committee of Marmara University Research Involving Animals.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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