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Capsaicin ameliorate the nephrotoxicity induced by methotrexate

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Abstract: Although methotrexate (MTX) is an effective immunosuppressive and anti-cancer agent, it is associated with side effects, including nephrotoxicity. Capsaicin, a component of hot chilli peppers, induces rapid desensitization of TRPV1 pain receptors and therefore has uses in pain treatment. Capsaicin also has anti-cancer activity, including anti-inflammatory properties. Thus, capsaicin may have potential in preventing MTX-induced nephrotoxicity. The purpose of this research work was to observe protective effects of capsaicin towards renal toxicity caused by methotrexate and mechanisms responsible for these effects. As expected, capsaicin had nephroprotective effects in MTX-intoxicated rats. Serum creatinine urea, nitric oxide (NO) and renal malondialdehyde (MDA) levels decreased significantly, with a concurrent increase in superoxide dismutase (SOD) and renal glutathione peroxidase (GPx) activities as compared to rats that had been untreated with nephrotoxic. Biochemical analyses confirmed the protective effects of capsaicin. We conclude that capsaicin provides protection against MTX-nephrotoxicity in rats via anti-inflammatory and antioxidant activities.

Keywords: Antioxidant, creatinine, capsaicin, methotrexate, nephrotoxicity.

INTRODUCTION

Previous research demonstrated the role of methotrexate (MTX) as a treatment for inflammatory diseases and malignancies (Cetin *et al.,* 2017). Methotrexate belongs to folic acid antagonist and an anti-cancer agent, which become unlike other agents; its administration allows for wide dose range as termed in rheumatoid treatment as high dose methotrexate (HDMTX) (Fukasawa *et al*., 2014). MTX is commonly combined with other disease-modifying anti-rheumatic drugs when additive therapeutic value has been clearly demonstrated. Liver toxicity is a major adverse effect of MTX, and guidelines for liver toxicity monitoring have been developed (Howard *et al*. 2016). The pathogenesis of MTX nephrotoxicity involves multiple pathways, which include both inflammation and oxidative stress (Mahmoud *et al.,* 2016). Different agents are used at varying degree of success to help ameliorate nephrotoxicity of MTX such as curcumin (Selvakumar *et al.,* 2009). Toxicity is a function of the drug concentration and duration of exposure with acute renal, liver and central nervous system toxicity occurring at millimolar concentrations (Yilmaz *et al*., 2018). MTX-induced toxicity is the result of high-dose therapy, with nephrotoxicity rare at low doses of the drug (Heidari *et al*. 2018). Previous research observed gastrointestinal epithelial and bone marrow toxicity with MTX exposure at concentrations as low as 0.005 and 0.01μM for >24h (Izzedine *et al*., 2005). Maximum dose of methotrexate used in the treatment of cancers. According to the literature, there is a risk of severe MTX toxicity as long as a high concentration of MTX persists in the circulation (Howard *et al*. 2016). Hence, there is a need to address MTX-induced nephrotoxicity.

Capsaicin is a component of hot chilli peppers. When administered orally, absorption was as high as 94% as determined using a Wistar rat model. As pain medication, capsaicin induces rapid desensitization of TRPV1 receptors (Rabi *et al.,* 2008). Therefore, it is used as a treatment for various diseases, such as peripheral neuropathy associated with diabetes and arthritis. Capsaicin possesses anti-cancer and anti-inflammatory properties and ameliorates nephrotoxicity induced by MTX and even cisplatin. Previous research has examined different agents and antioxidants (Fukasawa *et al.,* 2014). The curcumin and capsaicin possess protective effects against renal toxicity through their anti-inflammatory and antioxidant properties (Shehzad *et al.,* 2013). The purpose of this study was to explore the effect of capsaicin on methotrexate-induced renal toxicity.

In order to evaluate the protective effects of capsaicin against methotrexate renal toxicity, we worked on various parameters that included, serum creatinine, nitric oxide (NO), renal malondialdehyde (MDA) levels, total antioxidant capacity (TAC) and caspase-3 expression in the kidneys. We also evaluated renal histopathology in order to reveal the protective effects of capsaicin against methotrexate toxicity.

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Since curcumin and capsaicin both possess anti-inflammatory and antioxidant properties against methotrexate induced renal toxicity. So, further research work should be needed to observe the protective effects of curcumin and capsaicin against methotrexate induced renal toxicity at the same doses of curcumin and capsaicin and with similar research methodology.

MATERIALS AND METHODS

Drugs

Methotrexate along with capsaicin were purchased by Sigma-Aldrich, United State Of America. MTX was mixed in physiological saline, and capsaicin was prepared in a solution of carboxymethylcellulose (0.5%). Methotrexate and capsaicin doses were selected that based on the previous study in our lab (Aldossary *et al.,* 2019).

Animals

Male Sprague-Dawley rats (N=40, weight: 200-250 g), provided by the animal house, college of medicine, king faisal university. The animals were housed under a 12 hours light/12 hours dark cycle and 24ºC, 45% humidity. All animals had free access to tap water and commercial chow ad libitum. All experiments were conducted according to the standard guidelines for animal research, that were provided by Deanship of Scientific Research and accepted by local animal research committee (KFU-REC/2019-12-02, G12/01/2019).

Drug treatments

The animals were randomly divided into four treatment groups. Each group contains 10 animals. The animals of group 1 (control group) received physiological saline everyday via an intraperitoneal injection for 7 days. Group 2 received MTX (20 mg/kg) via intraperitoneal injection daily for 7 days, and CMC via oral route. In this group, oral CMC was given 1 day before the administration of methotrexate. The animals of group 3 received methotrexate and a final treatment along with capsaicin (10mg/kg/d, p.o.) in 5% CMC-Na (5ml/kg). As in group 2, group 3 animals received 5% CMC-Na (5 ml/kg) 1 day before administration of MTX. Finally, group 4 received capsaicin (10mg/kg/day, p.o) only in 5% CMC-Na (5ml/kg) for 7 days.

Sampling and biochemical processes

By the end of the experiment, the rats were euthanized by 70 mg/kg, i.p. thiopental. Blood samples were collected through left ventricular puncture. A commercial colorimetric kit (Biovision Inc., United State of America) was used to measure the serum creatinine level. The kidneys were dissected and subjected to different treatments. The right kidney was homogenized in potassium phosphate buffer (pH 7.4, 0.05M). The obtained homogenate was centrifuged at 5,000 rpm for 10 min at 4ºC. The levels of nitric oxide, malondialdehyde and total antioxidant capacity (TAC) in the supernatant were determined using colometric kits (Biovision Inc., USA). The activity of caspase-3 was also evaluated using a colorimetric kit (R&D Systems, USA).

The left kidneys were fixed in formalin 10% solution, and embedded in paraffin wax. Sections were cut at 5µm, stained with hematoxylin and eosin (H & E), and examined under light microscope.

STATISTICAL ANALYSIS

The experiment data obtained are expressed as mean S.E.M one way anova was used to analyses the data. The difference of statistical significance was placed under consideration of p 0.05. The software prism with version 8.4.3 was used for statistical analysis.

RESULTS

The results indicated that capsaicin treatment greatly reduced the urea and serum creatinine levels in rats intoxicated by methotrexate as compared with those in the control group and MTX-treated group (fig. 1). Otherwise, capsaicin on its own did not alter renal function.

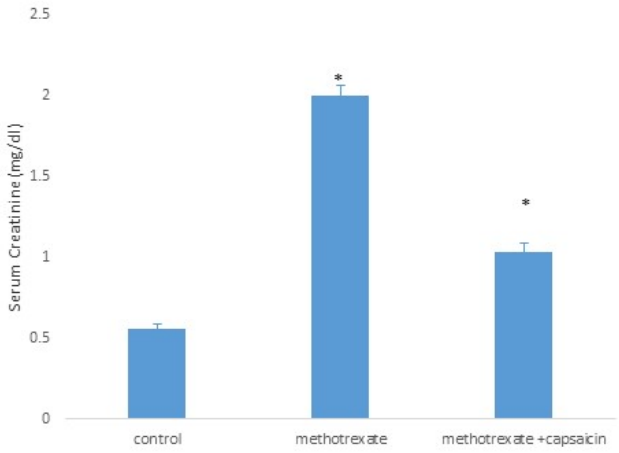

Fig. 1: Effect of capsaicin on serum creatinine in rats received methotrexate (MTX). * Significantly different p<0.05.

In addition, treated rats with MTX administration significantly elevated renal MDA, NO, and significantly reduced kidney total antioxidant capacity (TAC), when compared to the control group animals (p<0.05). However, pre treatment rats with capsaicin significantly reduced renal MDA and NO, and significantly increased total antioxidant capacity (TAC) in kidneys of rats intoxicated with methotrexate (p<0.05) (fig. 2). To assess lipid peroxidation, MDA levels were measured by an aliquot. They were evaluated through double heating method, that based on spectrophotometry use in

measurement of colour generated by reaction of MDA and thiobarbituric acid (TBA) at 532nm. The total antioxidant capacity (TAC) level was assessed with the help of ferric, which reduced tissue ability.

There was minimum caspase-3 expression in kidneys of control group and capsaicin-treated group. In methotrexate treated group, caspase-3 was significantly unregulated within the epithium that lined the kidney tubules. The combination of MTX and capsaicin significantly reduced caspase-3 expression when compared with methotrexate (MTX) only (fig. 3).

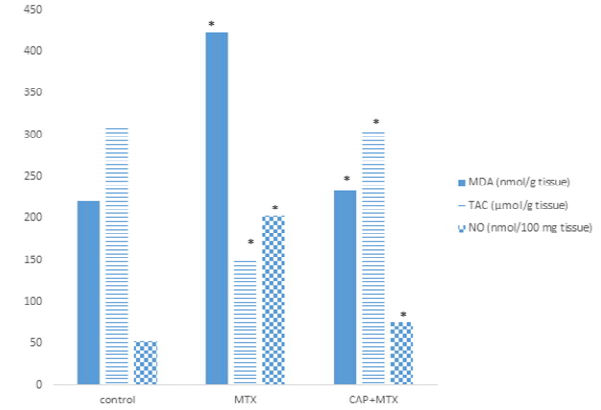

Fig. 2: Effects of capsaicin on kidney MDA, NO and TAC in rats received MTX. * Significantly different $p<0.05$.

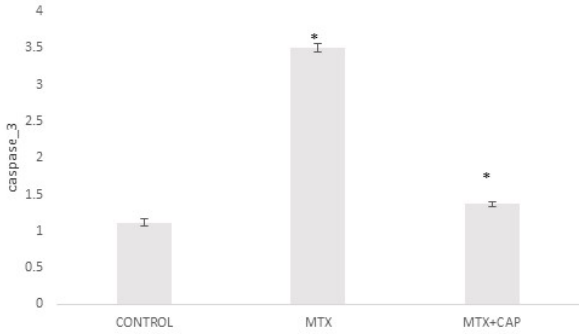

Fig. 3: Effects of capsaicin on caspase-3 in kidneys of rats received methotrexate. * Significantly different $p<0.05$.

Histopathological examination showed abundant necrosis of renal tubules, degeneration and desquamation of renal tubular epithelium, vacuolization, interstitial edema and infiltration of leukocytes in rat kidneys that treated with methotrexate. However, pre treatment with capsaicin significantly reduced renal injury caused by methotrexate (fig. 4).

DISCUSSION

After comparison with the animals of control group, it was seen that blood urea and creatinine levels were high in animals treated with methotrexate, possibly due to a reduction in the renal filtration rate caused by MTX toxicity. As a folate antagonist, MTX is capable of inhibiting malignant cell proliferation through dihydrofolatereductase enzyme inhibition. It is commonly used to treat rheumatoid. At very low doses, MTX can inhibit hepatotoxicity, toxicity and nephrotoxicity in the reproductive and respiratory system (Abo-Haded *et al*., 2017). At high concentrations, MTX is distributed widely in the body, including in the liver, kidney, skin and spleen (Yuksel *et al*., 2017). It may be retained for several weeks in the form of polyglutamates in the kidneys and for several months in the liver. MTX at higher doses may cause diarrhoea, especially with more water consumption (Çetin *et al.,* 2017). It was seen that treatment with methotrexate causes bowel motility changes. The irritant and corrosive effects on gastrointestinal mucosa are the side effects with methotrexate treatment. (Howard *et al*., 2016). In addition to the toxicity observed in the animals that were used in the experiment, MTX also causes weight loss.

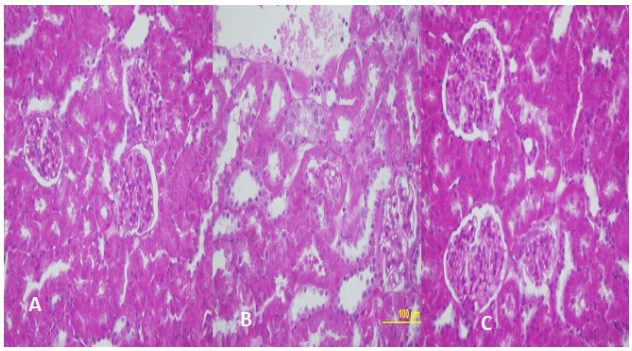

Fig. 4: H & E (40×) of rat kidneys from: (A) control group, showing normal renal histology; (B) methotrexate (MTX) treated group, showing marked distortion of kidney architecture, widespread necrosis of kidney tubules, dilatation of renal tubules, desquamation of renal epithelial cells, vacuolization, and coagulative necrosis; (C) Capsaicin + MTX showing that normal kidney structure is remained.

Increases in urea and renal levels in animals treated with MTX indicate nephrotoxicity (Izzedine *et al*., 2005). These changes, which are significant under the biochemical parameters, are a result of MTX on function and morphology of the kidney and liver (Kremer, 2004). However, the mechanism by which MTX induces hepatorenal damage remains unclear (Abo-Haded *et al*., 2017). Imparted levels of nitroxidate stress, oxidative stress parameters and downregulation of the TNF-α/NF-κB/COX-2 pathway of inflammation in rats treated with MTX have been linked to the development of hepatorenal damage (Abd *et al.,* 2016). The liver enzyme level increase, and that there is an increased rate of infiltration of inflammatory cell in pulmonary tissues of MTX-treated rats. The authors attributed this possibly to the Akt pathway and mitogen-activated protein kinase (Kolli *et*

al., 2009). Previous research indicated that capsaicin was well distributed in rat tissue and that it prevented MTX-induced nephrotoxicity by mitigating phase I enzymes affected by MTX, without any alteration in the outcome of MTX metabolism (Widemann & Adamson, 2006). Therefore, in addition to induce nephrotoxicity in rats, methotrexate also induces oxidative stress.

Although NO plays an important role in homeostasis and host defence, it can also be harmful, with involvement in the pathogenesis of numerous autoimmune and inflammatory diseases. The effects exerted by NO is either directly or via the formation of potent oxidants. The inflammatory reactions induced large amounts of super oxide and NO formations, resulting in the formation of peroxynitrite anions (Mahmoud *et al.,* 2017). This is able to nitrate the tyrosine residues phenolic ring in proteins. Animals treated with methotrexate showed intestinal injury through nitrosative stress (Kolli *et al.,* 2009). When treatment was done by MTX, the results showed increased nitrotyrosine staining and nitrate levels in samples of intestine with infiltration of neutrophils (Kandemir *et al*., 2017).

Hafez *et al*. (2015) reported that methotrexate treatment caused further degeneration of hepatic and renal functions by alteration of the creatinine levels. AL Haithloul *et al*. (2019) demonstrated that MTX caused oxidative stress, apparent by a decrease level of GSH and catalase activity with an increase in lipid peroxidation product MDA (Miyazono *et al.,* 2004). MTX also induced hepatorenal stress by increasing nitrate, a representative of the level of NO. Hepatorenal stress was confirmed due to upregulation of iNOS expression in liver and kidneys (Abd *et al.,* 2016). Previous reports demonstrated that increases in NO in the kidney were induced by MTX and MTX caused iNOS in small intestine. In one of the previous study, MTX exerted a proapoptotic effect by increasing caspase-3 expression in liver and kidney (Kolli *et al.,* 2008). Research also showed that capsaicin exhibited an antiapoptotic effect by decreasing the expression of the apoptotic marker caspase-3 induced by MTX. Capsaicin also mediated caspase-3 suppression in the liver caused by endotoxaemia (Armagan *et al*., 2015).

CONCLUSION

Capsaicin reduced nephrotoxicity induced by MTX and provided protection against oxidative damage. Capsaicin significantly reduced serum creatinine and levels of urea and alterations in MTX-intoxicated animals. Oxidative stress is important in development of methotrexate induced renal toxicity. NO plays a significant role in acute renal failure via the induction of free radicals, which contribute to tubular damage (Wiczer *et al*., 2016).

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