Reno-protective effect of mangiferin against methotrexate-induced kidney damage in male rats: PPARγ-mediated antioxidant activity

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ABSTRACT

Methotrexate (MTX) is an immunosuppressant used for the treatment of cancer and autoimmune diseases. MTX has a major adverse effect, acute kidney injury, which limits its use. Mangiferin (MF) is a natural bioactive xanthonoid used as a traditional herbal supplement to boost the immune system due to its potent anti-inflammatory and antioxidant activity. The present study evaluates the protective effect of MF against MTX-induced kidney damage. Male Wistar rats received MTX to induce nephrotoxicity or were pretreated with MF for 10 constitutive days before MTX administration. MF dose-dependently improved renal functions of MTX-treated rats and this activity was correlated with increased renal expression of PPARγ, a well-known transcriptional regulator of the immune response. Pretreating rats with PPARγ inhibitor, BADGE, reduced the reno-protective activity of MF. Furthermore, MF treatment significantly reduced MTX-induced upregulation of the pro-inflammatory (NFκB, interleukin-1β, TNF-α, and COX-2), oxidative stress (Nrf-2, hemoglobinase-1, glutathione, and malondialdehyde), and nitrosative stress (nitric oxide and iNOS) markers in the kidney. Importantly, BADGE treatment significantly reduced the anti-inflammatory and antioxidant activity of MF. Therefore, our data suggest that the reno-protective effect of MF against MTX-induced nephrotoxicity is due to inhibition of inflammation and oxidative stress in a PPARγ-dependent manner.

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1. Introduction

Mangiferin (MF) is a xanthonoid derivative found in the leaves of Mangifera indica Linn (Mango) and is traditionally used as an anti-inflammatory and antioxidant to protect against lifestyle and age-related diseases including diabetes, hyperlipidemia, cancer, cardiomyopathy, and nephropathy (Garrido et al., 2004; Khurana et al., 2016; Imran et al., 2017; Ding et al., 2018; Wang et al., 2018). The anti-inflammatory activity of MF is suggested to be mediated by; but not limited to, the downregulation of pro-inflammatory cytokines (IL-1β, TNF-α), suppression of cyclooxygenase-2 (COX2) and prostaglandins, along with inactivation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) activity (Imran et al., 2017). As a phenolic compound, MF has a potent antioxidant activity mediated by its iron chelating properties and free radical scavenging activities (Andreu et al., 2005). MF also increases the expression and activation of nuclear factor-erythroid factor 2-related factor 2 (Nrf2) and peroxisome proliferator-activated receptor γ (PPARγ), two key players in the antioxidant defense system (Zhang et al., 2015; Gold-Smith et al., 2016).

Nuclear factor-erythroid factor 2-related factor 2 (Nrf2) is a basic leucine-zipper transcription factor which binds and activates the antioxidant responsive element (ARE) in the promoter regions
of several cytoprotective genes. Nrf2 activation increases the expression of proteins regulating NADPH regeneration, reactive oxygen species (ROS) detoxification, thioredoxin-based antioxidant system, heme metabolism, and glutathione production (Tonelli et al., 2018). The expression and the activity of Nrf2, which is encoded by the gene nuclear factor erythroid 2 like 2 (NFE2L2), are highly regulated at the transcriptional and post-translational levels to control the cell response to different stressors (He et al., 2020). The promoter region of NFE2L2 gene contains binding sites for different transcription factors such as Nrf2 (positive feedback), aryl hydrocarbon receptor (AHR), and NFκB. Under normal condition, cytoplasmic Nrf2 protein remains inactive by binding to Kelch-like-ECH-associated protein 1 (KEAP1). Under stress condition, Nrf2 dissociates, and the active form translocates to the nucleus to bind and activate antioxidant gene expression (Tonelli et al., 2018).

Peroxisome proliferator-activated receptor-γ is a ligand activated transcription factor which regulates the expression of various genes involved in lipid metabolism, inflammatory response, and oxidative stress (Tyagi et al., 2011). Up on activation with endogenous or exogenous ligands, PPARγ heterodimerizes with retinoid X receptors (RXR) transcription factor then binds and activates PPARE response element upstream the target genes. PPARE target genes include Pparg (positive feedback), antioxidant genes such as heme oxygenase 1 (Hmox1) (Elshazly and Soliman, 2019), and inhibitor of NFκB, alpha (IkBα) (Scirpo et al., 2015), a negative regulator of NFκB activity. Previous studies suggested a connection between PPARγ and Nrf2 pathways against oxidative stress (Polvani et al., 2012; Lee 2017). PPARE agonists increased the expression of Nrf2 and Nrf2-regulated antioxidant genes such as glutathione-S-transferase (GST), suggesting that at least part of antioxidant activity of PPARE is mediated by Nrf2 (Park et al., 2004).

Methotrexate (MTX) is an antifolate immunosuppressant used for the treatment of cancer and autoimmune diseases (Widemann et al., 2004). MTX is highly cytotoxic and can cause damage to multiple organs due to the inhibition of purine metabolism (Tabassum et al., 2010). Acute kidney injury is the major adverse effect of MTX which limits its use. The mechanism of MTX-induced nephrotoxicity is not fully understood; however, it was suggested to be mediated by MTX crystallization, constriction of the afferent capillary, induction of inflammation, damage of mitochondria, and reduction of the antioxidant capacity in the kidney (Asci et al., 2017; Heidari et al., 2018; Kitamura et al., 2018). Prophylactic treatment is the most acceptable strategy to prevent MTX-induced kidney damage (Kitamura et al., 2018). Several animal studies have shown that natural products with antioxidant and anti-inflammatory properties may protect against MTX-induced kidney damage without affecting the therapeutic activity (Morsy et al., 2013; Yuksel et al., 2017; Younis et al., 2021). Based on its potent antioxidant and anti-inflammatory activities, we sought to determine if MF protects against MTX-induced nephrotoxicity and gain further insights into the underlying mechanism mediating this effect.

2. Materials and methods

2.1. Animals

Adult male albino Wistar rats weighing 180–250 g were used in the current study. Rats were obtained from the faculty of veterinary medicine, Zagazig University, Zagazig, Egypt. Experiments were performed on the rats after one week of acclimatization period. Rats were housed three per cage, food and water were available ad libitum, temperature was maintained at 23 ± 2 °C, and light/dark cycle was kept at 12/12 h.

2.2. Ethical considerations

Animal handling procedures and experimentation were approved by the Ethical Committee for Animal Handling at the Faculty of Pharmacy, Zagazig University, Zagazig, Egypt following the guidelines of the National Institutes of Health Guide for care and use of laboratory animals.

2.3. Chemicals

2.3.1. Drugs

Methotrexate (MTX) was obtained from Baxter Company (Cairo, Egypt). Bisphenol A diglycidyl ether (BADGE), Mangiferin (MF), and Kolliphor® EL were purchased from Milipore Sigma (St. Louis, USA).

2.4. Drug treatment and experimental design

Male Wistar albino rats were randomly divided into six groups. Group 1: Control group received the vehicle of the three drugs: MF (daily oral acacia gum solution (10% w/v)), BADGE [daily i.p. injection of Kolliphor® EL (9%): ethanol (9%): saline (1:1:9)], and MTX (single intraperitoneal (i.p.) injection of saline). Group 2: MTX group was injected i.p. with a single dose of MTX (20 mg/kg) to induce acute renal damage, daily oral acacia gum solution (10% w/v), and daily i.p. injection of Kolliphor® EL (9%): ethanol (9%): saline (1:1:9) (Abd El-Twab et al., 2016). Groups 3–5: Mangiferin (MF)-treated groups received daily oral doses of MF (10, 20, or 40 mg/kg) suspended in acacia gum solution (10% w/v) for 10 consecutive days starting at day 6 pre-MTX injection. Group 6: Bisphenol A diglycidyl ether (BADGE)-treated group received daily intraperitoneal doses of BADGE (15 mg/kg) dissolved in Kolliphor® EL (9%): ethanol (9%): saline (1:1:9) (Soliman et al., 2019) for 12 consecutive days starting at day 8 pre-MTX injection and daily oral doses of MF (20 mg/kg) for 10 consecutive days starting at day 6 pre-MTX injection (Fig. 1).

2.5. Sampling of blood and tissue

Three days post-MTX treatment, all animals were euthanized by isofluorane inhalation and blood samples were collected by cardiac puncture. Serum was separated by centrifugation of blood samples at 4000 rpm for 10 min and then stored at – 80 °C for biochemical analysis. The two kidneys of each rat were isolated, one kidney was flash frozen for preparation of renal homogenate and the other kidney was fixed in 10% formalin for the histopathological examinations.

2.6. Biochemical assays

2.6.1. Measurement of kidney function parameters

Blood urea nitrogen (BUN) and serum creatinine were measured in blood serum samples using colorimetric assay kits (Biodiagnostic, Giza, Egypt) following the manufacturer’s instructions.

2.6.2. Measurement of urinary excretion of NAG

Urine samples were collected and N-acetyl-β-D glucosaminidase (NAG) activity was measured using NAG activity colorimetric assay kit (Diazyme Laboratory, San Diego, CA, USA) following the manufacturer’s protocol.
2.6.3. Measurement of renal malondialdehyde and total glutathione levels

Renal malondialdehyde (MDA) and glutathione (GSH) contents were measured in kidney homogenates using quantitative colorimetric assay kits obtained from Bio-diagnostic Co. (Egypt) following the manufacturer’s instructions.

2.6.4. Measurement of TNF-α and IL-1β levels

The protein levels of TNF-α and IL-1β were measured in the kidney homogenate using Rat TNF- alpha ELISA kit (RayBiotech, Inc., Norcross, Georgia, USA) and IL-1β ELISA Kit (R&D System, Minneapolis, MN, USA), respectively, according to manufacturer’s instructions.

2.6.5. Measurement of phospho- and total NFκB protein levels

The protein levels of phospho- (pNFκB) and total NFκB p65 (tNFκB) levels were measured in the kidney homogenate using Rat NFκB p65 ELISA kit (Cusabio Biotech Co., Ltd., Wuhan, China) and phospho-NFκB p65 ELISA kit (Cell Signaling Technology, Inc., Beverly, MA, USA), respectively, according to manufacturer’s instructions.

2.6.6. Serum NO level

Nitric oxide levels were measured in the serum samples using colorimetric NO assay kit (Bio-diagnostic, Egypt) according to manufacturer protocol. This assay is an indirect method for determination of NO by measuring its stable decomposition products nitrite and nitrate using Griess reaction with a mixture of naphthyl ethylenediamine and sulfanilamide (Moshage et al., 1995; Bryan and Grisham 2007).

2.6.7. Quantitative polymerase chain reaction (qPCR) analysis

Total RNA was extracted from frozen kidney tissues using Trizol reagent (Invitrogen, Carlsbad, CA) and RNAeasy Mini Kit (Qiagen, USA). cDNA was synthesized using RevertAid Premium Reverse Transcriptase-Kit (Fermentas International Inc., Burlington, Canada) and amplified using Maxima SYBR Green qPCR Kit (Fermentas International Inc., Burlington, Canada), specific primers listed in Table 1, and ABI prism 7500 sequence detector system (Applied Biosystems, Foster City, CA). CT values were normalized to GAPDH control and the fold change was calculated using ΔΔCT method (Livak and Schmittgen 2001).

2.7. Histopathological examination for the kidney

Kidney samples fixed in 10% formalin were processed through the routine histological procedures being dehydrated by immersion in various concentrations of ethanol (70%, 90%, then 100%) 3 times for 30 min each at room temperature, cleared in xylene for 20 min at room temperature, then embedded in paraffin wax at 58 °C. Paraffinized tissues were then cut into 4 μm sections using a microtome (Leica RM 2155, England). Kidney sections were rehydrated gradually by immersion in xylene 2 times for 10 min each, then various ethanol concentrations (100%, 95%, 70%, then 50%) 5 min each. Slides were then rinsed in deionized water, washed with PBS, and stained with hematoxylin and eosin (H&E). Stained slides were blindly examined and scored by expert pathologists.

2.8. Statistical analysis

Data were analyzed by one-way analysis of variants (ANOVA) followed by Tukey’s multiple comparison test using GraphPad.
3. Results

3.1. Mangiferin improves kidney function of MTX treated rats in a dose-dependent manner

Serum creatinine, blood urea nitrogen (BUN), and urinary N-acetyl-beta-D-glucosaminidase (NAG) were measured to determine the effect of MF on kidney and renal tubular functions. MTX injection significantly increased serum creatinine, BUN, and urinary NAG levels ($p < 0.05$) when compared to control group; indicating MTX-induced renal damage. Treatment with different doses of MF (10, 20 and 40 mg/kg) improved kidney functions in a dose dependent manner. MF decreased BUN (Fig. 2A, $p < 0.05$), serum creatinine (Fig. 2B, $p < 0.05$), and urinary NAG (Fig. 2C, $p < 0.05$); when compared to the MTX group.

3.2. Mangiferin increases renal PPAR$\gamma$ expression in MTX treated rats in a dose-dependent manner

PPAR$\gamma$ is highly expressed in major renal cells and plays a fundamental important role in regulating the inflammatory response in the kidney (Ma et al., 2020). In the present study, MTX-induced nephrotoxicity was associated with a significant reduction in PPAR$\gamma$ mRNA expression in the kidney. MF treatment showed an elevation in PPAR$\gamma$ expression in a dose-dependent manner. A dose of 10 mg/kg MF significantly increased PPAR$\gamma$ expression when compared to MTX group; however, 20 mg/kg and 40 mg/kg doses restored PPAR$\gamma$ to the normal levels (Fig. 3). No significant difference in PPAR$\gamma$ expression was observed between 20 and 40 mg/kg; therefore, 20 mg/kg was selected to determine the role of PPAR$\gamma$ in the reno-protective effect of MF against MTX-induced nephrotoxicity.

3.3. PPAR$\gamma$ inhibitor reduces the reno-protective effect of mangiferin

To determine if the reno-protective effect of MF is mediated by PPAR$\gamma$, animals were pretreated with a selective PPAR$\gamma$ antagonist, BADGE (15 mg/kg) (Soliman et al., 2019), before MF (20 mg/kg) treatment. BADGE significantly reduced MF-induced upregulation of PPAR$\gamma$ expression (Fig. 4A) and diminished the effect of MF on renal functions. Fig. 4 showed that BUN (Fig. 4B), serum creatinine (Fig. 4C), and urinary NAG levels (Fig. 4D) were significantly higher in rats treated with both BADGE and MF when compared to MF group. The levels of BUN, serum creatinine, and urinary NAG in the BADGE/MF group were comparable to the untreated MTX group indicating that the reno-protective effect of MF is PPAR$\gamma$-dependent.
3.4. Mangiferin reduced MTX-induced renal inflammation

MTX-induced nephrotoxicity was associated with renal inflammation as indicated by increased expression of NFκB (at both mRNA and protein levels), IL-1β, TNF-α, and COX2 in the kidney when compared to control group (Fig. 5A-E). MF treatment (20 mg/kg) significantly reduced the expression of these pro-inflammatory markers when compared to the MTX group. Importantly, concurrent treatment with MF and BADGE significantly increased the expression of NFκB (phosphorylated, pNFκB, and total, tNFκB, forms), IL-1β, TNF-α, and COX2 when compared to MF-treated animals. The level of IL-1β, pNFκB, and tNFκB protein expression in the MF + BADGE group was comparable to the untreated MTX group (Fig. 5B); while the levels of TNF-α, and COX2 were significantly less than MTX group (Fig. 5A, 5C, and 5D, respectively). Our data suggest that the anti-inflammatory activity of MF was mediated, at least in part, by PPARγ activation.

3.5. Mangiferin enhanced Nrf2 antioxidant defense in a PPARγ-dependent manner

MTX treatment reduced Nrf2 antioxidant response in the kidney as indicated by increased MDA content (a marker of lipid peroxidation), downregulated Nrf2 and Hmox1 expression, and reduced glutathione content (Fig. 6). The MF-treated group showed significant reduction in MDA content and upregulation of both Nrf2 and Nrf2 downstream targets (Hmox1 and GSH) (Fig. 6A-D). Concurrent treatment with MF and BADGE significantly diminished the effect MF on Nrf2 and Hmox1 expression as well as GSH content indicating that the antioxidant activity of MF is PPARγ-dependent.
3.6. Mangiferin reduced MTX-induced nitrosative stress in a PPAR-δ-dependent manner

MTX induced nitrosative stress in the kidney as indicated by increased NO production and iNOS expression. The MTX-treated group showed a significant elevation in the levels NO and iNOS mRNA when compared to the control group (Fig. 7A-B). The MF-treated group showed a significant reduction in these nitrosative stress markers when compared to the MTX group. Concurrent treatment with MF and BADGE significantly increased the levels of NO and iNOS (Fig. 7A-B) when compared to the MF group. NO and iNOS expression in the MF + BADGE group was comparable to the untreated MTX group suggesting that the activity of MF against nitrosative stress was PPAR-δ-dependent.

3.7. Histopathological findings

Examination of MTX group renal sections showed lobulated and atrophic glomeruli with desquamated epithelium for the disorganized renal tubules (Fig. 8B, C), compared to the control group with normal glomerular and tubular structure (Fig. 8A). MF treatment at different doses (10, 20, and 40 mg/kg) improved the histopathological alterations elicited by MTX (Fig. 8D-8F). The ameliorative effect of MF (20 mg/kg) on renal pathology was abrogated in animals co-treated with BADGE (Fig. 8G and Table 2.).

4. Discussion

Methotrexate (MTX) is one of the most common and effective immunosuppressant used for treatment of autoimmune diseases such as rheumatoid arthritis. MTX has multiple serious adverse effects including acute renal damage. The mechanism of MTX-induced nephrotoxicity involves the induction of oxidative stress and inflammation (Asci et al., 2017; Heidari et al., 2018; Kitamura et al., 2018). MF, a natural C-glucosyl xanthone derivative isolated from different parts of Mangifera indica, is a potent antioxidant and anti-inflammatory traditional medicine used to protect against age and lifestyle-related disorders including nephropathy (Imran et al., 2017; Wang et al., 2018). The present study demonstrated that MF protects against MTX-induced renal damage and this effect was associated with inhibition of oxidative stress and inflammation. Importantly, the use of BADGE, a well-known selective antagonist for the nuclear receptor PPAR-δ, diminished the antioxidant and anti-inflammatory activities of MF in MTX-treated rats. Therefore, our data reveals, for the first time, the reno-protective activity of MF against MTX-induced nephrotoxicity and this effect is PPAR-δ dependent.

Acute kidney injury is one of the major adverse effects of MTX limiting its use. Renal excretion is the primary route of MTX elimination; therefore, kidneys are vulnerable to the toxic effect of MTX and its metabolites. Previous studies have reported the precipitation of MTX and its metabolite, 7-hydroxy-MTX, in the renal tubules resulting in tubular necrosis, inflammation, and histological damage (Abdel-Raheem and Khedr 2014; El-Sheikh et al., 2015; Erboga et al., 2015). In addition, MTX triggers a renal accumulation of reactive oxygen (ROS) and reactive nitrogen (RNS) species due to the disruption of antioxidant defense system (Morsy et al., 2013; Yuksel et al., 2017). In agreement with these findings, the present study demonstrates that a single dose of MTX induced acute kidney damage which is indicated by histological damage and impaired functions. MTX-mediated nephrotoxicity was accompanied with upregulation of the inflammatory mediators, IL-1β, TNF-α, and COX2, downregulation of the antioxidant defense mediators, Nrf2, Hmox1, and GSH, as well as augmentation of nitrosative stress markers, iNOS and NO. Notably, MTX reduced the expression of PPAR-γ, a key regulator of inflammatory and immune response, and this effect may correlate with the pathological outcomes (Mahmoud et al., 2019).
Previous studies have revealed the renoprotective effect of MF in medical conditions associated with increased risk of renal failure. In diabetic rat model, MF improved the renal structure and function as indicated by the inhibition of glomerular extracellular matrix expansion, amelioration of renal interstitial fibrosis, and reduction of BUN, serum creatinine, and urine protein (Li et al.,...
In hyperuricemic nephropathy rat model, MF reduced renal inflammation and apoptosis (Li et al., 2020). In sepsis mouse model, MF attenuated the acute kidney injury induced by cecal-ligation and puncture (He et al., 2014). Moreover, MF protects against drug-induced nephrotoxicity. Sadhukhan and colleagues reported that MF attenuated cisplatin induced nephrotoxicity both in-vivo and in-vitro (Sadhukhan et al., 2018). Consistently, the present study showed that MF ameliorated MTX-induced nephrotoxicity as indicated by the improvement of renal function and histology. This effect was associated with an upregulation of PPAR-\(\gamma\), a master regulator of the cellular response to oxidative stress and inflammation (Kokeny et al., 2021).

Mangiferin is traditionally used to boost the immune system and reduce the oxidative stress. Previous studies have shown that the reno-protective effect of MF is related to its anti-inflammatory and antioxidant activity (Sadhukhan et al., 2018). In the current study we found that MF reduced the expression of inflammatory cytokines and oxidative stress markers in the kidney of MTX-treated animals. Importantly, this effect was reversed in animals co-treated with BADGE, a well-known selective PPAR-\(\gamma\) antagonist, indicating that the reno-protective effect of MF in MTX-induced kidney damage is PPAR-\(\gamma\)-dependent. Our findings are in line with previous findings showing that the anti-inflammatory effect of MF may be mediated by PPAR-\(\gamma\). In human osteoarthritis chondrocytes, MF inhibits IL-1\(\beta\)-induced inflammatory response, and this response was abrogated in the presence of PPAR-\(\gamma\) inhibitor (Qu et al., 2017). In addition, molecular docking studies performed by Singh and colleagues suggest a direct interaction between MF and PPAR-\(\gamma\) receptor (Singh et al., 2018). Moreover, several studies have shown that MF modulates PPAR-\(\gamma\) downstream signaling specifically linked to NF\(\kappa\)B activation (Mahmoud-Awny et al., 2015; Mei et al., 2021).

NF\(\kappa\)B is a transcription regulator activated by ROS and several inflammatory cytokines to promote the pro-oxidant and pro-inflammatory gene expression (Liu et al., 2017). In resting condi-

Table 2

| Histological changes          | Control | MTX  | MF 10 | MF 20 | MF 40 | MF 20 + BADGE |
|------------------------------|---------|------|-------|-------|-------|---------------|
| Damaged glomeruli            | --      | ***  | ++    | ++    | +     | +++           |
| Luminal casts                | --      | ++   | --    | --    | --    | ++            |
| Disorganized tubules         | --      | ***  | +     | +     | +     | +++           |
| Extravasated haemorrhage     | --      | ++   | +     | --    | --    | ++            |

Histopathological scores were designed as follows: (−, negative; *, weak; ++, moderate; ++++, severe).
tions, inactive NFκB is located in the cytoplasm bound to the inhibitory molecule (IκB). Upon activation, IκB is phosphorylated by IκB kinase (IκK) releasing the active form of NFκB which then translocates to the nucleus and increases the expression of pro-oxidant genes such as iNOS and pro-inflammatory genes such as IL-1β, TNF-α, and COX2 (Liu et al., 2017). PPAR-γ is a fundamental repressor for NFκB activity (Ma et al., 2020). Activation of PPAR-γ inhibits the transcriptional activity of NFκB and increases the expression of inhibitor of NFκB, alpha (IκBα), a negative regulator of NFκB activity (Scirpo et al., 2015). Previous studies have shown that MF inhibits NFκB activity in various inflammatory conditions including kidney disorders (Mahmoud-Awany et al., 2015; Sadhukhan et al., 2018; Mei et al., 2021). Nonetheless, the present study reports for the first time that the MF effect on NFκB is mediated by the activation of PPAR-γ since the use of BADGE, PPAR-γ antagonist, reversed MF-induced downregulation of NFκB (both total and phosphorylated forms) and its targets, iNOS, IL-1β, TNF-α, and COX2. Therefore, the anti-inflammatory activity of MF in MTX-induced kidney damage is attributed, at least in part, to PPAR-γ/NFκB signaling pathway.

PPARγ and Nrf2 are two fundamental synergistic players in establishing the intracellular antioxidative defense system against drug-induced oxidative damage (Lee 2017). Previous studies have suggested the positive feedback loop connecting PPARγ and Nrf2 signaling to reinforce the expression of one another and maintain their activity against oxidative stress. Huang et al (2010) and other researchers reported that PPARγ is a target gene for Nrf2 transcription activity since Nrf2 directly bind to ARE identified in the promoter region of the PPARγ gene (Huang et al., 2010; Lee 2017). On the other hand, Park et al. reported that PPARγ activation induces the transcription of Nrf2-regulated antioxidant genes such as Hmox1 and GST (Park et al., 2004; Chorley et al., 2012). In the present study, MF reduced MTX-induced oxidative stress in kidney and this effect correlated with upregulation of Nrf2, Hmox1, and GST. Interestingly, PPARγ antagonist abrogated the antioxidant activity MF and reduced the expression of these antioxidant mediators. Therefore, our data suggests that the antioxidant activity of MF in MTX-induced nephrotoxicity is mediated by PPARγ/Nrf2 signaling pathway.

5. Conclusion

Our study revealed the protective effect of MF against MTX-induced renal damage. This reno-protective effect correlates with the powerful anti-inflammatory and antioxidant activity of MF. Importantly, our results provide some evidence that MF activity is mediated by the activity of PPARγ/Nrf2 signaling. The limitation of our study was that we did not investigate how MF activates PPARγ. Therefore, future studies are still required to determine whether MF directly activates PPARγ by binding the active site or indirectly by increasing the expression of PPARγ endogenous ligands.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Consent to Participate

We confirm that all authors have participated, reviewed, approved, and consented to the submission of this manuscript.

Consent to Publish

We confirm that all authors consented to publish this manuscript.

Authors contributions

Authors contributed equally to the manuscript. SME, MMA, SHA, and ES conceived and designed the research. SME, MMA, and SHA conducted experiments and contributed to the research methodology. ES, SHA, SME, and MAA analyzed data. ES and SHA wrote the initial version of the manuscript. All authors read, reviewed, and approved the manuscript.

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