Dimethyl Fumarate attenuates Di-(2-ethylhexyl) phthalate-induced Nephrotoxicity, Crosstalk between Nrf2 and NFκB Signaling Pathways

Sorour Ashari (sorour.ashari424@gmail.com)
Mazandaran University of Medical Sciences

Nasrin Ghassemi Barghi
The Institute of Pharmaceutical Sciences(TIPS), Tehran university of Medical sciences

Abouzar Bagheri
Mazandaran University of Medical Sciences

Research Article

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Abstract

**Introduction:** Dimethyl fumarate (DMF), the methyl ester of fumaric acid, exhibited various pharmacological effects. The aim of this study was to clarify the potential nephroprotective effect of DMF against DEHP-induced nephrotoxicity.

**Methods:** HEK-293 cells were exposed to different concentrations of DMF plus IC50 concentrations of monoethylhexyl phthalate (MEHP) to evaluate possible cytoprotective effect of DMF. For more evaluation, rats were divided into 7 groups (n=6 per group). Corn oil group, DEHP group (200 mg/kg dissolved in corn oil by gavage). DMF (15, 30, and 60 mg/kg) plus DEHP (200 mg/kg) groups, vitamin E (20 mg/kg) plus DEHP (200 mg/kg) group and DMF (60 mg/kg) alone (45 days).

**Results:** DMF significantly decrease MEHP-induced cytotoxicity DMF improved BUN and creatinine levels compared to DEHP in a dose-dependent manner. Furthermore, DEHP-induced mitochondrial dysfunction was reduced by DMF. To determine the molecular signaling involved in the nephroprotective effects of DMF, the expression of the p65 nuclear factor kappa B (NFκB), tumor necrosis factor alpha (TNFα), nuclear factor E2-related factor 2 (Nrf2), and heme oxygenase-1 (HO-1) genes was measured by real-time PCR. p65NFκB and TNFα expression was increased, whereas Nrf2 and HO-1 expression was decreased in the DEHP group compared to the control group. These effects were reversed by using DMF.

**Conclusions:** The findings of the current study revealed that DMF can act as a nephroprotective agent against kidney damage induced by DEHP partly through inhibiting NFκB and improving Nrf2 signaling pathways.

Introduction

Di-(2-ethylhexyl) phthalate (DEHP) is a main plasticizer present in polyvinyl chloride (PVC) products to increase the flexibility of plastics [1]. DEHP, due to its noncovalent binding to plastics, can easily separate from products and contaminate the surrounding milieu [2]. The use of medical devices and contaminant food (mainly through packaging processes) are the most common exposure routes to DEHP [3]. The daily intake reported for DEHP by U.S. Environmental Protection Agency (EPA) was 20 µg/kg bw/day [4], whereas the tolerable daily intake (TDI) of DEHP was 50 µg/kg bw, as reported by the European Food Safety Authority (EFSA) [5]. Daily exposure to DEHP increased in persons who were on dialysis (4 mg/kg/day) [6]. After exposure, DEHP converts to a main metabolite, monoethylhexyl phthalate (MEHP), by intestinal lipases and esterase [7]. Renal elimination is the main elimination route for DEHP metabolites; therefore, the kidney can be considered a main target for DEHP [8].

DEHP and its metabolite induced or promoted renal system injury. Tubular injury [9], disruption of kidney development [10], proteinuria, glomerulonephritis [11], and progression of Wilms tumor (an embryonic cancer of the kidney) [12] were indicated following exposure to DEHP or MEHP. In addition, DEHP induced histopathological changes in kidney tissue, including glomeruli shrinkage, tubule cell dilation [13] glomerular swelling and a narrower renal capsular cavity [14].
Oxidative stress is one of the involved mechanisms in DEHP-induced nephrotoxicity [15]. The production of reactive oxygen species (ROS) following exposure to DEHP has been observed in various studies [16–18]. ROS can induce damage in cells and tissues mostly by influencing cellular macromolecules, including lipids, proteins, and DNA [19]. Nuclear factor erythroid 2-related factor 2 (Nrf2) is an important transcription factor in living cells that defends against oxidative stress [20]. Activation of the Nrf2/Keap1/ARE pathway promotes the expression of antioxidants and detoxifying enzymes such as superoxide dismutase (SOD), catalase (CAT), NAD(P)H-quinone oxidoreductase 1 (NQO1), glutathione (GSH) and heme oxygenase-1 (HO-1) [21, 22]. Nrf2 stimulation has been shown following exposure to both protective agents, including sulforaphane and fumaric acid esters, and nonprotective components, especially environmental pollutants [23–25]. Nrf2 signaling has a protective role in kidney disease [26].

Activation of the Nrf2 pathway by DEHP has been indicated in some previous studies [14, 27, 28]. Mitochondrial dysfunction is reported as another toxic mechanism in DEHP-induced nephrotoxicity [29, 30]. Mitochondria, as a main target for exogenous components, are triggered by both DEHP and MEHP [30–32]. This organelle is not only the main source of ROS production but also the main target of produced ROS [33]. The loss of mitochondrial membrane potential (MMP) induced following ROS attack to the mitochondria led to one of the main phenomena in cells, called programmed cell death or apoptosis [34, 35].

Nuclear factor-κB (NF-κB), a ubiquitous transcription factor, participates in the regulation of inflammatory processes by impacting the expression of various genes, such as those encoding tumor necrosis factor alpha (TNF-α), interleukins and cyclooxygenase-2 [36]. Activation of the NF-κB signaling pathway is a probable mechanism underlying the toxic effects induced by DEHP [18, 37]. DEHP increased the activation of NF-κB in mouse liver cells and in the rat mast cell RBL-2H3 cell line [38, 39]. The translocation of p65NF-κB into the nucleus was significantly enhanced in macrophage-like THP-1 cells after exposure to DEHP [40]. In addition, MEHP increased the nuclear translocation and expression of NF-κB in Wilms' tumor cells and in the testes of rats [12, 41]. By regarding Nrf2 and NF-κB signaling as pathways involved in DEHP/MEHP-induced nephrotoxicity, the use of agents that influence these pathways can be a useful strategy to improve DEHP/MEHP-induced nephrotoxicity.

Dimethyl fumarate (DMF, BG-12) is a novel oral therapeutic agent to treat multiple sclerosis (MS) disease. DMF has been shown to have various pharmacological effects, such as anti-inflammatory, anticancer, and antioxidant effects, via activation of the Nrf2 pathway [42–44]. Furthermore, DMF can induce its pharmacological effects by inhibiting NF-κB activity [45, 46]. The nephroprotective effect of DMF has been reported previously [47].

However, the effect of DMF against DEHP-induced nephrotoxicity has not been clarified. The aim of the present study was to determine the effect of DMF against DEHP-induced nephrotoxicity and to reveal its underlying mechanisms.

**Material And Methods**
Chemicals

Embryonic human kidney cells (HEK293 cell line) were obtained from the Institut Pasteur of Iran, Tehran. Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, and fetal bovine serum (FBS) were obtained from Gibco. All other chemicals used were purchased from Sigma–Aldrich company.

In vitro

Cell culture

The HEK293 cell line was cultured in DMEM containing 10% FBS and 100 IU/ml penicillin–streptomycin at 37°C in a humidified atmosphere (5% CO$_2$). 5×10$^4$ cells were placed in per well of 96 well plate. HEK293 cells were treated with MEHP (dissolved in 1% DMSO) at different concentrations (50, 100, 200, and 400 µM) to obtain the IC50 concentration, and various concentrations of DMF were dissolved in normal saline 2 h before MEHP treatment. DMF concentrations (25, 50, 100, and 200 µM) were selected based on previous studies [48, 49]. Untreated cells were considered the control group.

MTT assay

Cell viability was measured via an MTT method [50]. The treated cells were incubated with an MTT solution (0.5 mg/ml) and maintained for 4 hr at 37°C and 5% CO2. The supernatant was separated, and 200 µl DMSO was added to the cells. The absorbance was determined via an ELISA reader (Tecan, Rainbow Thermo, Austria) at 570 nm.

Oxidative stress parameters assay

In this study, GSH content and ROS and MDA levels were evaluated as oxidative stress parameters by using 50,50-dithio-bis(2-nitrobenzoic acid) (DTNB) [51], 20,70-dichlorofluorescein diacetate (DCFHDA) [52], and thiobarbituric acid (TBA) [53], respectively.

In vivo

Animals

Male Wistar rats weighing 250-300 g were obtained from the Animal Laboratory of Mazandaran University of Medical Sciences (Sari, Iran). The animals (42 rats) were kept under standard conditions (temperature 23 ± 2°C, humidity 55 ± 5%) with free access to water and food. The study procedures were in accordance with the approved instructions of the Committee of Animal Experimentation of Mazandaran University of Medical Sciences (Sari, Iran), which was in agreement with the ethical guidelines of the National Council for the Control of Animal Experimentation.

1.1 Experimental design
The animals were divided into seven groups with six animals in each group as follows: corn oil group (DEHP vehicle); DEHP group (200 mg/kg); DEHP (200 mg/kg) plus DMF (15, 30, and 60 mg/kg); DMF alone (30, 60 mg/kg); and DEHP (200 mg/kg) plus vitamin E (20 mg/kg). The selected doses were based on previous studies [29, 54]. The rats received treatments by gavage for 45 days. After treatment duration, the animals were killed by chloroform, blood was collected for biochemical assays, and kidney tissues were separated for more assessments.

Biochemical assay

The blood was centrifuged at 3000 rpm for 15 min for serum collection. Biochemical biomarkers of kidney injury, including creatinine and blood urea nitrogen (BUN), were measured in serum by using a commercial kit (Parsazmoon, Iran).

Isolation of kidney mitochondria

The mitochondria of the kidney were isolated by a previously described method [29]. Briefly, the kidney tissues were washed with cold mannitol buffer including 0.225 M D-mannitol, 75 mM sucrose, and 0.2 mM EDTA. The kidneys were homogenized by using a homogenizer device and centrifuged to remove nuclei and cell remains (1000 ×g, 10 min, 4°C). Mitochondria were centrifuged at 15,000 ×g for 10 min. The mitochondria were resuspended in mannitol buffer and centrifuged at 10 000 ×g for 10 min. This step was carried out twice. Regarding the type of assessment, the mitochondria were suspended in specific buffer solutions, such as Tris buffer (0.05 M Tris–HCl, 0.25 M sucrose, 20 mM KCl, 2.0 mM MgCl$_2$, and 1.0 mM Na$_2$HPO$_4$, pH = 7.4), respiration buffer (125 mM sucrose, 65 mM KCl, 10 mM HEPES, 20 mM Ca$^{2+}$, 5 mM sodium succinate), swelling buffer (125 mM sucrose, 65 mM KCl, 10 mM Hepes-KOH, 20 mM Ca$^{2+}$), and MMP buffer (68 mM mannitol, 220 mM sucrose, 10 mM KCl, 5 mM KH$_2$PO$_4$, 50 μM EGTA, 2 mM MgCl$_2$ and 10 mM HEPES). The cold chain was preserved in all mitochondrial isolation processes.

Protein concentration assay

The Bradford method was used to measure the protein concentration of samples described previously [55]. In brief, Coomassie blue was added to the mitochondria and different concentrations of bovine serum albumin (BSA). The samples were incubated for 10 min, and then the absorbance of the samples was determined by spectrophotometry (UV-1601 PC, Shimadzu, Japan).

Mitochondrial function assay

Mitochondrial function was evaluated by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [56]. The mitochondrial samples were incubated with 0.4% MTT (37°C, 30 min). MTT produces purple formazan in the presence of the succinate dehydrogenase enzyme. After adding DMSO (1 ml), the absorbance of the samples was measured by an ELISA reader at 570 nm (Tecan, Rainbow Thermo, Austria).
Mitochondrial membrane potential assay

Rhodamine 123 was used for MMP assessment [57]. Briefly, the mitochondria were suspended in MMP buffer (pH 7.2). After that, the samples were incubated with 10 µM rhodamine 123 (37°C, 30 min). The samples were centrifuged at 16,000×g for 5 min at 4°C. The fluorescence of the supernatant was determined at 490/535 nm (excitation wavelength and emission wavelengths) by using a spectrofluorometer (Jasco, FP6200, Japan).

Mitochondrial swelling assay

To evaluate mitochondrial swelling, the mitochondria were suspended in swelling buffer (pH 7.2), and the absorbance of mitochondrial fractions was measured for 60 min by an ELISA reader at 540 nm (Tecan, Rainbow Thermo, Austria). Mitochondrial swelling is shown by a decrease in absorbance [29].

Mitochondrial oxidative stress assay

To measure the level of ROS, 20,70-dichlorofluorescein diacetate (DCFHDA) was applied based on the method described previously [58]. Malondialdehyde (MDA) levels were measured as the lipid peroxidation index by using TBA as described previously [55]. The GSH contents were measured by using DTNB [59].

Real time PCR

Total RNA extraction of kidney tissue was carried out using an RNA extraction kit (Yekta tajhiz company, Iran). Quantitative and qualitative evaluations of extracted RNA were determined by NanoDrop and agarose gel electrophoresis, respectively. cDNA synthesis from total RNA was performed using a commercial kit (Yekta tajhiz company, Iran). A StepOne Plus Real-time PCR system (Applied Biosystems, USA) with RealQ Plus 2x Master Mix Green (Ampliqon, Denmark) was used to determine the mRNA expression of Nrf2, HO-1, p65NFκB, and TNFα. The primers sequences are indicated in Table 1. Alternations in the expression of each mRNA were normalized to β-actin as the endogenous control gene. The mRNA fold change was calculated by the $2^{-\Delta\Delta CT}$ method.

Table 1. Primers sequences
**Gene** | **Forward** | **Reverse**
--- | --- | ---
Nrf2 | 5´-CACCCACATTCCAAACAAAGATG-3´ | 5´-TATCCAGGGCAAGCGACTCA-3´
HO-1 | 5´-GCACAGGGTGACAGAAGAGG-3´ | 5´-AGGTAGTATCTTGACCAGGCT-3´
TNFα | 5´-ATGGGCTCCCTCTCATCAGT-3´ | 5´-GCTTGGTGGTTGCCAGGAC-3´
p65NFκB | 5´-CCTCATTTTCCCTCAGAGCC-3´ | 5´-TGCTTCTCTCAGGAAATAC-3´
β-actin | 5´-ACGGTCAGGTCATCCTATCG-3´ | 5´-CCACAGGGATTCCATACC-3´

**Statistical analysis**

The data analysis was carried out via Prism (version 8.0.2) statistical software. The results are shown as the mean ± SEM and were statistically analyzed through one-way ANOVA multiple comparison test followed by Tukey's multiple comparisons test. The statistical significance level was considered P < 0.05.

**Results**

2.1 **DMF decreased cytotoxicity induced by MEHP**

The cytotoxic effects of MEHP were evaluated by the MTT method, and the findings are shown in Fig 1A. The IC50 value of MEHP was determined after treating cells with various concentrations of MEHP (IC50=256.6 μM). To evaluate the effect of DMF on cytotoxicity induced by MEHP, the cells were treated with different concentrations of DMF 24 h before MEHP administration (IC50 concentration). Cell viability was significantly (p < 0.01) decreased in the MEHP group compared to the control group. However, pretreatment with DMF significantly (p < 0.05) increased cell viability at high concentrations compared with the MEHP group (Fig 1B). These findings indicated that DMF could decrease MEHP-induced cytotoxicity in a dose-dependent manner.

2.2 **DMF improved MEHP-oxidative damage in HEK293 cells**

ROS, MDA, and GSH levels were measured in HEK-293 cells as oxidative stress parameters. As shown in Fig 2A, B, the ROS and MDA levels were significantly (p < 0.001, p < 0.01, respectively) increased in the MEHP group compared with the control group and were significantly decreased in the high concentration of DMF compared with the MEHP group. The GSH content was significantly (p < 0.05) decreased in the MEHP group compared with the control group and was increased in the DMF group compared with the MEHP group (Fig 2C).
DMF improved DEHP–induced renal dysfunction

BUN and creatinine levels were measured as biomarkers of renal function. As shown in Fig 3A and B, BUN and creatinine levels were significantly \((p < 0.05\) and \(p < 0.01\), respectively) increased in the DEHP group compared with the control group. Pretreatment with DMF significantly decreased BUN and creatinine levels at a dose of 60 mg/kg compared with the DEHP group. There was no significant change in the mentioned biomarkers in the vitamin E group compared with the DEHP group.

DMF improved mitochondrial dysfunction, MMP collapse, and mitochondrial swelling induced by DEHP

Mitochondrial function was assayed by measuring succinate dehydrogenase activity. As shown in Fig 4A, the activity of succinate dehydrogenase was significantly \((p < 0.05)\) decreased in the DEHP treatment group in isolated kidney mitochondria compared with the control group and was significantly \((p < 0.05)\) increased in both the high-dose DMF treatment group and vitamin E group compared with the DEHP treatment alone.

The findings of MMP assay are indicated in Fig 4B. A significant decrease \((p < 0.05)\) in fluorescence intensity was observed in the DEHP group compared with the control group. DMF at doses of 30 and 60 mg/kg significantly increased the fluorescence intensity compared with the DEHP group. However, the change was not significant in the vitamin E group compared with the DEHP group. These findings confirmed the improvement of MMP collapse by DMF.

The mitochondrial swelling findings are shown in Fig 4C. Mitochondrial swelling was significantly \((p < 0.0001)\) increased in the DEHP group compared with the control group. DMF at doses of 30 and 60 mg/kg and vitamin E significantly \((p < 0.0001)\) decreased mitochondrial swelling compared with the DEHP group.

DMF improved DEHP–induced mitochondrial oxidative damage

The level of ROS is shown in Fig 5A. The ROS levels were significantly \((p < 0.01)\) increased in the DEHP group compared with the control group. Pretreatment with 30 and 60 mg/kg DMF and 20 mg/kg vitamin E significantly \((p < 0.01)\) decreased ROS levels compared with the DEHP group.

As shown in Fig 5B, a significant \((p < 0.01)\) increase was observed in MDA levels in the DEHP group compared with the control group. Pretreatment with 60 mg/kg DMF significantly \((p < 0.01)\) decreased MDA levels compared with DEHP alone. In addition, vitamin E treatment significantly \((p < 0.05)\) decreased MDA levels compared with the DEHP group.

As shown in Fig 5C, a significant \((p < 0.01)\) decrease in GSH content was observed in the DEHP group compared with the control group. A significant \((p < 0.05)\) increase was shown in the GSH content of 60 mg/kg DMF compared with DEHP alone. Vitamin E treatment had no significant effect on the GSH content compared with the DEHP group.
DMF improved the nephrotoxic effects of DEHP through activation of Nrf2 and inhibition of NFκB signaling pathways

To determine whether DMF induced its nephroprotective effect against DEHP through the Nrf2 and NFκB pathways, the gene expression levels of Nrf2, HO-1, p65NFκB, and TNFα were determined by real-time PCR. Because the most protective role of DMF was at a dose of 60 mg/kg, the mentioned dose was used to evaluate gene expression. As shown in figure 6, the mRNA expression levels of Nrf2 (Fig 6A) and HO-1 (Fig 6B) were significantly reduced in the DEHP group compared to the control group and were reversed in the DMF group. Furthermore, p65NFκB (Fig 6C) and TNFα (Fig 6D) mRNA expression was significantly increased in the DEHP group compared to the control group. However, DMF significantly decreased p65NFκB and TNFα mRNA expression compared to the DEHP group. It can be concluded that DMF probably improved DEHP-induced nephrotoxicity by influencing such signaling pathways.

Discussion

DEHP is well known as a food contaminant component that has different deleterious impacts on organism health. Most of these effects are due to long-term exposure to DEHP, and acute toxic effects following exposure to DEHP are low. In previous studies, the potential of DEHP to induce nephrotoxicity has been shown. In recent years, researchers have focused on determining the involved mechanisms in DEHP-induced nephrotoxicity and finding an effective way to address such toxicities. By considering previous studies, we aimed to evaluate the role of DMF against nephrotoxicity induced following subchronic exposure to DEHP.

In the current study, BUN and creatinine, as kidney functional biomarkers, were increased in the DEHP group, which was in agreement with the Aydemir et al. study that indicated urea level enhancement in 200 and 400 mg/kg doses of DEHP [60].

In our previous study, the role of oxidative stress and mitochondrial dysfunction in DEHP-induced nephrotoxicity was indicated [61]. In agreement with our previous study, in this study, oxidative stress, mitochondrial dysfunction and mitochondrial oxidative damage were observed in HEK-293 cells and kidney tissue of rats treated with MEHP and DEHP, respectively. ROS can attack mitochondria and trigger the lipid structure of the mitochondrial membrane, disturbing hemostasis of mitochondria and leading to MMP collapse and mitochondrial swelling, which are involved in the initiation of mitochondrial apoptosis [62]. Therefore, improvement of the antioxidant system of kidney tissue can be helpful to address DEHP toxic effects in the kidney.

In this study, the use of DMF improved oxidative stress, as shown by a reduction in ROS production and MDA levels and enhancement of GSH in MEHP-treated cells, and reduced mitochondrial dysfunction, as indicated by a reduction in mitochondrial swelling, MMP collapse, and mitochondrial oxidative damage in kidney tissues exposed to DEHP. DMF has high potential to deal with oxidative stress due to its role as an Nrf2 inducer.
Nrf2, as a main transcription factor, is vital to maintain hemostasis of the antioxidant system in cells due to its role in the expression of detoxification and cytoprotective agents such as HO-1, NQO1, and GSH. The effects of DEHP on the Nrf2 system have been evaluated previously [63–65]. The expression and protein content of Nrf2 and HO-1 were reduced in HEK-293 cells following exposure to DEHP [66]. DEHP reduced the protein level of Nrf2 in the kidneys of mice [67]. DEHP affected the Nrf2 system in the kidneys of quail in a dose-dependent manner [68]. DEHP at a dose of 250 mg/kg suppressed the Nrf2 pathway, while high doses of DEHP activated the mentioned signaling [68]. In this study, on the one hand, the expression of Nrf2 and HO-1 was reduced in the kidney tissue of rats treated with 200 mg/kg DEHP, and on the other hand, Nrf2 and HO-1 expression levels were increased in the DMF group compared to the DEHP group. Therefore, it can be concluded that the nephroprotective effect of DMF, in part, is because of its potential to activate the Nrf2 pathway.

HO-1 is a main target of Nrf2 signaling, and its nephroprotective effect has been shown previously [69, 70]. HO-1 is involved in heme degradation, and metabolites of this reaction, including bilirubin and carbon monoxide (CO), have ROS scavenger potential and therefore can decrease oxidative stress. Furthermore, HO-1 can reduce inflammation by suppressing the NF-κB pathway directly through proinflammatory cytokine inhibition and anti-inflammatory cytokine activation or indirectly through CO, which acts as a proinflammatory cytokine inhibitor [71]. Actually, there is cross talk between the Nrf2 and NF-κB pathways.

In this study, to determine the possible crosstalk between Nrf2 and NF-κB signaling to reduce DEHP-induced nephrotoxicity by DMF, the expression levels of p65NF-κB and its downstream gene TNF-α were evaluated. In previous studies, activation of the NF-κB signaling pathway was reported following exposure to DEHP and MEHP [18, 72–75]. In the current study, the findings indicated that DEHP could increase the p65NF-κB and TNF-α expression levels in the kidney tissue of rats. All of these findings were reversed by using DMF. Therefore, it can be a strong hypothesis that HO-1 enhancement following Nrf2 signaling stimulation by DMF could be involved in NF-κB signaling suppression.

In conclusion, this study, in agreement with other studies, confirmed the involvement of Nrf2 signaling in DEHP-induced nephrotoxicity. Furthermore, NF-κB involvement in DEHP-induced nephrotoxicity was revealed. In this study, DMF improved oxidative stress and mitochondrial dysfunction induced by DEHP and its metabolite, probably due to its potential to stimulate the Nrf2 signaling pathway and inhibit the NF-κB signaling pathway.

**Declarations**

**Ethics approval and consent to participate**

This study procedures were in accordance with the approved instructions of the Committee of Animal Experimentation of Mazandaran University of Medical Sciences (Sari, Iran), which was in agreement with the ethical guidelines of the National Council for the Control of Animal Experimentation.
Consent for publication

Not Applicable

Availability of data and materials

The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

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Authors’ contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Sorour Ashari], [Nasrin Ghassemi Barghi], and [Abouzar Bagheri]. The first draft of the manuscript was written by [Sorour Ashari] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not Applicable

References


**Figures**

**Figure 1**

The viability of HEK293 cells determined using MTT assay. Values are expressed as mean ± SEM for at least three experiments. Data were analysed by one-way ANOVA multiple comparison test followed by a tukey's multiple comparisons test. ##p < 0.01 versus control group. *p < 0.05 versus DEHP group.
Figure 2

The effect of DMF pretreatment on ROS, MDA, and GSH levels in HEK293 cells exposed MEHP. Values are expressed as mean ± SEM for at least three experiments. Data were analysed by one-way ANOVA multiple comparison test followed by a tukey’s multiple comparisons test. # p < 0.05, ## p < 0.01, ### p < 0.001 versus control group. * p < 0.05, ** p < 0.01, *** p < 0.001 versus DEHP group.
Figure 3

Effect of DMF treatment on levels of serum BUN, and creatinine in rats received DEHP. Values are expressed as mean ± SEM for at least three experiments. Data were analysed by one-way ANOVA multiple comparison test followed by a tukey’s multiple comparisons test. *p < 0.05, **p < 0.01 versus control group. *p < 0.05, **p < 0.01 versus DEHP group.
Figure 4

The effect of DMF treatment on kidney mitochondrial function (A), MMP (B), and swelling (C) in rats received DEHP. Values are expressed as mean ± SEM for at least three experiments. Data were analysed by one-way ANOVA multiple comparison test followed by a tukey's multiple comparisons test. #p < 0.05, ##p < 0.01 versus control group. *p < 0.05, **p < 0.01 versus DEHP group.
Figure 5

The effect of DMF treatment on kidney mitochondria level of ROS (A), MDA (B), and GSH (C) in rats received DEHP. Values are expressed as mean ± SEM for at least three experiments. Data were analysed by one-way ANOVA multiple comparison test followed by a tukey’s multiple comparisons test. *p < 0.05, **p < 0.01, ***p < 0.001 versus DEHP group. ## p < 0.05, ### p < 0.01, #### p < 0.001 versus control group.
Figure 6

The effects of DEHP and 60 mg/kg of DMF on mRNA expressions of Nrf2 (A), HO-1 (B), p65NFκB (C), and TNFα (D) in kidney tissue of rats. Values are expressed as mean ± SEM for at least three experiments. Data were analysed by one-way ANOVA multiple comparison test followed by a tukey's multiple comparisons test. *p < 0.05 versus control group. *p < 0.05 versus DEHP group.
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