ORIGINAL ARTICLE

ROLE OF DIMETHYL FUMARATE (NRF2 ACTIVATOR) IN REDUCING OF CIPROFLOXACIN-INDUCED HEPATOTOXICITY IN RATS VIA THE NRF2/HO-1 PATHWAY

DOI: 10.36740/WLek202305117

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ABSTRACT

The aim: The present study aims to study the effect of DMF on ciprofloxacin-induced liver damage as assessed by liver function and liver pathology and to study this effect if it is thought to activate the Nrf2 antioxidant defense mechanism.

Materials and methods: G1 (control), G2 (ciprofloxacin group), G3 and G4 (two DMF groups rats treated with DMF 50mg and 100mg), and G5 and G6 (two DMF groups rats treated with DMF 50mg and 100mg) (two ciprofloxacin Plus DMF at 50 mg and 100 mg). The tests included study of liver function, Nrf2 analysis, and anti-oxidant enzyme analysis.

Results: The serum blood Nrf2, HO-1, and tissue anti-oxidant enzymes all increased after ciprofloxacin treatment. The serum levels of Nrf2 and HO-1 were higher in the ciprofloxacin plus DMF groups, but anti-oxidant enzymes were lower. DMF increased Nrf2 expression in rats when ciprofloxacin caused hepatotoxicity.

Conclusions: DMF lowers experimental hepatotoxicity in vivo. This effect is thought to activate the Nrf2 antioxidant defense mechanism.

KEY WORDS: Nrf2, ciprofloxacin, HO-1, anti-oxidants

Wiad Lek. 2023;76(5 p.1):1001-1006

INTRODUCTION

Fluoroquinolones are a class of antibiotics that have a broad spectrum of activity against Gram-negative and Gram-positive bacteria. It is well accepted by patients, although it has been linked to many side effects, including unfavorable central nervous system effects and oxidative stress [1]. Oxidative stress not only causes of liver damage by causing of permanent damage to lipids, proteins, and DNA, but it also affects biological functioning through modifying pathways. Since these pathways of control gene transcription, protein expression, cell death, and hepatic stellate cell activation, so, the oxidative stress is one of the most important pathophysiological mechanisms behind the onset and progression of many liver illnesses [2]. Ciprofloxacin's hepatotoxicity results could be due to oxidative stress in the liver, induced by the drug's production of reactive radicals, which causes protein depletion in hepatocytes due to nucleic acid loss and DNA damage. This may lead to a significant decrease of mitochondrial number and its degeneration, what is responsible for energy production [3]. Many naturally occurring antioxidants are controlled by a family of transcription factors, including the nuclear factor-erythroid 2 related factor 2 (Nrf2). The antioxidant response element (ARE) is located in the promoters of several chemoprotective genes, including those that involved in the response to oxidative stress, and Nrf-proteins bind to it. The binding of Nrf2 to ARE boosts the transcription of numerous antioxidant genes, such as Super-Oxide Dismutase (SOD), Catalase (CAT), and Glutathione (GST) [4, 5]. Ciprofloxacin-induced hepatotoxicity was characterized by the elevation of liver damage hallmarks: Alanine aminotransferase (ALT), aspartate transferase (AST), and alkaline phosphatase (ALP). Amino-transferase is the most specific and routinely utilized indicator of hepatocellular necrosis. High levels of these enzymes may indicate liver damage. Anti-oxidant enzymes such SOD, and GSH, mainly derived from hepatocytes, biliary epithelial cells, liver tubules, pancreas, and intestine [6]. Elevated levels of serum enzyme activity have been considered as a sensible marker of hepatic disorders. The NRF2 field developed from biochemical and molecular discoveries on chemical transcription factors of hepatic detoxication enzymes, as well as

Table I. Distribution of anti-oxidant enzymes among study groups.

Groups/ Markers	Nrf2 ng/g of protein	HO-1pg/ g of protein	Catalase (U/l)	SOD (U/l)	GSH (U/l)	*p-value
G1	23.67 ± 0.72	15.39 ± 0.52	14.82 ± 0.29	19.97 ± 0.32	22.37 ± 0.94	0.00
G2	32.49 ± 0.43	16.34 ± 0.82	6.69 ± 0.35	11.84 ± 0.35	13.97 ± 0.51	
G3	42.66 ± 0.64	19.31 ± 0.60	14.83 ± 0.34	19.87 ± 3.25	22.50 ± 0.47	
G4	43.45 ± 1.99	19.94 ± 0.74	14.88 ± 0.25	19.81 ± 0.12	22.92 ± 0.69	
G5	38.03 ± 0.56	18.47 ± 0.66	12.31 ± 0.25	17.26 ± 0.18	19.23 ± 0.40	
G6	39.02 ± 0.50	18.95 ± 0.52	12.93 ± 0.09	19.48 ± 0.21	21.23 ± 0.56	

Each value is a mean of six animals ± SD, *=one-way ANOVA

subsequent characterization of the modulation of drug/toxicant-induced hepatotoxicity [7]. As a result, using of the Nrf2 pathway to stimulate the production of cytoprotective genes could be employed to treat liver diseases.

THE AIM

The aim of the study was to see, if DMF could protect the liver in an acute Cipro-induced hepatotoxicity chemical model.

MATERIALS AND METHODS

The hepatoprotective effects of DMF against Cipro were examined, because it has been found, that DMF activate Nrf2. Hepatotoxicity was studied in rats.

ANIMALS AND STUDY DESIGN

Six groups of 36 Sprague Dawley male rats will be formed. For a period of 30 days, each group has six rats. Drug preparation: Dimethyl fumarate dissolves in DMSO and is given intravenously at doses of 50 and 100 mg/kg, respectively. The animals were maintained in the animal house at the University of Kufa's Faculty of Medicine. The University of Kufa's Animal Care and Research Committee accepted the experiment, and the investigation followed the Laboratory Animals Guide Care. The animals has unlimited access to clean water and will be divided into the following groups: G1: DMSO is given for 30 days, G2**:** administered medication (ciprofloxacin) once daily by oral delivery at a dose of 100 mg/kg for 30 days [8], G3**:** IP administration of DMF at a dosage of 50 mg/kg once daily for a period of 30 days [9], G4**:** IP administration of DMF at a dosage of 100 mg/kg once daily for a period of 30 days [10], G5: For 30 days, ciprofloxacin 100 mg/kg was administered orally once daily, adding DMF IP at a dosage of 50 mg/kg starting on day 10 [11, 12], and G6: For 30 days, ciprofloxacin 100 mg/kg was administered orally once daily, adding DMF IP at a dosage of 100 mg/kg starting on day 10. Animals will be sacrificed by heart puncture under ketamine (25 mg/kg) and xylazine (5 to 10 mg/kg) anesthesia at the end of the experiment [13]. The animals were euthanized so that blood and liver tissues can be collected for further analysis [14].

MEASUREMENT OF OXIDANT PARAMETERS

Two commercial detection kits were used to assess the amounts of CAT, SOD, and GSH enzymes, as well as Nrf2 (*Nanjing Jiancheng Bioengineering Institute*) according to the manufacturer's procedure and a recent study [15].

STATISTICAL ANALYSIS

The data are presented as means with standard deviations (SD). The significance of differences in multiple group comparisons was established using the SPSS program 26.0 and one-way analysis of variance (ANOVA).

RESULTS

DMF-ASSOCIATED MINIMIZING OF THE OXIDATIVE STRESS AND INCREASING OF THE PRODUCTION OF ANTIOXIDANT ENZYMES IN LIVER TISSUES

DMF administration has previously been seen to improve the expression of Nrf2 and its downstream protein such as HO-1 [16, 17]. To investigate is the DMF's reno-protective impact in ciprofloxacin-induced hepatotoxicity is linked to Nrf2 activation (researchers increased the gene expression of Nrf2 and HO-1). According to our findings, DMF greatly protected the kidney against ciprofloxacin-induced damage via the Nrf2-HO-1 pathway. The levels of Nrf2 and HO-1 in tissue were considerably higher in the treated group than in the untreated done (Table I, Figure 1).

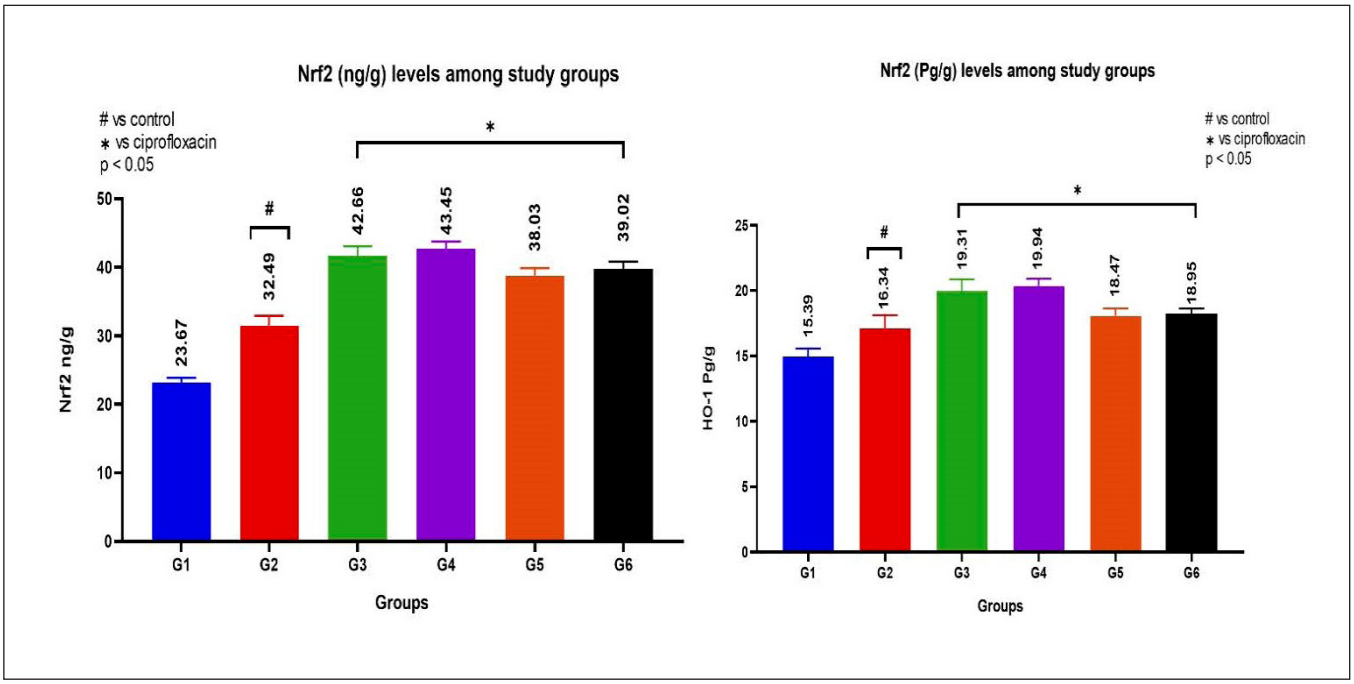

Fig. 1. Represent the Nrf2 and HO-1 enzyme tissue levels among groups.

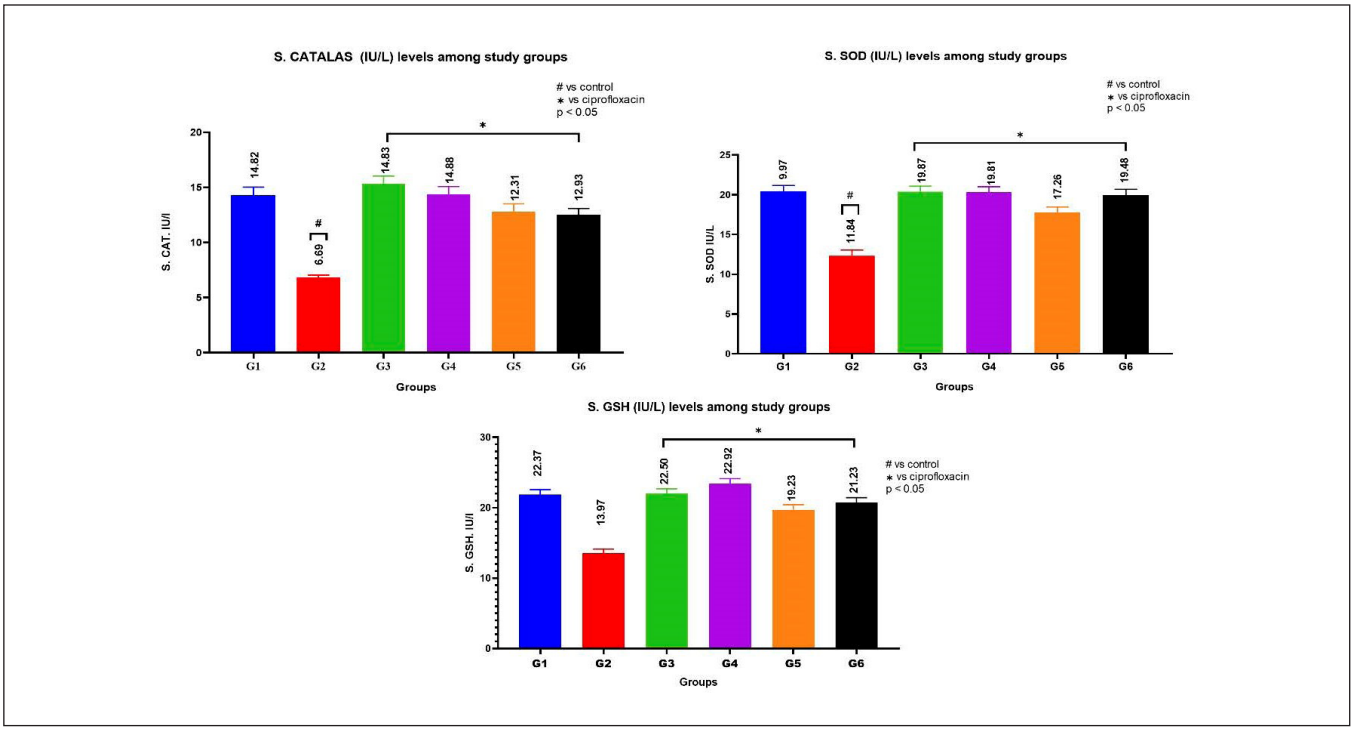

Fig. 2. Representation of the anti-oxidant enzymes serum levels (U/L) among study groups.

The antibacterial ciprofloxacin lowered the activity of the CAT, SOD, and GSH enzymes, but this effect was reduced by DMF treatment. Furthermore, the levels of antioxidant enzymes in the serum were altered in six groups, as shown in Table I and Figure 2.

The levels of ciprofloxacin in serum were considerably lower (P<0.05) in the G2 group than in the G1 group. In the ciprofloxacin plus DMF groups, DMF significantly increased these decreases in anti-oxidant levels, while anti-oxidant levels did not differ significantly between the control and ciprofloxacin plus DMF groups. In addition, there is no statistically significant difference (p>0.05) between the DMF groups with both G5 and G6 groups. There is, however, a significant increase (p<0.05) when we compared treated groups with the G2.

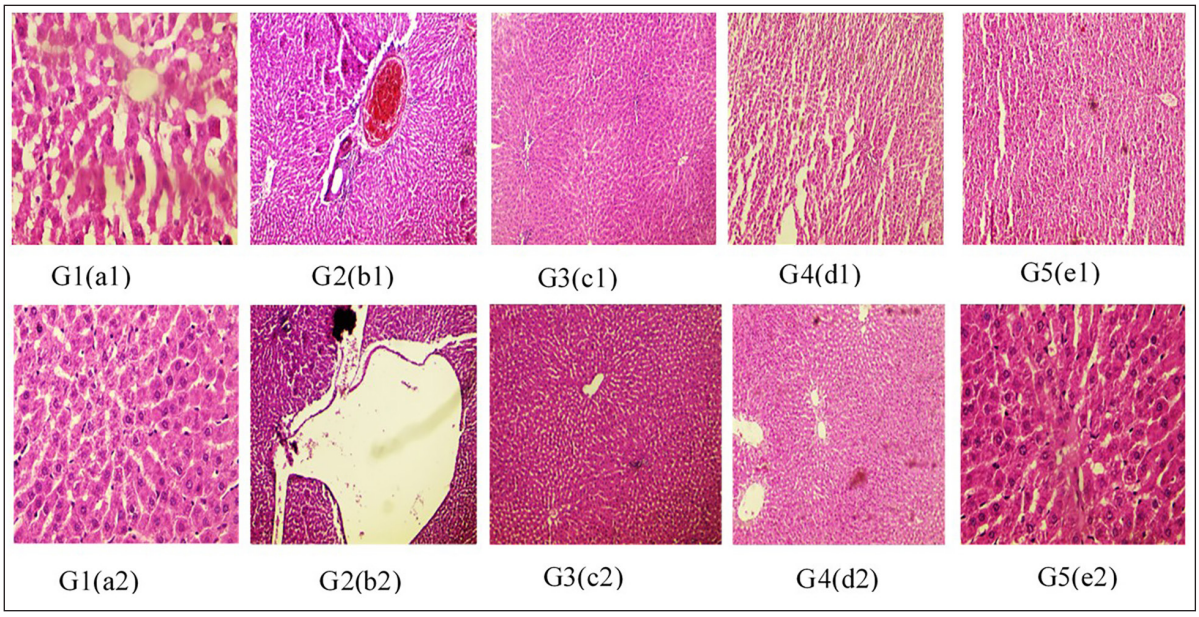

Fig. 3. Representative histologic samples from several groups, G1(a1, a2), G2(b1,b2), G3(c1,c2), G4(d1,d2), and G5 (e1, e2). Magnification: X40.

DMF-ASSOCIATED REDUCTION IN LIVER TISSUE DAMAGE

The degenerative alterations in liver slices from all groups are depicted in Figure 3. The liver sections of the control and DMF groups of rats showed no significant histological changes. The liver sections from the ciprofloxacin group had substantial damage, including epithelial necrosis, and hemorrhagic foci. The development of these lesions and tissue damage is dramatically reduced in the ciprofloxacin plus DMF group. According to this liver pathological finding, DMF may prevent ciprofloxacin induce liver damage in rats.

DISCUSSION

The liver seems to be the major organ for maintaining homeostasis. Drugs are thought to be a primary cause of liver failure, and the majority of idiosyncratic drug reactions resulting in negative consequences, including liver transplantation. It has been reported, that the fundamental mechanism, that causing by ciprofloxacin-induced ALF is the oxidative stress. Indeed, the main signal in the evolution of several liver illnesses is an oxidant/antioxidant imbalance [18, 19]. DMF protects the cells from harmful influence, that caused by nuclear Nrf2 upregulation, inflammation and oxidative stress modulation, and its down-regulation [20]. According to our findings, using of ciprofloxacin caused the immediate liver injury: the rats' liver function had deteriorated, and histological damage had occurred. Furthermore, the ciprofloxacin group had significantly higher levels of ROS in liver tissue. In contrast, providing of DMF without an inducible agent, boosted of an anti-oxidative enzyme activity via increasing the expression of the antioxidant transcription factor Nrf2, demonstrating that DMF has a protective role in the liver. This protection, demonstrated after induction, drastically reduced of liver damage and decreased ROS levels in the ciprofloxacin plus DMF group. The reduced CAT, SOD, and GSH enzymes in the liver tissues of the ciprofloxacin group in this study also indicated the role of oxidative damage in the ciprofloxacin inducing liver damage in animals. In rats without damage, treatment with DMF elevated antioxidant enzyme activity to protect against oxidative damage, which can be seen in figure 3. Furthermore, in rats with damage, DMF treatment immediately scavenged ROS and unregulated anti-oxidant enzymes. DMF's cytoprotective characteristics are tightly associated with the removal of excess ROS. Previous research by Naguib et al. 2021 reported that Nrf2 could have therapeutic potential in the treatment of hepatotoxicity by inhibiting oxidative stress-induced hepatocytes cell membrane disruption during ciprofloxacin-induced changes [21]. In addition, Xu D et al. showed that Nrf2 increased autophagy in hepatocytes results in increased clearance of damaged mitochondria, reduced mtDNA release, and reduced ROS production, resulting in a reduction in DAMP-induced inflammatory responses and consequent secondary hepatocytes injury [21]. Finally, we have reported DMF's antioxidant properties, which are consistent with our findings. In addition, DMF improved the viability of ciprofloxacin-induced injuries.

CONCLUSIONS

According to our findings, DMF improves ciprofloxacin and induces liver damage as assessed by liver function and liver pathology. The anti-oxidant protein Nrf2 nuclear translation was dramatically enhanced by DMF. These favorable effects are mostly due to improved antioxidant defense in the liver and activation of the Nrf2 pathway. DMF may thus be an effective treatment for CIN prevention, while more research and randomized clinical trials are needed to prove this.

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Conflict of interest:
The Authors declare no conflict of interest.

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Received: 08.06.2022
Accepted: 30.03.2023

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article