ORIGINAL ARTICLE

Chrysin mitigates diclofenac-induced hepatotoxicity by modulating oxidative stress, apoptosis, autophagy and endoplasmic reticulum stress in rats

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Abstract

Background Diclofenac (DF) is a non-steroidal anti-inflammatory drug (NSAID) generally prescribed for the treatment of pain. In spite of the widespread use of DF, hepatotoxicity has been reported after its administration. The current study discloses new evidence as regards of the curative effects of chrysin (CHR) on DF-induced hepatotoxicity by regulating oxidative stress, apoptosis, autophagy, and endoplasmic reticulum (ER) stress.

Methods The animals were separated into five different groups. Group-I was in control. Group-II received CHR-only (50 mg/kg bw, p.o.) on all 5 days. Group-III received DF-only (50 mg/kg bw, i.p.) on 4th and 5th day. Group-IV received DF (50 mg/kg bw) + CHR (25 mg/kg, bw) and group-V received DF (50 mg/kg, bw) + CHR (50 mg/kg, bw) for 5 days.

Results DF injection was associated with increased MDA while reduced GSH level, activities of superoxide dismutase, glutathione peroxidase, and catalase and mRNA levels of *HO-1* and *Nrf2* in the liver. DF injection caused apoptosis and autophagy in the liver by up-regulating *caspase-3, Bax*, *LC3A*, and *LC3B* levels and down-regulating Bcl-2. DF also caused ER stress by increasing mRNA transcript levels of *ATF-6, IRE1*, *PERK*, and *GRP78*. Additionally, it was observed that DF administration up-regulated *MMP2* and *MMP9*. However, treatment with CHR at a dose of 25 and 50 mg/kg considerably ameliorated oxidative stress, apoptosis, autophagy, and ER stress in liver tissue.

Conclusion Overall, the data of this study indicate that liver damage associated with DF toxicity could be ameliorated by CHR administration.

Keywords Apoptosis · Autophagy · Chrysin · Diclofenac · Endoplasmic reticulum stress

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most prescribed drugs that are clinically used to manage pain and inflammation [1]. One important group of

NSAID family drug is diclofenac (DF). Similar to the most NSAIDs, DF possesses analgesic, anti-inflammatory, and anti-pyretic activities [2]. DF has mostly been considered as well-tolerated drug. Nonetheless, considering its enormous global consumption, cases of side effects and toxicities in

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the gastrointestinal tract [3], kidney [4], and liver [5] have been reported. This restricts their use in the treatment of inflammation and pain. In spite of this fact, DF remains to be in over-spread use owing to its critical role an efficient anti-inflammatory and analgesic agent in the management of chronic conditions of pain and inflammation [6]. The molecular and biochemical mechanisms of DF-induced hepatotoxicity have been partly accredited to the mitochondrial damage, generation of oxidative stress, impairment of anti-oxidant defence system and immune mediated mechanisms [7]. Since toxic effects DF mainly caused toxicities through mechanisms induced by oxidative damage, researchers have highlighted the importance of anti-oxidants as natural agents for the treatment of DF-induced toxicities [8, 9].

Flavonoids containing hydroxyl moieties are essential contents of a daily diet of humans; consequently, they are most comprehensively examined with regard to their impact on human. Hydroxyflavones possess a wide range of biological activities containing anti-oxidant, anti-inflammatory anti-allergic, hepatoprotective and neuroprotective ones [10–12]. Chrysin (CHR; 5,7-dihydroxyflavone) is a natural polyphenolic compound which is abundantly found in bee products (i.e. honey, propolis) and several medicinal plants [13, 14]. CHR is well-thought-out to be a favourable natural compound to be used in the avoidance of several diseases containing cancers, diabetes, and neurodegenerative diseases such as Alzheimer or Parkinson disease [15, 16]. In addition, it has been reported that CHR is effective against liver and other tissue damage and reduces oxidative stress due to its antioxidant properties [17–19].

Because of the likelihood protective impacts of CHR on DF-induced hepatotoxicity, we aimed to explore whether treatment of rats with CHR could prevent the hepatotoxic effects of DF on the liver and suppress oxidative stress, apoptosis, autophagy and endoplasmic reticulum stress.

Materials and methods

Drugs and chemicals

Chrysin (CAS no: 480-40-0) and all other chemicals were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Diclofenac sodium (75 mg/3 ml, injectable solution, dikloron®) was purchased from Deva Company.

Animals

Thirty-five Wistar albino male rats (250–300 g) purchased from Experimental Research Center, Bingol University was used in the experiment. They were kept under laboratory conditions (25 ± 1 °C, % 50 ± 5 humidity and 12:12 h light/dark cycle). During the experiment, standard rat feed and water ad libitum were given to the animals. This study was approved by Local Animal Experimentation Ethics Committee of the Bingol University (Bingol, Turkey) (2022-E.53,632).

Experimental design

In this study, rats were randomly divided into 5 groups with 7 rats in each group.

Group-1 Normal control.

Group-2 CHR (50 mg/kg b.w./day, p.o.) on all 5 days [14].

Group-3 DF (50 mg/kg b.w./day, i.p.) on 4th and 5th days [8].

Group-4 DF (50 mg/kg b.w./day, i.p.) on 4th and 5th day + CHR (25 mg/kg b.w./day, p.o.) on all 5 days.

Group-5 DF (50 mg/kg b.w./day, i.p.) on 4th and 5th day + CHR (50 mg/kg. b.w./day, p.o.) on all 5 days.

All animals were killed under mild sevoflurane anesthesia 24 h after the last treatment. Blood samples were collected from the jugular veins of rats. The blood samples were left to coagulate at room temperature and then centrifuged at 3000 rpm for 10 min. Dissected liver tissues were flash-frozen in liquid nitrogen and stored at − 20 °C until analyzed.

Determination of liver function markers

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) levels were analyzed in Mindray Perfect Plus 400 autoanalyzer device. These enzyme results were given as U/L.

Determination of antioxidant markers and lipid peroxidation in liver

To determine the antioxidant capacity in liver tissues, SOD, CAT, and GPx activities as well as GSH levels were investigated. Liver homogenates required for antioxidant biomarkers and lipid peroxidation assays were obtained as described in our previous study [20]. SOD activity was assayed by the method of Sun et al. [21]. GPx activity was determined according to the method of Lawrence and Burk [22]. These enzymes were expressed as U/g of protein. CAT activity was estimated according to Aebi [23] method and the results were expressed as katal/g protein. GSH levels

Table 1 Primer sequences

Gene	Sequences (5'-3')	Length (bp)	Accession no
Nrf2	F: TTTGTAGATGACCAT-GAGTCGC R: TCCTGCCAAACTT-GCTCCAT	161	NM_031789.2
HO-1	F: ATGTCCCAGGATTT-GTCCGA R: ATGGTACAAGGAGGC-CATCA	144	NM_012580.2
Bax	F: TTTCATCCAGGATC-GAGCAG R: AATCATCCTCTG-CAGCTCCA	154	NM_017059.2
Bcl-2	F: GACTTTGCAGAGAT-GTCCAG R: TCAGGTACTCAGT-CATCCAC	214	NM_016993.2
Cas-pase-3	F: ACTGGAATGT-CAGCTCGCAA R: GCAGTAGTCGCCTCT-GAAGA	270	NM_012922.2
ATF-6	F: TCAACTCAGCAC-GTTCCTGA R: GACCAGTGACAG-GCTTCTCT	130	NM_001107196.1
PERK	F: GATGCCGAGAAT-CATGGGAA R: AGATTCGAGA-AGGGACTCCA	198	NM_031599.2
IRE1	F: GCAGTTCCAGTACATT-GCCATTG R: CAGGTCTCTGTGAA-CAATGTTGA	163	NM_001191926.1
GRP78	F: CATGCAGTTGTGACT-GTACCAG R: CTCTTATCCAGGC-CATATGCAA	143	NM_013083.2
LC3A	F: GACCATGTTAACAT-GAGCGA R: CCTGTTCATAGATGT-CAGCG	139	NM_199500.2
LC3B	F: GAGCTTCGAA-CAAAGAGTGG R: CGCTCATATTCACGT-GATCA	152	NM_022867.2
MMP2	F: CTCTAGGAGAAGGA-CAAGTG R: CTCAAAGTTGTAC-GTGGTGG	158	NM_031054.2
MMP9	F: AGCTGGCAGAGGAT-TACCTG R: ATGATGGTGCCACTT-GAGGT	230	NM_031055.2
β-Actin	F: CAGCCTTCCTTCTTGGG-TATG R: AGCTCAGTAACAGTC-CGCCT	360	NM_031144.3

were measured by the method of Sedlak and Lindsay [24]. MDA was determined in the liver homogenate following the method of Placer et al. [25]. Total protein content of liver tissues was measured to calculate enzyme activities. To determine this, Lowry et al. [26] method was used.

Analysis of mRNA transcript levels of oxidative stress, apoptosis, ER stress, autophagy and metalloproteinases in liver tissue

The mRNA transcript levels of oxidative stress (Nrf2 and HO-1), apoptosis (Bax, Bcl-2, and caspase-3), ER stress (ATF-6, PERK, IRE1, and GRP78), autophagy markers (LC3A and LC3B) and metalloproteinases (MMP2 and MMP9) in liver tissues of rats were analyzed by the RT-PCR method. First of all, total RNA was isolated from liver tissues by the Trizol method. QIAzol Lysis Reagent (Qiagen, Cat: 79,306, Germany) was used in the Trizol method, and the applications were carried out according to the manufacturer's instructions. Total RNA concentrations were measured in NanoDrop (Epoch Microplate Spectrophotometer, USA). Next, double-stranded cDNAs were synthesized from RNAs. High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™ Cat: 4,368,814, USA) was used for this. The cDNAs obtained in the last step were mixed with reverse and forward primers of the relevant genes and iTaq Universal SYBR Green Supermix (BIORAD) and the reaction was started in the ROTOR-GENE Q (Qiagen, Germany) device. Reaction conditions were established according to the instructions included with the iTaq Universal SYBR Green Supermix (BIORAD) kit. At the end of the procedure, the CT values obtained from the device were used to calculate the mRNA transcript levels of the genes whose sequences are given in Table 1. β-actin was used as the housekeeping gene and calculations were made according to the $2^{-\Delta\Delta CT}$ method [27].

Statistical analysis

The results were given as mean ± standard deviation. Graph-Pad Prism 5.0 software was used for the data analysis. Data were analysed using one-way ANOVA with Tukey's post hoc tests for multiple comparisons. $P < 0.05$ was considered as significantly different.

Results

Effects of CHR on serum ALT, AST and ALP levels in DF-induced hepatotoxicity in rats

Table 1 shows the effects of CHR and DF on serum ALT, AST, and ALP levels. The results show that DF injection caused liver damage and significantly increased ($p < 0.05$)

Table 2 Effect of chrysin (CHR) on hepatic serum markers and oxidative stress biomarkers in DF-induced hepatotoxicity

Parameters	Control	CHR	DF	DF + CHR 25	DF + CHR 50
ALP (U/L)	111.57 ± 10.98^a	118.29 ± 7.29^a	316.29 ± 18.64^c	168.43 ± 6.07^b	123.71 ± 7.11^a
ALT(U/L)	43.00 ± 7.26^a	46.86 ± 5.25^a	87.03 ± 8.86^c	58.57 ± 7.34^b	45.86 ± 5.24^a
AST (U/L)	181.19 ± 11.68^a	188.10 ± 13.18^a	434.37 ± 20.45^d	318.24 ± 13.79^c	230.31 ± 18.49^b
MDA(nmol/g tissue)	37.47 ± 2.20^a	37.08 ± 2.49^a	55.64 ± 2.24^d	48.40 ± 1.62^c	44.16 ± 1.47^b
GSH (nmol/g tissue)	7.57 ± 0.45^d	7.78 ± 0.16^d	3.60 ± 0.15^a	4.66 ± 0.18^b	5.94 ± 0.60^c
CAT(katal/g protein)	55.41 ± 3.24^d	57.58 ± 2.39^d	33.30 ± 2.27^a	40.90 ± 1.66^b	48.60 ± 1.18^c
SOD (U/g tissue)	27.49 ± 1.74^d	27.99 ± 1.65^d	15.44 ± 1.48^a	19.84 ± 0.93^b	23.31 ± 1.46^c
GPx (U/g tissue)	41.93 ± 2.23^d	43.55 ± 2.61^d	27.83 ± 1.37^a	32.42 ± 1.81^b	37.15 ± 1.66^c

Different superscripts (a–d) in the same row indicate significant difference ($p < 0.05$) among groups
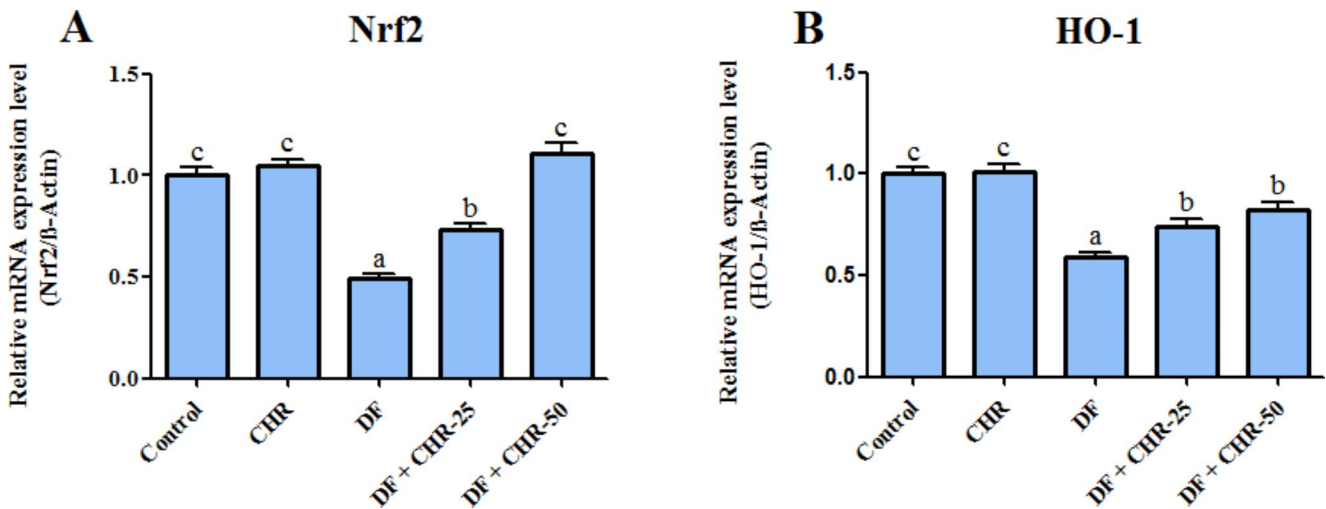
Fig. 1 Effects of CHR and DF treatments on Nrf-2/HO-1 mRNA expression levels in liver tissue. (A) Nrf-2 mRNA transcript levels, (B) HO-1 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a–c) on the columns show a statistical difference ($p < 0.05$)

serum ALP, ALT, and AST levels compared to the control group. Treatment with CHR significantly improved liver function in DF-induced rats by significantly attenuating serum ALP, ALT, and AST activities ($p < 0.05$).

## Effects of CHR on antioxidant status in DF-induced hepatotoxicity in rats

The curative effects of CHR against DF-induced oxidative stress in the liver tissue were evaluated through assessment of the antioxidant biomarkers (SOD, CAT, GPx, and GSH) and MDA levels. The activities of SOD, CAT, GPx, and level of GSH significantly ($p < 0.05$) decreased in the DF-induced group in comparison to the normal control group. Conversely, the MDA levels of liver tissue were remarkably increased ($p < 0.05$). It was determined that CHR treatment dose-dependently increased antioxidant enzyme activities and GSH levels and decreased MDA levels in the liver tissue (Table 2).

Effects of CHR on mRNA Transcript Levels of Nrf2 and HO-1 in DF-induced Hepatotoxicity in rats

According to the data obtained after RT-PCR analysis, it was determined that DF significantly suppressed Nrf2 and HO-1 expressions compared to the control group ($p < 0.05$). It was also observed that Nrf2 expression was dose-dependently up-regulated after CHR administration ($p < 0.05$), while HO-1 expression did not make a significant difference between the doses of CHR. The results obtained are presented in Fig. 1.

Effects of CHR on mRNA Transcript Levels of ATF-6, PERK, IRE1 and GRP78 in DF-induced Hepatotoxicity in rats

ATF-6, PERK, IRE1, and GRP78 mRNA transcript levels were analyzed by the RT-PCR method to determine the degree of ER stress after DF and CHR applications in liver tissue, and the results are given in Fig. 2. According to the results, DF caused ER stress in liver tissues of rats and
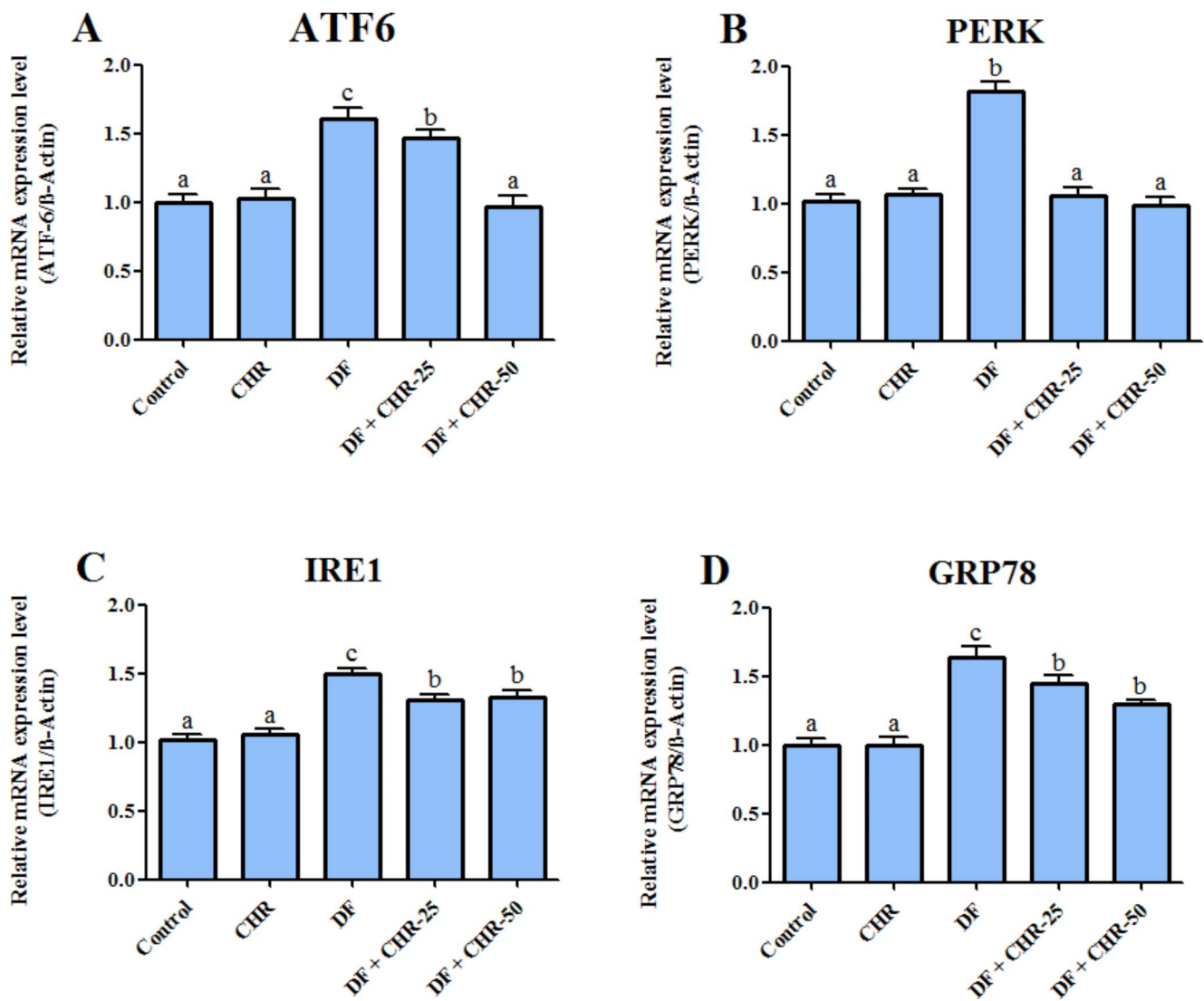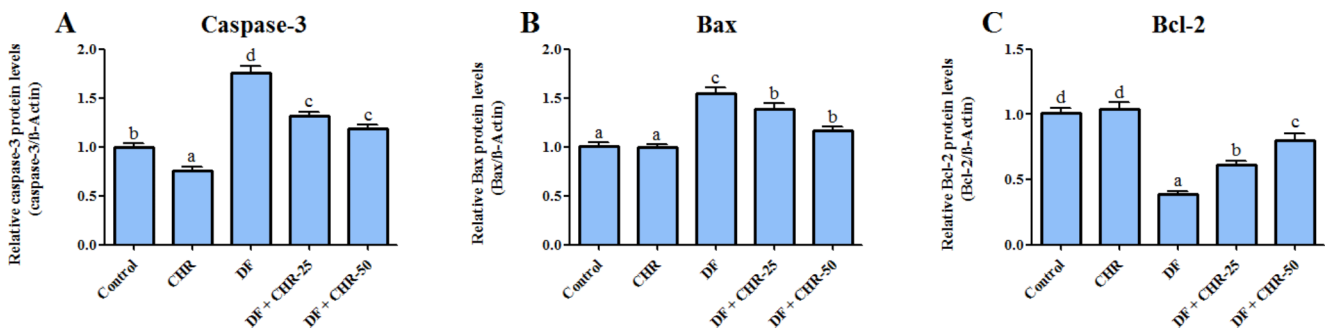
Fig. 2 Effects of CHR and DF treatments on ATF-6, PERK, IRE1 and GRP78 mRNA transcript levels in liver tissue. (A) ATF-6 mRNA transcript levels, (B) PERK mRNA transcript levels, (C) IRE1 mRNA transcript levels, (D) GRP78 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a–c) on the columns show a statistical difference ($p < 0.05$)

up-regulated ATF-6, PERK, IRE1, and GRP78 expressions ($p < 0.05$). Although the 25 mg/kg dose of CHR suppressed ATF-6 and GRP78 expressions compared to the DF group ($p < 0.05$), it was not as effective as the 50 mg/kg dose. On the other hand, mRNA transcript levels of IRE1 and PERK genes did not make a statistical difference in both low and high doses of CHR. Moreover, the mRNA transcript levels of the PERK gene decreased to the levels of the control group in all groups given CHR.

Effects of CHR on mRNA Transcript Levels of Bax, Bcl-2, and caspase-3 in DF-induced Hepatotoxicity in rats

According to the results summarized in Fig. 3, it was observed that DF might cause apoptosis by activating Bax and Caspase-3 genes and suppressing the Bcl-2 gene in liver tissue ($p < 0.05$). However, it was determined that CHR application increased Bcl-2 expression dose-dependently and decreased Bax expression dose-dependently. When Caspase-3 expression was evaluated after CHR treatment, it was detected that this gene was significantly suppressed ($p < 0.05$) compared to the DF group, but there was no significant difference between doses.

Fig. 3 Effects of CHR and DF treatments on Caspase-3, Bax and Bcl-2 mRNA transcript levels in liver tissue. (A) Caspase-3 mRNA transcript levels, (B) Bax mRNA transcript levels, (C) Bcl-2 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a–d) on the columns show a statistical difference (p < 0.05)

Effects of CHR on mRNA Transcript Levels of LC3A and LC3B in DF-induced Hepatotoxicity in rats

Autophagic status in liver tissue was estimated by analysis of mRNA transcript levels of LC3A and LC3B genes. Our findings showed that DF could trigger autophagy by causing activation of LC3A and LC3B genes in liver tissue. CHR treatment, on the other hand, displayed anti-autophagic properties and down-regulated DF-induced LC3A and LC3B expressions. When the doses of CHR were compared, it was found that the higher dose was more effective. The results are presented in Fig. 4 A and B.
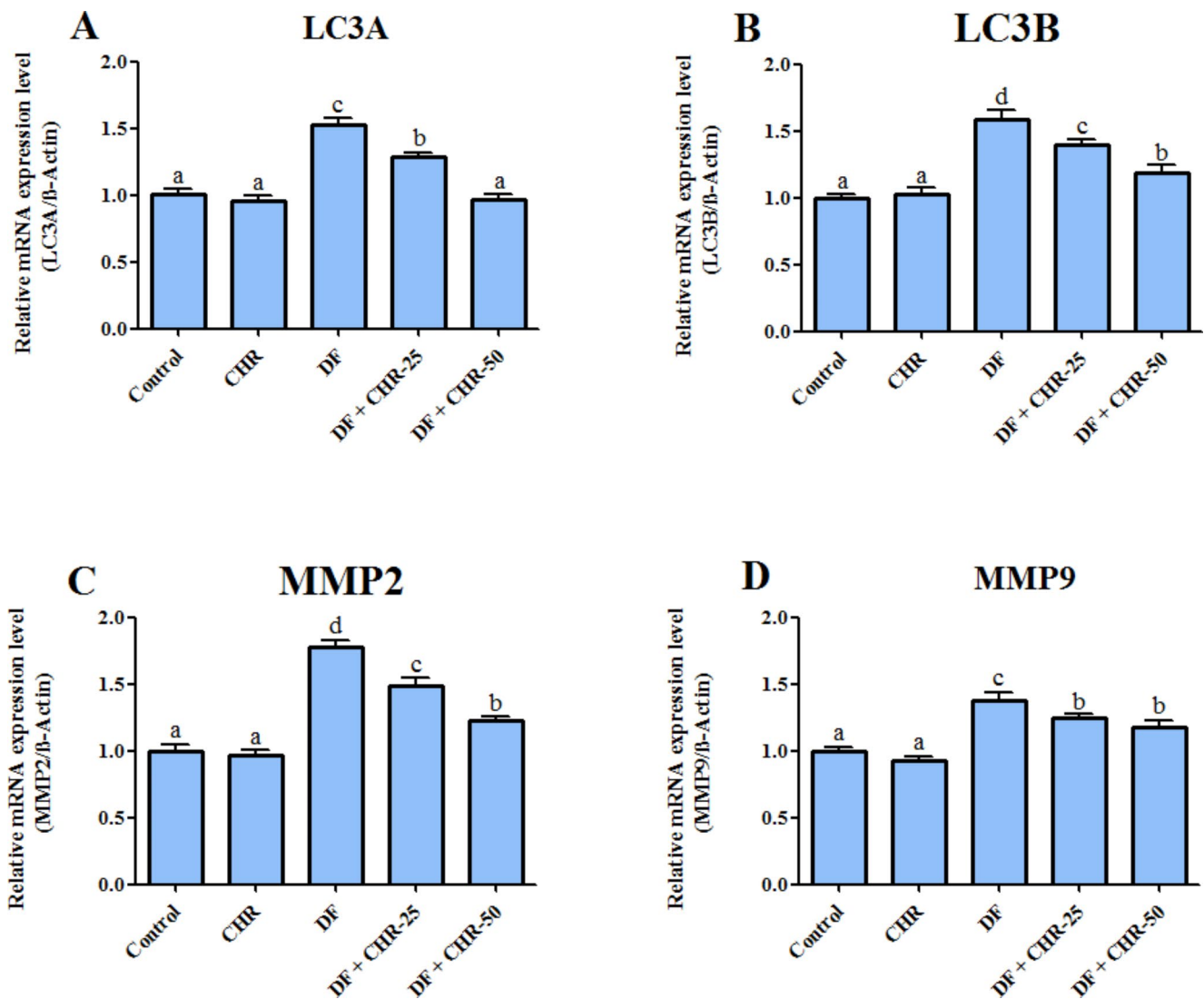
Effects of CHR on mRNA Transcript Levels of MMP2 and MMP9 in DF-induced Hepatotoxicity in rats

In order to evaluate the efficiency of matrix metalloproteinases (MMPs) in liver tissue, MMP2 and MMP9 mRNA transcript levels were analyzed by the RT-PCR method. The results (see Fig. 4 C and D) showed that DF triggered the expression of MMP2 and MMP9 in liver tissue, whereas the expression of these genes was suppressed by CHR treatment. It was determined that high dose was more effective on MMP2, whereas both low and a high doses had similar effects on MMP9.

Discussion

Diclofenac is an NSAID generally used for the treatment of pain. In spite of its common prescription, hepatotoxicity has been reported after the administration of DF. The generation of reactive metabolites and reactive oxygen species has been postulated as the main convict fundamental DF hepatotoxicity [5]. The current study discloses new evidence as regards the curative effects of CHR on DF-induced hepatotoxicity by regulating oxidative stress, apoptosis, autophagy, and ER stress.

Increased activities of hepatic function enzymes (AST, ALT, and ALP) imitate severe liver cell injuries with consequent leakage of the cytosolic enzymes into the blood circulation [28]. In our study, DF injection led to a significant increase in the activities of hepatic liver enzymes AST, ALT, and ALP indicating that DF administration caused plain damage in the membrane of hepatic cells in harmony with previous studies [5, 7]. In the current study, administration of CHR (25 and 50 mg/kg) considerably decreased serum ALT, ALP, and AST activities in DF administrated rats revealing that CHR prevented hepatic enzyme leakage into the circulation, maintained hepatocyte membrane integrity hence provided hepatoprotective effects. Earlier studies

Fig. 4 Effects of CHR and DF treatments on LC3A, LC3B, MMP2 and MMP9 mRNA transcript levels in liver tissue. (A) LC3A mRNA transcript levels, (B) LC3B mRNA transcript levels, (C) MMP2 mRNA transcript levels, (D) MMP2 mRNA transcript levels. Values are expressed as mean ± SD. Different letters (a–d) on the columns show a statistical difference ($p < 0.05$)

have also reported that CHR showed marked modulatory effects on hepatic tissue [17, 19].

Drug-prompted damage of hepatic tissues in humans and animals is generally triggered by oxidative damage that emerges as a consequence of the effects of excessive ROS generated in the liver tissue [29]. The obvious reduction in serum liver SOD, CAT, GSH, and GPx activities following DF treatment in our study might be an indication of oxidative damage that leads to the decrease in activities of antioxidant enzymes could be related to the side effects of the drug. DF has been reported to form reactive metabolites such as 5-hydroxyl diclofenac and N, 5-dihydroxyl DF, both of which might induce oxidative damage in the liver of animals [30]. The findings of our study are in close agreement with recent reports that demonstrated ROS generated

by drug metabolites could lead to an imbalance in the production and eradication of ROS, causing oxidative damage [31]. However, the substantial rise in SOD, CAT and GPx activities and GSH level, after CHR co-treatment, might put forward modulatory effects of CHR against oxidative damage, thus avoiding moderate reduction in the activities of antioxidant enzymes in rats. These findings are in harmony with reports of earlier studies that showed that CHR could improve the tissue antioxidant status of drug-exposed rats [32]. The remarkable elevations in serum liver levels of MDA and reduction in levels of GSH, after the administration of DF, could also point to oxidative damage and lipid peroxidation that might be attributed to the side effects of DF. The attack of ROS on the lipoproteins or membrane lipids of the cells during oxidative stress initiates

lipid peroxidation reactions that is commonly observed in the development of various diseases and anomalies containing drug-induced liver damage [33]. A study carried out by Nouri et al. reported that there was a significant elevation in the levels MDA and a reduction in GSH levels after administration of DF [34]. Nevertheless, the considerable decrease in levels of MDA and increase in GSH levels after treatment of DF-exposed rats with CHR could suggest its ameliorative effects towards on liver damage that could be attributed to the antioxidant activities of CHR.

The injection of DF resulted in decreased mRNA transcript levels of HO-1 in the liver. We presumed that down-regulation of HO-1 could be related to DF-induced hepatotoxicity and its up-regulation after CHR treatment possesses a significant role in ameliorating oxidative damage. Considering that the mRNA expression of HO-1 is regulated by Nrf2 [35], the down-regulation of HO-1 could be credited to DF-induced reduction mRNA levels of Nrf2. Additionally, excessive and continuous ROS generation induced by DF treatment could suppress Nrf2 signaling and then HO-1. This assumption was supported by researches indicating that the excess generation of ROS down-regulated Nrf2 and HO-1 expression in the hepatorenal tissues of rats [35–37]. In our study, treatment with CHR increased the expression of HO-1 and Nrf2 in the liver of DF-induced rats. CHR was shown to modulate the toxic effects of gibberellic acid at the molecular level through the repression of the HO-1 and Nrf2 [38].

Endoplasmic reticulum (ER) is an organelle responsible for biosynthesis, folding and maturation of several types of proteins [39]. Disruption of ER homeostasis by microbial infections, calcium imbalance, and build-up of unfolded/misfolded proteins may ultimately cause the ER stress, and subsequently activates an unfolded protein response (UPR) in order to restore ER function. The UPR is relayed via three ER transmembrane sensors containing PERK, IRE1, and ATF-6. These sensory biomolecules are mostly bound to and separated by a fundamental ER chaperone GRP78 [40, 41]. Current research reported that ER stress might be a significant event participated in drug burden, as well as other crucial mechanisms including oxidative damage and mitochondrial dysfunction. Actually, drug-stimulated ER stress may possibly give rise to numerous harmful effects inside cells and tissues containing an accumulation of lipids, cytolysis, cellular death, and inflammation [39]. According to the results of our study, it was observed that DF up-regulated ATF-6, PERK, IRE1, and GRP78 genes in liver tissue and triggered ER stress in agreement with the literature [42]. However, after CHR administration, expressions of these genes were suppressed. Our studies have also identified that DF injection caused the generation of ROS via inhibiting antioxidant enzymes. For that reason, we speculated that

ROS overgeneration may be a trigger of DF-induced ER stress.

The biomolecular mechanism by which CHR treatment reduced the levels of DF-induced apoptosis in liver was also examined within this study. Bax and Bcl 2 possess significant roles in regulating apoptosis [43]. The release of Cyt C in the intrinsic pathway of apoptosis is connected with permeabilization of the mitochondria wall by Bcl-2 family proteins. The antiapoptotic protein Bcl-2 localizes in the mitochondrial wall and prevents release of Cyt C [44]. This prompts the apoptosome generation then causing activation of caspase-9 that activates caspase-3 and induces apoptosis. In this study, DF triggered cell death by the down-regulation of Bcl-2 and the up-regulation of Bax along with caspase-3 levels while treatment with CHR inhibited DF-induced apoptosis via ameliorating the expression level of these genes. These findings confirm the involvement of mitochondria in DF-induced apoptosis. In a different study, exposure to DF triggered cell death by the up-regulation of Bax and caspase 3 levels [45].

Recent studies have demonstrated that DF can cause activation of autophagic response [4, 46]. The main events that determined DF-triggered liver toxicity have been reported as lysosomal dysfunction and autophagic flux deterioration facilitated by mitochondrial ROS [46]. LC3A and LC3B are among the essential autophagic markers in case of oxidative injury [47]. The results of current study revealed that DF exposure up-regulated mRNA transcript levels of LC3A, and LC3B, while CHR treatment reduced the DF effects. Moreover, DF was reported to reduce autophagy in hepatic cells via down-regulation of mRNA levels of LC3B [4]. In a different study, CHR administration was shown to ameliorate liver autophagy through down-regulation of LC3B in paracetamol-induced liver cells [14].

Matrix metalloproteinases (MMPs) have been reported as widespread family of zinc dependent proteinase enzymes that are crucial for extracellular matrix degradation [48, 49]. MMPs are sub-categorized in gelatinases, collagenases and several other examples of MMPs are among others [50]. They have been reported to conduce to the cell proliferation, apoptosis and angiogenesis [51]. MMP-2 (gelatinase A) is an MP involved in the maintenance of liver vascular homeostasis. It is expressed by various liver cells, most abundantly by hepatic stellate cells and kupffer cells, and is one of the widely studied enzymes in liver fibrosis. MMP-9 (gelatinase B) is an important multi-faceted MP with a significant role in destruction liver regeneration [52]. The data of the current study revealed that expression levels of MMP-2 and MMP-9 in the DF-induced liver were up-regulated while CHR treatment down-regulated levels of MMP-2 and MMP-9 further demonstrating ameliorative effects of CHR in DF induced liver toxicity.

Conclusion

In this study, we demonstrated that CHR ameliorative effects against DF-induced hepatotoxicity, which was characterized by increased hepatic serum biomarkers, in addition to increased tissue oxidative stress, apoptosis, autophagy and endoplasmic reticulum stress. Therefore, the findings from this study suggest that CHR as a natural antioxidant may be formulated for use as a safe nutraceutical or dietary supplement in the near future. It is thought that more studies are still needed to clarify its role in clinical practice.

Declarations

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