Life Sciences 217 (2019) 251–260

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Dimethyl fumarate ameliorates acetaminophen-induced hepatic injury in mice dependent of Nrf-2/HO-1 pathway

Rehab S. Abdelrahman[a], Noha Abdel-Rahman[b,*]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt
[b] Department of Biochemistry, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

ARTICLE INFO

Keywords:
Drug-inducd liver toxicity
Dimethyl fumarate
Nuclear erythroid-related factor 2
Oxidative stress

ABSTRACT

Drug-induced liver toxicity is the most frequent cause of acute liver failure worldwide. Hepatotoxicity caused by acetaminophen (ACT) overdose is mediated by its metabolic product promoting oxidative stress and activation of inflammatory mediators. Nuclear factor erythroid-related factor-2 (Nrf-2) induces the release of cytoprotective enzymes in response to electrophilic or oxidative stress and is considered a promising therapeutic target. Dimethyl fumarate (DMF) is a potent activator of (Nrf-2), its anti-inflammatory and antioxidant properties of DMF have been highlighted recently. We designed this study to explore the effect of DMF (100 mg/kg, orally) administered once and twice on hepatotoxicity induced by acetaminophen (ACT, 500 mg/kg, i.p.) in mice. DMF administration enhanced ACT-induced parameters in liver function, inhibited apoptosis and ameliorated the antioxidant machinery and inflammatory markers in a Nrf-2-dependent fashion. DMF elevated Nrf-2 and HO-1 levels and ameliorated liver injury as indicated by lowered levels of serum aminotransferases, ALP, GGT and bilirubin levels. Hepatic (Bcl-2) was elevated whereas hepatic caspase-3, NFκ-B, TNF-α and MPO were reduced. Hepatic levels of GSH, SOD, MDA and NO were altered promoting the antioxidant machinery. Histological examination of liver has further supported these results. These findings suggest that DMF can be employed in the treatment ACT-induced liver injury acting primarily through targeting Nrf-2/HO-1 pathway.

1. Introduction

Acetaminophen (ACT), a widely administered analgesic and antipyretic, is safe and effective at therapeutic doses. However, ACT overdose causes severe hepatic injury and has been estimated to be the primary cause of acute liver failure in western countries [1–3]. Acute hepatotoxicity is mainly caused by the ACT metabolite, *N*-acetyl-p-benzoquinoneimine (NAPQI), which is generated by the cytochrome P450 system. If the highly reactive metabolite NAPQ1 is not detoxified adequately, it covalently modifies liver proteins and depletes the intracellular pool of glutathione (GSH) [4]. Regulating the oxidative stress status and scavenging electrophiles is one strategy to treat ACT-induced hepatotoxicity.

Nuclear erythroid-related factor 2 (Nrf2) has emerged as a distinct oxidative-mediated transcription factor that regulates downstream cytoprotective targets. Nrf2 binds with antioxidant response elements (ARE) located upstream to genes of detoxifying enzymes [5,6]. Heme oxygenase-1(HO-1) is one important detoxifying enzyme that is upregulated during oxidative stress. HO-1 initiates the oxidative degradation of heme to carbon monoxide and bilirubin which are both powerful antioxidants [7]. The activity of the transcription factor Nrf-2 is regulated by Kelch-like ECH-associated protein-1(KEAP-1) which sequesters Nrf-2 in the cytosol through physical attachment. Exposure to electrophilic and oxidative stress destabilizes this interaction. Nrf-2 is then translocated to the nucleus where it triggers transcription of downstream cytoprotective genes such as HO-1. Nrf2 can be considered a plausible drug target for alleviating drug-induced liver injury where oxidative stress is implicated.

Nrf2 enhancers are a promising therapeutic class that have progressed to clinical trials and have been indicated for treatment of a

Abbreviations: ACT, acetaminophen; NAPQI, *N*-acetyl-p-benzoquinoneimine; GSH, glutathione; Nrf-2, Nuclear erythroid-related factor 2; ARE, antioxidant response elements; HO-1, heme oxygenase-1; DMF, dimethyl fumarate; CMC, carboxy methyl cellulose; SGOT, Serum glutamic oxaloacetic transaminase; SGPT, Serum glutamic pyruvic transaminase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; SOD, superoxide dismutase; MDA, malondialdeyhde; TCA, Trichloroacetic acid; NOx, nitrite/nitrate; NFκ-B, Nuclear factor kappa B; TNF-α, tumour necrosis factor-alpha; Bcl-2, B-cell lymphoma 2; H& E, hematoxylin and eosin

* Corresponding author.
E-mail address: noha82@mans.edu.eg (N. Abdel-Rahman).

https://doi.org/10.1016/j.lfs.2018.12.013
Received 13 October 2018; Received in revised form 7 December 2018; Accepted 9 December 2018
Available online 11 December 2018
0024-3205/

number of neurodegenerative and autoimmune diseases. Dimethyl fumarate (DMF) is FDA-approved for managing relapsing multiple sclerosis and psoriasis and works primarily through potentiating Nrf2 activity [8–10]. DMF also possesses cytoprotective and antiinflammatory properties which have proven effective in inflammatory-mediated conditions such as asthma [11] or experimental colitis [12]. DMF was also shown to ameliorate liver ischemia/reperfusion injury [13].

Herein, we explore the role of DMF in the treatment of ACT-induced liver toxicity emphasizing its effect on Nrf-2 pathway, inflammation, apoptosis and the oxiditave stress status in order to elucidate the mechanism by which DMF exerts its activity.

2. Materials and methods

2.1. Drugs and chemicals

Paracetamol (10 mg/ml, solution for infusion) was purchased as the pharmaceutical drug from Bristol-Myers-Squibb Co. Dimethyl fumarate (DMF) was purchased from (Sigma-Aldrich, St. Louis, MO, USA), and was suspended in 0.5% carboxymethylcellulose (CMC) to be administered orally. All other chemicals used in this study were of high analytical grade.

2.2. Animals

Adult BALB/c mice weighing (25–30 g) were housed under standard conditions. They were permitted free access to food and water throughout the experimental process. The research ethics committee criteria for care of laboratory animals at Mansoura University approved the project and experiments were conducted in compliance with its criteria for experimental animals.

2.3. Induction of acetaminophen hepatotoxicity and experimental design

All animals were allowed to fast overnight and were divided into 5 groups: [1] control group received a vehicle control (0.5% CMC orally) twice a day [2] DMF group received DMF (100 mg/kg, orally) twice a day [3] diseased group received a single dose (500 mg/kg) of ACT intraperitoneally [4] diseased group received ACT (500 mg/kg) followed by one-dose of DMF (100 mg/kg) after 1 h (**ACT + once DMF**) [5] diseased group received ACT (500 mg/kg) followed by two doses of DMF (100 mg/kg) after 1 h and 12 h of ACT treatment (**ACT + twice DMF**). The dose was chosen based on a previous study (Schulze-Topphoff et al., 2016). After 24 h of ACT administration mice were anaesthetized using thiopental and blood was collected from retro-orbital puncture. The right lobe of liver tissue was homogenized as (10% w/v) in 20 mM Tris-HCl (containing 1 mM EDTA, pH 7.4). The left lobe of liver samples was fixed in 10% buffered formalin for histopathalogical analysis.

2.4. Assessment of biochemical markers of liver function

The parameters SGOT, SGPT, GGT, ALP, bilirubin and albumin were measured photometrically in serum using commercial kits (SPINREACT, Sant Esteve de Bas, Spain) according to the manufacturer's instructions: The transaminases are proportional to the amount of oxalate (for SGOT) or pyruvate (for SGPT) formed which can be quantified by reacting with the developer 2,4 dinitrophenylhydrazine. Briefly, 0.5 ml of the substrates were mixed with 100 µl serum and incubated at 37 °C for 30 min. Then 2,4 dinitrophenylhydrazine in alkaline medium was added and incubated at room temperature for 20 min. Absorbances were measured at 505 nm and transaminase activities were estimated from a plotted calibration curve.

The rate of 2-nitro-5-aminobenzoic acid formation is proportional to the catalytic concentration of gamma-glutamyl transferase (GGT). 100 µl of serum was mixed with 1 ml of solution (100 mM Tris pH 8.6, 10 mM glycylglycine: 3 mM L-γ-glutamyl-3-carboxy-4-nitroanilide at 4:1 ratio). The absorbance was measured every minute over 3 min period at 405 nm. The average absorbance per minute was multiplied by 1190 to calculate the GGT concentration as U/L.

The rate of p-nitrophenol formation is proportional to the catalytic concentration of alkaline phosphatase (ALP) present in the sample. 20 µl of serum was mixed with 1 ml solution (0.35 M 2-amino-2-methyl-1-propanol, 1 mM $ZnSO_4$, 2 mM Magnesium acetate, 1 mM *N*-hydroxy-EDTA: 10 mM *p*-nitrophenylphosphate at 4:1 ratio). Absorbances at 405 nm were measured every minute for 3 min and the average absorbance was multiplied by 2764 to determine ALP activity as U/L.

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and the intensity of the color formed is proportional to the bilirubin concentration. 100 µl of serum or standard was mixed with 1.5 ml of (30 mM sulfanilic acid, 50 mM HCl, 7 M dimethylsulfoxide) and 50 µl 29 mM sodium nitrite. The mixture was incubated at 25 °C for 5 min and absorbances were measured at 555 nm.

Albumin in the presence of bromcresol green at a slightly acidic pH produces a color change of the indicator from yellow-green to green-blue. The intensity of the color formed is proportional to the albumin concentration in the sample. 10 µl of serum or standard were mixed with 1 ml of bromocresol green pH 4.2 and incubated at room temperature for 5 min. Absorbances were measured at 620 nm and concentrations were calculated compared to standard. Blank reactions were performed without adding standard or sample.

Lactate dehydrogenase (LDH) was measured using Human Diagnostics kit (Wiesbaden, Germany) by monitoring the disappearance of the reduced form of nicotinamide dinucleotide. According to the manufacturer's protocol, 20 µl of serum was mixed with 1 ml (50 mM Tris pH 7.4, 1.5 mM pyruvate) then incubated for 5 min at 30 °C. 250 µl of 0.8 mM NADH was added and absorbances were measured every 1 min for 3 min period at 340 nm. The mean change of absorbance per minute was measured and multiplied by 10,080 to calculate LDH activity.

2.5. Measurement of hepatic malonaldehyde, superoxide dismutase, glutathione and nitric oxide

The following parameters were estimated in liver homogenate. Superoxide dismutase (SOD) activity was measured by the degree of inhibition of the auto-oxidation of pyrogallol at an alkaline pH according to the method of Marklund and Marklund [14]. The reaction mixture was composed of 0.1 ml of liver homogenate with 1.5 ml of 20 mM Tris-HCl, 1 mM EDTA, pH 8.2 then 0.1 ml of 15 mM pyrogallol was added. The change of OD at 412 nm per minute was monitored over a three minute period. Controls with no samples were run under the same conditions in order to compute the rate of inhibition. The enzyme activity was expressed as U/100 mg where one unit represents the amount of enzymes that suppresses pyrogallol autooxidation by 50%.

Hepatic malonaldeyhyde (MDA) was measured employing a method described previously [15]. Briefly, 0.4 ml of liver homogenate was mixed with 8 ml of 10.3 mM 1-methyl-2-phenyl-indole in acetonitrile diluted with methanol containing 32 µM $FeCl_3$ (3:1). The reaction was incubated at 45 °C for 60 min followed by centrifugation at 4000 × *g* for 10 min. Absorbances were measured at 586 nm.

We utilized a previously described method for GSH measurement [16]. Briefly, 0.05 ml of 50% (w/v) trichloroacetic acid was used to preceiptate the protein in liver homogenate. The supernatant was collected after centrifugation at 1000 × *g* for 5 min and 0.25 ml of the supernatant was mixed with 1 ml of solution composing of 0.2 M Tris-HCl and 1 mM EDTA, pH 8.9 and 0.05 ml of 10 mM 5,5′-dithiobis-(2-nitrobenzoic acid) in absolute methanol. The reaction was kept at room temperature for 5 min. A yellow color was formed and measured spectrophotometrically at 412 nm. GSH concentration expressed as

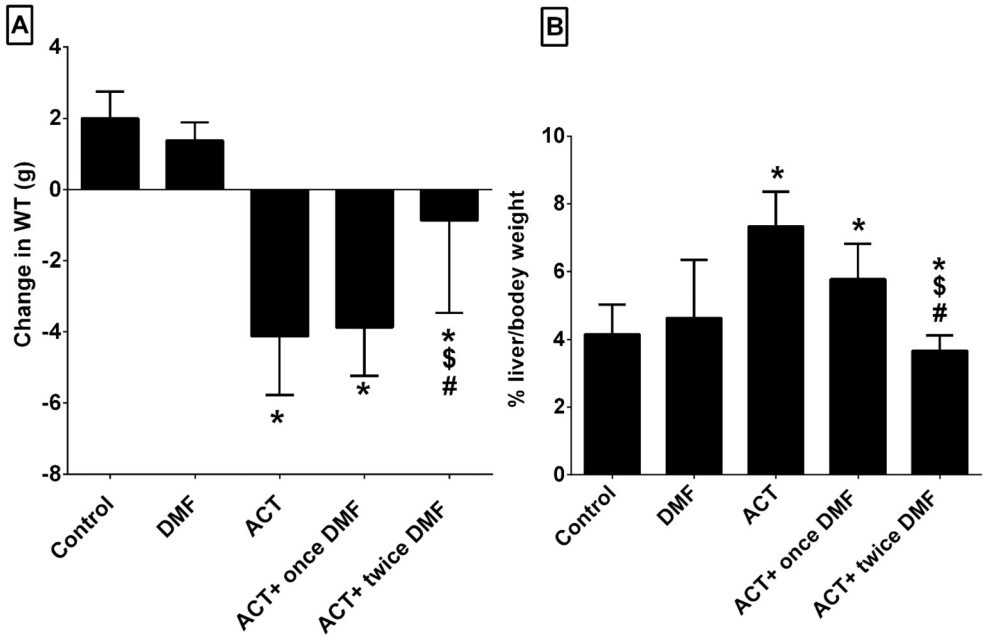

Fig. 1. Effect of DMF on body weight and liver/body weight ratio. A) ACT treatment caused a marked decrease in body weight compared to control and control drug groups ($p < 0.05$). DMF significantly increased body weight in ACT-treated mice. B) A signficant increase in liver/body weight ratio was observed in mice treated with ACT compared to control and control drug groups. DMF reversed it values comparable to normal control and control drug mice. Data is expressed as mean ± SD, n = 8. Different symbols indicate statistical significance of $p < 0.05$ (* compared to control group, $ compared to ACT group, # compared to one-dose treatment group) using regular one-way ANOVA followed by Tukey's multiple comparisons test.

nmole/100 mg tissue was calculated from a standard calibration curve (0–500 nmol/ml).

Total nitrite/nitrate (NOx) products, an indicator of NO synthesis, were measured according to the method described previously [17]. Briefly, 0.5 ml of liver homogenate was mixed with 0.25 ml of 0.3 N NaOH and the reaction was incubated at room temperature for 5 min. This was followed by deprotonization using 0.25 ml of 5% (w/v) $ZnSO_4$. The reaction was centrifuged at 3000 ×*g* for 20 min at 4 °C. 0.5 ml of the supernatant was mixed with 0.3 ml VCl_3 (8 mg/ml) in 1 M HCl and 0.3 ml Griess reagent composed of the following: 0.15 ml of 2% (w/v) sulphanilamide in 5% (v/v) HCl and 0.15 ml of 0.1% (w/v) *N*-(1-naphthyl)-ethylenediamine dihydrochloride in distilled water. The reaction mixture was incubated at 37 °C for 45 min and the product was measured spectrophotometrically at 540 nm. Concentrations of NOx was computed from a calibration curve constructed $NaNO_3$ (0–100 nmol/ml). NOx levels were expressed as nmol/100 mg tissue.

2.6. Determination of hepatic Nuclear erythroid-related factor 2 (Nrf-2) and heme oxygenase-1 (HO-1)

Liver homogenates were used to measure hepatic Nuclear erythroid-related factor 2 (Nrf-2) and heme oxygenase-1 (HO-1) using sandwich enzyme-linked immunosorbent assay (sandwich ELISA) by commercial kits (MyBioSource, Inc. San Diego, USA) and (CUSABIO) respectively following the manufacturer's instructions. The wells of a polyvinyl chloride microtiter plate were coated with monoclonal antibody specific for Nrf-2 and HO-1 respectively.

2.7. Evaluation of inflammatory mediators

Hepatic NFκ-B concentrations were determined by sandwich ELISA using a commercial kit (CUSABIO,China), hepatic tumour necrosis factor-alpha levels (TNF-α) were measured by sandwich ELISA employing a commercial kit (AssayPro, St. Charles, MO) following the manufacturer's instructions. The wells of a polyvinyl chloride microtiter plate were coated with antibody specific for NFκ-B and TNF-α respectively.

2.8. Measurement of the apoptotic regulator Bcl-2 and caspase-3 in hepatic tissue

Hepatic Bcl-2 and caspase-3 levels were measured by sandwich ELISA using commercial kits (MyBiosource San Diego, CA, USA), (EIAab Wuhan, China) respectively following the manufacturer's instructions. A monoclonal antibody specific for Bcl-2 and caspase-3 have been pre-coated onto a microplate respectively.

2.9. Histochemical analysis

The fixed tissues were embedded in a paraffin wax and sectioned (5 μm thick). Each section was stained with hematoxylin and eosin (H&E). Histopathological assessment was performed blindly by the pathologist. Inflammation was scored according to Knodell score [18].

2.10. Assessment of myeloperoxidase (MPO) expression in liver tissue

Liver tissue sections were stained using myeloperoxidase stain kit (Biodagnostics,Giza, Egypt,) according to the manufacturer's instructions. Briefly, liver sections were fixed using ethanol then incuabted with the substrate at room temperature. Copper sulphate was added as an enhancer. Finally, a counterstain methyl green is added, washed and allowed to dehydrate. The level of MPO intensity was scored 0, negative; 1, weak; 2, moderate and 3, strong staining. All readings were blindly performed by a pathologist.

2.11. Statistical analysis

Results were expressed as means ± SD. Statistics and graphical representation were carried out using Graphpad Prism V 6.01 (Graphpad Software Inc., San Diego, CA, USA) by regular one-way ANOVA followed by Tukey's *post-hoc* test for multiple comparisons. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Effect of DMF on body weight and liver/body weight ratio of mice

Hepatic lesions and damage can be initially estimated by examining liver weight/body weight ratios. Administration of ACT was associated

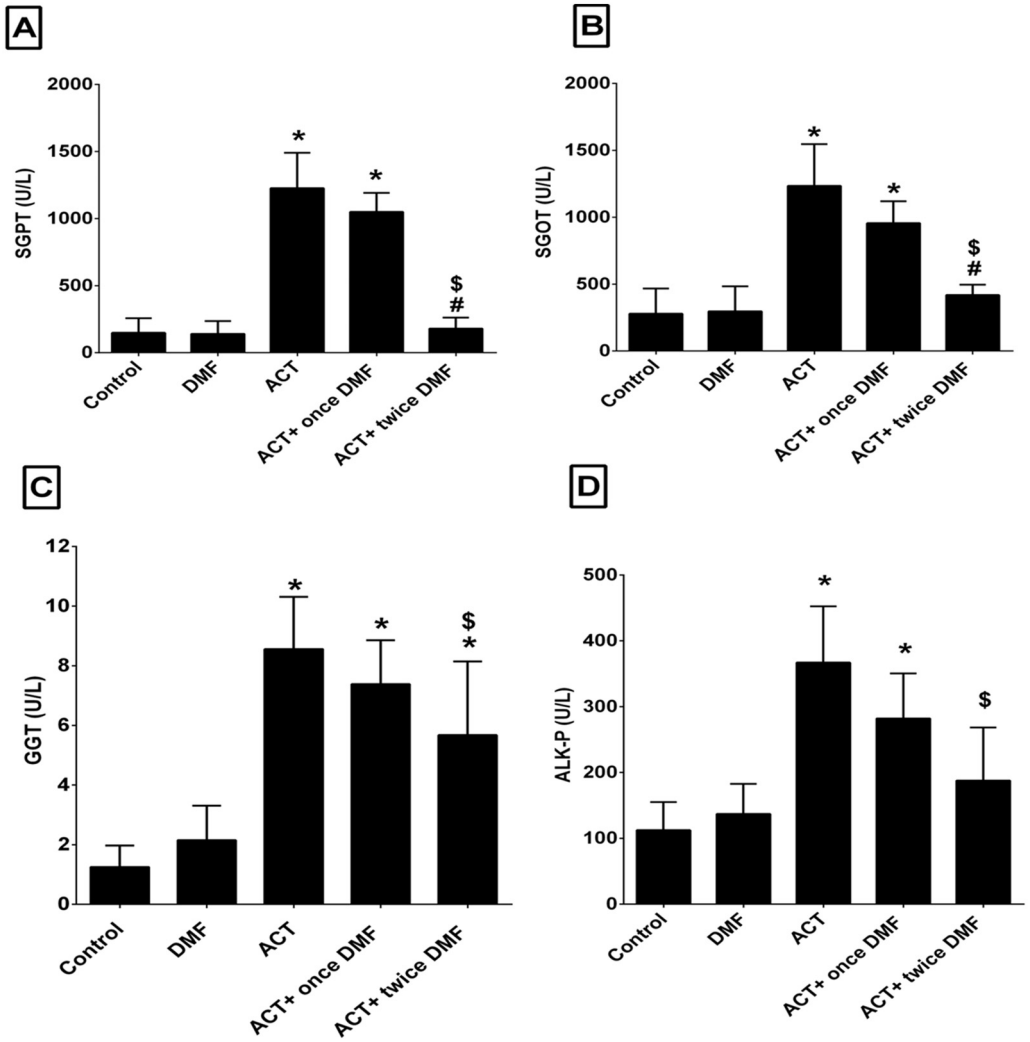

Fig. 2. Effect of DMF on biochemical markers SGPT, SGOT, ALK-P and GGT in serum. Serum levels of (A) SGPT and (B) SGOT, (C) GGT and (D) ALK-P were markedly elevated in ACT- treated mice group compared to control and control drug groups. DMF signficantly lowered serum levels of the former markers indicating an improvement in liver function. Data is expressed as mean ± SD, n = 8. Different symbols indicate statistical significance of $p < 0.05$ (* compared to control group, $ compared to ACT group, # compared to one-dose treatment group) using regular one-way ANOVA followed by Tukey's multiple comparisons test.

with a marked decrease in body weight and a notable increase in mean relative liver weights (liver weight/body weight) ($p < 0.05$) (Fig. 1A, B). DMF treatment significantly increased body weight compared to ACT group (Fig. 1A). The DMF treated group showed a significant reduction in mean relative liver weights ($p < 0.05$) (Fig. 1B).The improvement in liver/body weight was more pronounced after two doses compared to one-dose DMF. The control drug group did not show any significant change.

3.2. DMF ameliorated ACT-induced hepatotoxicity

In order to precisely measure the extent of liver damage, hepatic markers which include transaminases, GGT and ALK-P that are normally expressed in liver tissues were measured in serum. Elevated serum levels of the former markers indicate cell membrane damage and confirm liver injury. A single (500 mg/kg) dose of ACT caused more than a 6-fold increase in SGPT serum levels. Treatment with DMF significantly opposed elevated SGPT levels and restored it to levels comparable to the control group: one-dose treatment reduced to 0.85 fold of ACT group and two doses reduced dramatically to 0.14 fold of ACT group (Fig. 2A). Similarly, SGOT levels were significantly higher after ACT treatment: about 4.4 fold of that in control groups (Fig. 2B). DMF treatment reduced SGOT levels to 0.7 fold and 0.3 fold of ACT group in one and two dose groups respectively. Levels of GGT were also significantly elevated in ACT-treated group (6.8 fold of normal control group) and were reduced, although did not reach normal levels, after treating with DMF (0.8 fold and 0.6 fold in one and two-dose treatments respectively) (Fig. 2C). ALK-P increased 3.2 fold of normal group and declined to 0.7 fold in one-dose group and was lowered significantly to 0.5 fold of ACT groups two dose groups (Fig. 2D).

Serum albumin and bilirubin levels reflect the liver synthetic and excretory capacity respectively. ACT caused a decrease in albumin (0.5 fold of normal control group) that was reversed by DMF treatment. One-dose treatment raised albumin levels to 1.3 fold of ACT group and the two-dose treatment caused a 1.8 fold increase (Fig. 3A). Bilirubin was also elevated in ACT group (3.7 fold that of normal control group). Bilirubin levels were lowered 0.7 fold and 0.4 fold of ACT group in one and two-dose groups respectively (Fig. 3B).

Serum LDH levels were also evaluated as a measure of cell integrity and viability. Leakage of the cytoplasmic LDH from damaged cells is an indication of necrotic cell death [19]. LDH levels were markedly increased in ACT rats (2.9 fold the normal control group. LDH levels were reduced 0.8 fold and 0.5 fold of ACT group in one-dose and two-dose groups respectively (Fig. 3C). Control drug group did not show any significant changes in levels of SGOT, SGPT, GGT, ALK-P, albumin, bilirubin and LDH.

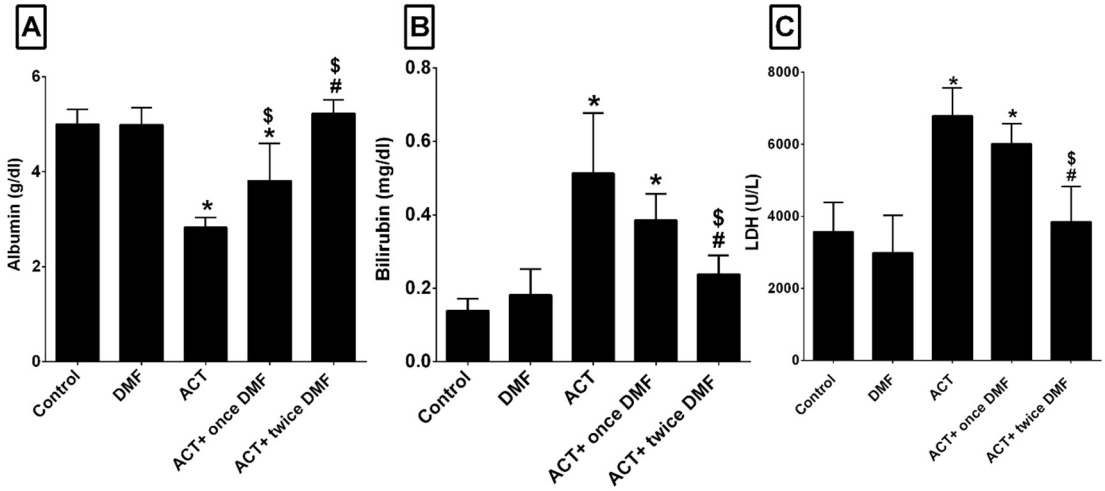

Fig. 3. Effect of DMF on serum levels of albumin, bilirubin and LDH. (A) DMF significantly elevated the albumin levels after being decreased upon ACT treatment. Serum (B) bilirubin and (C) LDH levels were augmented by ACT treatment and lowered by DMF. Data is expressed as mean ± SD, n = 8. Different symbols indicate statistical significance of $p < 0.05$ (* compared to control group, $ compared to ACT group, # compared to one-dose treatment group) using regular one-way ANOVA followed by Tukey's multiple comparisons test.

3.3. DMF promotes hepatocellular antioxidant defense

ACT exerts its hepatotoxicity primarily through oxidative stress [20]. To investigate the role of DMF in enhancing the antioxidant status, enzyme levels of SOD and MDA were measured in liver homogenate. SOD is an important enzyme in the cellular antioxidant machinery that scavenges free radicals and eventually causing depletion of GSH. MDA is a free radical metabolite and a measure of lipid peroxidation. As expected, SOD levels were reduced in ACT group (0.4 fold of normal control group) whereas DMF elevated SOD levels significantly to 1.5 fold and 2.2 fold of ACT group in one-dose and two-dose groups respectively. Serum MDA levels increased in ACT group 2.7 fold of normal control group and was attenuated after DMF treatment (0.7 fold and 0.4 fold in one-dose and two-dose groups respectively). Another parameter indicative of the oxidative stress status is NO levels released in response to hepatocellular injury. Our results clearly demonstrated that the NO levels increased by ACT were suppressed by DMF (0.9 fold and 0.5 fold of ACT group in one-dose and two-dose respectively). ACT has also resulted in severe depletion of GSH in hepatic cells which was significantly recovered by treatment with DMF (2.7 fold and a significant 6.9 fold elevation in one-dose and two-dose groups respectively) (Table 1). Again, DMF did not cause any significant variations in the former markers in the control drug group (Table 1).

3.4. DMF enhances antiinflammatory activity

Inflammation plays a complex role in ACT-induced hepatotoxicity. TNF-α is a pleitropic inflammatory mediator that stimulates many pathways involved in eliciting inflammatory response. NF-κB is another key regulator in inflammation that is triggered in response to hepatocellular injury. We evaluated the levels of NF-κB and TNF-α in liver homogenate. ACT induced the activation of NF-κB which was attenuated upon DMF treatment (0.8 fold and 0.5 fold of ACT group in one-dose and two-dose groups respectively) (Fig. 4A). Similarly, TNF-α levels were enhanced by ACT treatment and suppressed by DMF (non significant 0.8 fold and a significant 0.4 fold of ACT group in one-dose and two-dose groups respectively) (Fig. 4B). No significant changes in the control drug group were observed.

MPO is an inflammatory enzyme expressed abundantly in neutrophils. MPO initiates inflammatory reactions and is also linked to oxidative stress. Liver sections were stained to detect myeloperoxidase (MPO) expression (Fig. 6B). Liver tissue in the ACT group showed strong positive staining indicating elevated expression of MPO with a score 3 compared to the control group of a score 1. A single dose of DMF partially lowered MPO expression with a staining score of 2. Two doses significantly ameliorated the inflammatory status through lowering MPO expression with a staining score of 1.

3.5. DMF alleviates ACT-induced hepatotoxicity dependent of Nrf-2 pathway

DMF is a strong potentiator of Nrf-2 pathway and in order to investigate its role in ameliorating ACT-induced hepatotoxicity, Nrf-2 (Fig. 4C) and its downstream target HO-1 (Fig. 4D) have been evaluated in mice liver homogenates. Administration of DMF once and twice enhanced Nrf-2 and HO-1 expression levels in liver. Similarly, the control drug group did not show any significant variations.

Table 1
Anti-oxidant parameters.

Groups	Control	DMF	ACT	ACT + once DMF	ACT + twice DMF
MDA (nmol/100 mg tissue)	5.37 ± 2.53	3.63 ± 1.50	14.88 ± 4.19*	11.48 ± 4.197*	6.68 ± 2.79$^{\$\#}$
GSH (nmol/100 mg tissue)	0.55 ± 0.10	0.63 ± 0.29	0.20 ± 0.08*	0.5427 ± 0.20$^{\$}$	1.38 ± 0.24$^{*\$\#}$
SOD (U/100 mg tissue)	55.23 ± 1.63	47.73 ± 15.7	26.59 ± 7.88*	39.00 ± 5.71*	58.30 ± 8.43$^{\$\ \#}$
NO (nmol/100 mg tissue)	2.98 ± 0.84	3.35 ± 0.69	11.72 ± 1.77*	10.69 ± 2.99*	6.27 ± 1.90$^{*\$\#}$

ACT, acetaminophen (500 mg/kg, i.p.); DMF, dimethyl fumarate (100 mg/kg, once or twice orally). Data as mean ± SD, n = 8.
Malondialdehyde (MDA); reduced glutathione (GSH), superoxide dismutase (SOD), total nitrate/nitrite (NOx).
*, $, # $p < 0.05$, significantly different from control, ACT, or ACT + once DMF group respectively using one-way ANOVA followed by Tukey-Kramer multiple comparisons post-hoc test.

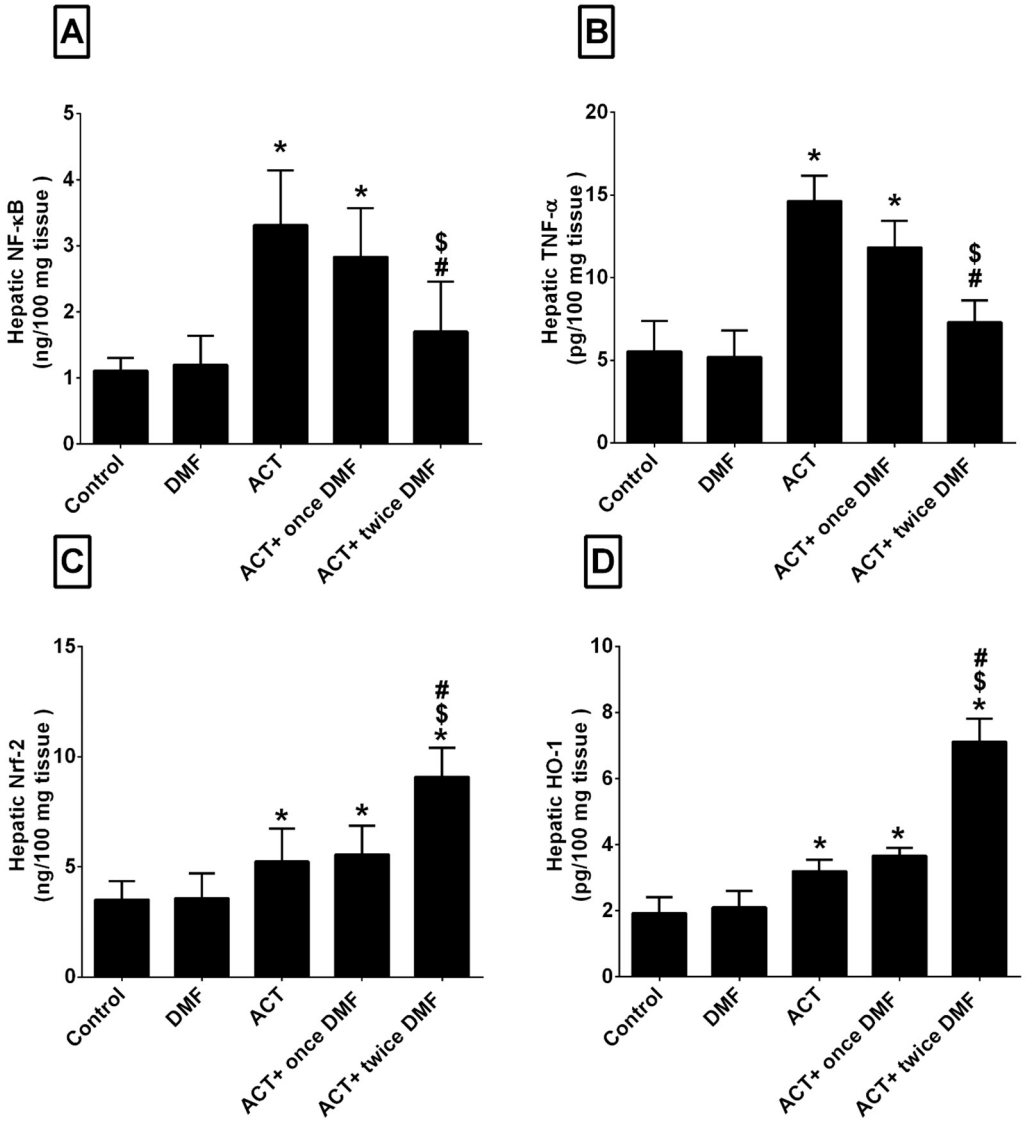

Fig. 4. Effect of DMF treatment on hepatic NF-κB and hepatic TNF-α and Nrf-2 pathway. DMF reduced elevated hepatic levels of (A) NF-κB and (B) TNF-α. ACT elevated hepatic levels of (C) Nrf-2 and (D) HO-1. Data is expressed as mean ± SD, n = 6. Different symbols indicate statistical significance of $p < 0.05$ (* compared to control group, $ compared to ACT group, # compared to one-dose treatment group) using regular one-way ANOVA followed by Tukey's multiple comparisons test.

3.6. DMF induced apoptosis through elevation of Bcl-2 antiapoptotic regulator and caspase-3

Apoptosis can be mediated through members of the Bcl-2 family which then recruit effector caspases including caspase-3 to degrade target proteins resulting in apoptotic death. Hepatic Bcl-2 levels were markedly reduced in ACT-group. Bcl-2 levels were elevated in both one-dose and two-dose groups. However, only the two-dose group was significantly higher than the ACT group ($p < 0.05$) (Fig. 5A). Caspase-3 levels were elevated in ACT-group. DMF treatment reduced caspase-3 levels and it was more pronounced when DMF administered twice compared to a single dose administration (Fig. 5B).

3.7. Histochemical analysis

Inflammation of liver was scored according to Knodell score [18]. The control group showed normal liver histology while the ACT group showed marked inflammation of score (+3). Additionally, hydropic degeneration of liver cells was observed (score = +3). Liver cells also exhibited necrosis. DMF treatment showed very mild inflammation (score = 0–1). No necrosis and mild to no ballooning were also observed. The control drug group also showed very mild inflammation (score = +1) with no hepatocellular ballooning or necrosis (Fig. 6A).

4. Discussion

Liver is the principle metabolic organ which detoxifies various toxins and chemicals. Yet, intoxication by drug overdosing is the prime reason for hepatotoxicity. Excessive consumption of ACT has been estimated to be the most frequent causative agent of drug-induced hepatotoxicity [21]. Several studies have demonstrated protective activities of therapeutic drugs or natural compounds against ACT-induced toxicity [22–25]. We here assess the curative potential of DMF, a safely-marketed drug, to treat ACT-induced liver injury in a mouse model. DMF, a fumaric acid ester indicated for psoriasis and multiple sclerosis, has proven to have antiinflammatory and antioxidant properties [12,26–28]. We have tested DMF at 100 mg/kg in two groups: one group was only treated once and the second group recieved two doses 12 h apart. Our results clearly showcase that the two-dose treatment was more effective than one single dose in ameliorating liver injury and improving various biochemical markers.

A marked enlargement of liver weight as represented by liver body

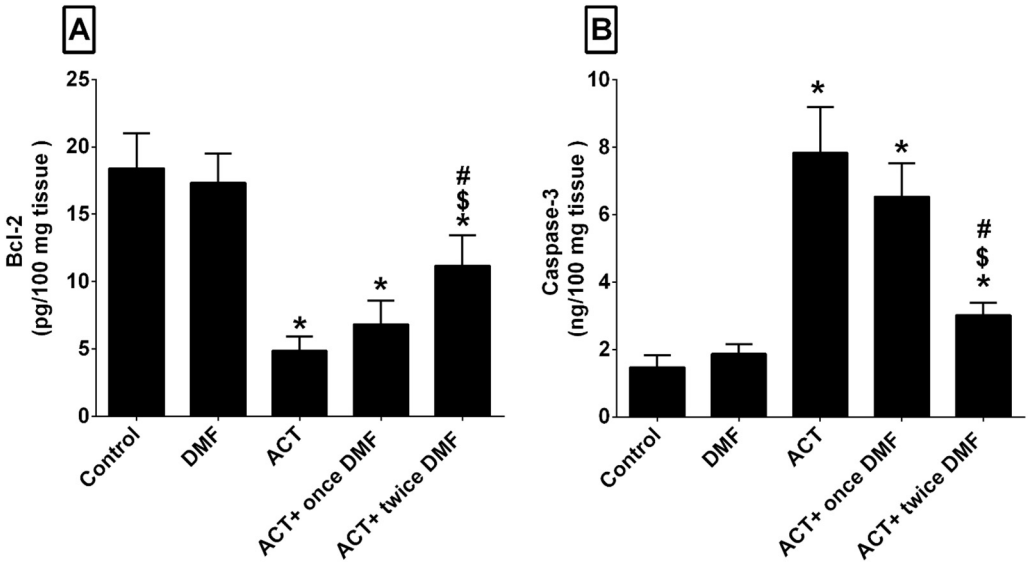

Fig. 5. Effect of DMF on hepatic Bcl-2 and caspase-3. Administration of DMF elevated levels of A) hepatic Bcl-2 and reduced B) caspase-3 levels. Data is expressed as mean ± SD, n = 6. Different symbols indicate statistical significance of $p < 0.05$ (* compared to control group, $ compared to ACT group, # compared to one-dose treatment group) using regular one-way ANOVA followed by Tukey's multiple comparisons test.

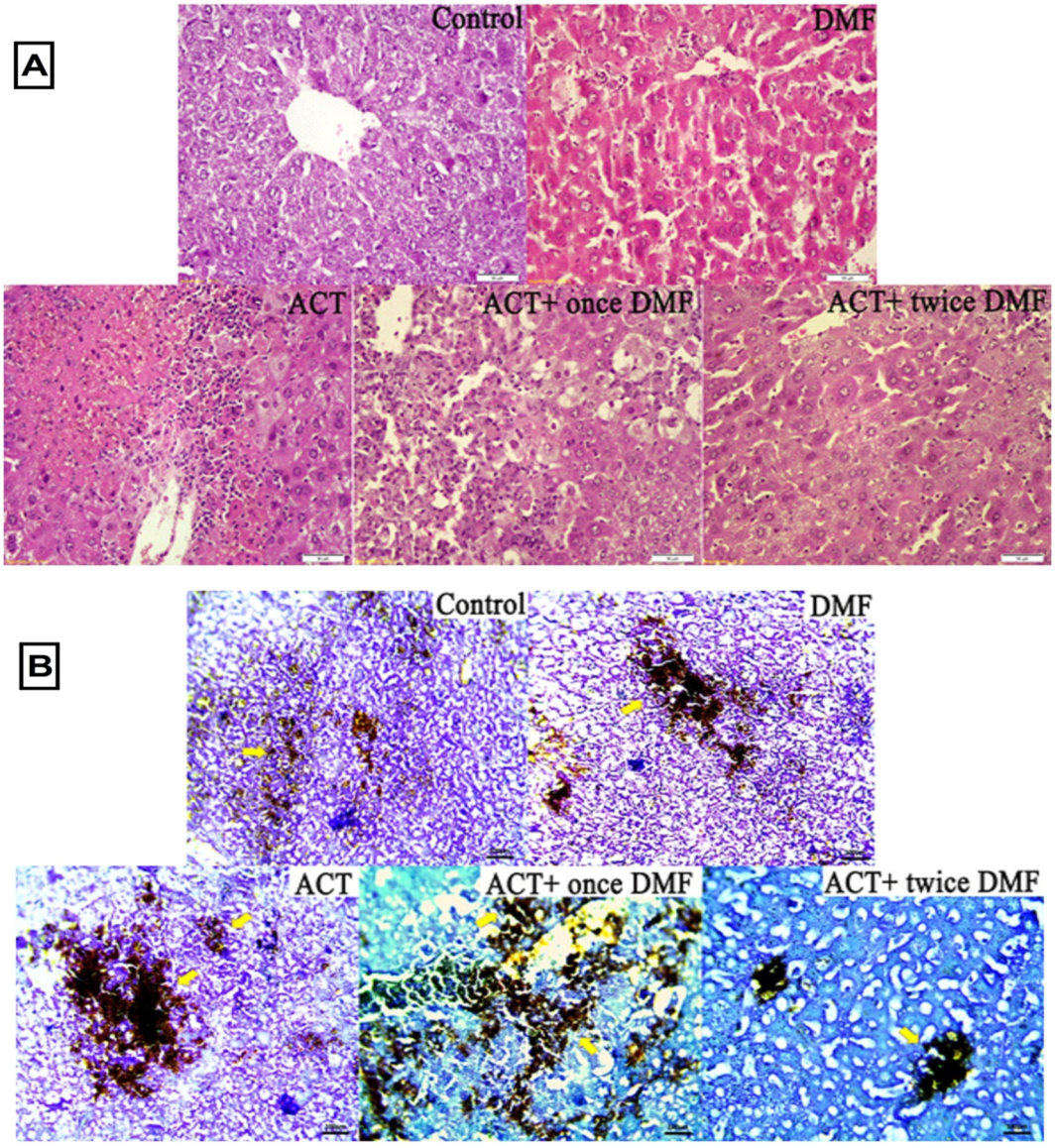

Fig. 6. Representative photomicrographs of (A) H&E stained and (B) MPO-stained liver tissue. Control group, control drug group, ACT group, ACT + one-dose DMF group and ACT + two-dose DMF groups.

weight ratio indicated hepatic lesions and liver injury after ACT treatment. The aminotransferases SGPT and SGOT located in the hepatocytes are leaked to the extracellular milieu after cellular damage caused by ACT. Hence, their levels were markedly elevated. The microsomal enzyme GGT is present in hepatocytes and biliary epithelial cells and is induced by a variety of drugs among which is ACT. Bilirubin is normally conjugated in the liver with glucuronic acid. Loss of hepatic function reduces the excretory capacity of the liver and is associated with augmented bilirubin serum levels as we have seen here after ACT toxicity. DMF was able to restore the former markers to normal values and ameliorate hepatotoxicity.

In order to pinpoint the exact mechanism of antihepatic activity of DMF we investigated its role in modulating the Nrf-2/ARE pathway. DMF is a well-known Nrf-2 poteniatior and exerts its cytoprotective role against oxidative stress through activating the Nrf-2 pathway and upregulation of antioxidant genes [29]. The Nrf-2/ARE pathway has been a recent attractive target in addressing ACT-induced toxicity. Several studies have reported the hepatoprotective role of various therapeutic agents through targeting the Nrf-2 pathway. Only one study described curative treatments after ACT administration using a natural compound [30]. DMF interacts with KEAP-1 and stabilizes Nrf-2 by inhibiting KEAP-1 interaction with Nrf-2. Indeed, in our study DMF elevated Nrf-2 levels and its downstream target HO-1 after administering one and two doses (100 mg/kg) post ACT treatment. HO-1 is a ubiquitously expressed heat shock protein that is activated during oxidative stress as another protective mechanism against various stimuli. The antioxidant enzyme HO-1 induces the formation of bilirubin which is capable of sequestering reactive oxygen species (ROS) [31]. This suggests that the antihepatic acitivy of DMF may occur through the Nrf-1/ARE cytoprotective pathway.

We also assessed the antioxidant role of DMF by evaluating NO, MDA, GSH and SOD levels in liver homogenates. It is well-established that NO is involved in the pathophysiology of ACT-induced hepatoxicity [32]. Accumulation of NO results in altered protein and DNA synthesis contributing to tissue injury [33,34]. DMF treatment has reduced serum NO levels potentially through suppressing NO synthase enzymes as it has been previously observed in microglia and astrocytes [35]. Drug-induced toxicity evokes lipid peroxidation leading to cellular death. Accumulation of MDA is considered a significant indicator of oxidative damage in the cell and its levels have been elevated upon acute ACT exposure. DMF treatment has significantly reduced MDA, elevated SOD levels and alleviated the oxidative stress status. Toxic doses of ACT metabolize into NAPQ1 which rapidly depletes hepatic GSH. DMF elevated supressed GSH levels potentially through either the Nrf2 pathway or through inducing GSH recycling by upregulating glutathione reductase as it has been recently reported [36]. Elevated GSH levels contribute to the cellular antioxidant defense system in the liver through scavenging hydroxyl and superoxide radicals, conjugating with endogenous electrophiles and toxic xenobiotics to facilitate their safe elimination from the body [37] or maintaining the cellular redox homeostasis with its oxidized form, glutathione disulphide [38]. Although the exact mechanism of antioxidant activity of DMF is not clearly understood, DMF may potentially exert its action through potentiating the antioxidant Nrf-2 pathway. One study investigated the antioxidant activity of DMF in oligodendrocytes by metabolic profiling and found that DMF altered the citric acid cycle intermediates, GSH and lipid metabolism which in turn influenced mitochondrial functions and activated antioxidant molecules [39].

We also sought to investigate the antiinflammmatory role of DMF against ACT toxicity. Multiple *in vivo* studies have shown that Nrf-2 has a protective effect in inflammatory diseases [40–44]. Protein adduct formation and extensive glutathione depletion cause mitochondrial oxidative stress triggering sequential events that lead eventually to transcriptional activation of proinflammatory cytokines such as Tumor Necrosis factor alpha (TNF-α) [45]. Overdoses of ACT caused a marked elevation of TNF-α that was diminished by DMF treatment. DMF has shown to reduce TNF-α levels in glial cells [35] and after liver ischemic/reperfusion [13]. NFκ-B is a key transcription factor that controls the expression of genes regulating liver regeneration. Following ACT administration, NFκ-B is upregulated and is translocated to the nucleus where it induces inflammatory mediators consistent with previous reports [46,47]. DMF reduced NFκ-B potentially through abolishing its phosphorylation and nuclear translocation [48]. Additionally, uncontrolled neutrophil migration contributes to the hepatic inflammatory response to ACT overdose [49]. Neutrophil accumulation triggers hepatic damage through the generation of reactive oxygen species [50]. This was clearly demonstrated in ACT-group which showed a marked elevation of MPO expression in mice liver tissue sections. MPO is a distinct marker of inflammation and neutrophil inflammation. DMF reduced MPO expression in liver sections which showcases its role in attenuation the inflammatory stress evoked by ACT-induced liver injury. It is believed that both Nrf-2 and NFκ-B pathways crosstalk to control the transcription of downstream target proteins. In prostate cancer cells, elevated expression of HO-1—the downstream target of Nrf-2—inhibited NFκ-B nuclear translocation and thus impeding NFκ-B activity [51]. Nrf-2 was also found to negatively regulate NFκ-B in Nrf-2 knockout murine lupus nephritis [52]. Additionally, Nrf-2 played a role in host viral infection of respiratory syncytial virus through inhibition of NF-κB and suppression of lung protein and lipid oxidation [53]. Moreover, activation of the antioxidant downstream genes of Nrf-2 may maintain reactive oxygen species at levels insufficient to trigger inflammation.

Mounting evidence highlighted the role of apoptosis in ACT-induced liver injury. It has been observed that ACT caused the translocation of Bcl-2 family proteins [54,55] and the elevation of the apoptotic effector caspase-3 activating the mitochondrial pathway [55,56]. The antiapoptotic Bcl-2 regulator hepatic levels were reduced and further elevated upon DMF treatment. DMF was shown to have dual effect on survival of breast cancer cells in a dose-dependent manner [57]. However, the apoptotic activity of DMF has been shown through the induction of apoptosis in human T cells in a concentration-dependent manner [58]. Additionally, DMF restored the apoptotic sensitivity in cuticle T-cell lymphoma [59]. Nrf-2 pathway seems to crosstalk with different elements of the apoptotic pathways as reviewed previously [60]. It was found that Nrf2 upregulated gene transcription of *Bcl-2* gene and elevated Bcl-2 protein and consequently elevated caspase-3 levels in lung cancer cell lines [61].

The present results confirm that DMF has ameliorated ACT-induced liver injury primarily through its anti-inflammatory, antiapoptotic and antioxidant activity in a Nrf-2 dependent manner. This study demonstrated the treatment efficacy of DMF, a marketed drug that has a well-defined safety profile, against ACT-induced hepatotoxicity. Such efficacy was evident by enhancing antioxidant defense machinery and reducing the generation of inflammatory cytokines and suppressing apoptosis. A suggested mechanism of DMF is illustrated in the graphical abstaract. Amelioration of the liver morphology confirmed the biochemical findings. Therefore, DMF may represent a promising treatment agent against ACT-induced liver injury. The experimental data presented here indeed provide a novel indication for the antipsoriatic drug, DMF.

Declarations

- Ethics approval

An ethical approval form of the Ethical Research Committee at the Faculty of Pharmacy, Mansoura University for the use of experimental animals in this study is submitted with this manuscript. The committee is headed by Prof.Dr. Ahmed F. Halim. Committee reference number: 2018-12

- Consent for publication

Not applicable.

- Availability of data and materials

All data generated or analyzed during this study are either included in this published article (and its Supplementary information files) or available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding.

Contributions

R.A designed the experiments; R.A and N.A performed the experiments, analyzed the data and wrote the manuscript.

Acknowledgments

The authors would like to acknowledge Dr. MA El-Hamid, fellow of pathology at the Urology and Nephrology Center at Mansoura University, for providing assistance in the histopathological examination.

References

[1] P.J. Gow, R.M. Jones, J.L. Dobson, P.W. Angus, Etiology and outcome of fulminant hepatic failure managed at an Australian liver transplant unit, J. Gastroenterol. Hepatol. 19 (2) (2004) 154–159.
[2] W. Bernal, Changing patterns of causation and the use of transplantation in the United Kingdom, Semin. Liver Dis. 23 (03) (2003) 227–238.
[3] A.M. Larson, J. Polson, R.J. Fontana, T.J. Davern, E. Lalani, L.S. Hynan, et al., Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study, Hepatology 42 (6) (2005) 1364–1372.
[4] S.S. Lee, J.T. Buters, T. Pineau, P. Fernandez-Salguero, F.J. Gonzalez, Role of CYP2E1 in the hepatotoxicity of acetaminophen, J. Biol. Chem. 271 (20) (1996) 12063–12067.
[5] R. Venugopal, A.K. Jaiswal, Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene, Proc. Natl. Acad. Sci. U. S. A. 93 (25) (1996) 14960–14965.
[6] R. Venugopal, A.K. Jaiswal, Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes, Oncogene 17 (24) (1998) 3145–3156.
[7] J.E. Clark, R. Foresti, C.J. Green, R. Motterlini, Dynamics of haem oxygenase-1 expression and bilirubin production in cellular protection against oxidative stress, Biochem. J. 348 (Pt 3) (2000) 615–619.
[8] L. Kappos, R. Gold, D.H. Miller, D.G. Macmanus, E. Havrdova, V. Limmroth, et al., Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study, Lancet 372 (9648) (2008) 1463–1472.
[9] R. Gold, L. Kappos, D.L. Arnold, A. Bar-Or, G. Giovannoni, K. Selmaj, et al., Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis, N. Engl. J. Med. 367 (12) (2012) 1098–1107.
[10] M. Stangel, R.A. Linker, Dimethyl fumarate (BG-12) for the treatment of multiple sclerosis, Expert. Rev. Clin. Pharmacol. 6 (4) (2013) 355–362.
[11] P. Seidel, M. Roth, Anti-inflammatory dimethylfumarate: a potential new therapy for asthma? Mediat. Inflamm. 2013 (2013) 875403.
[12] G. Casili, M. Cordaro, D. Impellizzeri, G. Bruschetta, I. Paterniti, S. Cuzzocrea, et al., Dimethyl fumarate reduces inflammatory responses in experimental colitis, J. Crohns Colitis 10 (4) (2016) 472–483.
[13] C. Takasu, N.D. Vaziri, S. Li, L. Robles, K. Vo, M. Takasu, et al., Treatment with dimethyl fumarate ameliorates liver ischemia/reperfusion injury, World J. Gastroenterol. 23 (25) (2017) 4508–4516.
[14] S. Marklund, G. Marklund, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur. J. Biochem. 47 (3) (1974) 469–474.
[15] D. Gerard-Monnier, I. Erdelmeier, K. Regnard, N. Moze-Henry, J.C. Yadan, J. Chaudiere, Reactions of 1-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Analytical applications to a colorimetric assay of lipid peroxidation, Chem. Res. Toxicol. 11 (10) (1998) 1176–1183.
[16] M.E. Shaker, Nilotinib interferes with the signalling pathways implicated in acetaminophen hepatotoxicity, Basic Clin. Pharmacol. Toxicol. 114 (3) (2014) 263–270.
[17] K.M. Miranda, M.G. Espey, D.A. Wink, A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite, Nitric Oxide 5 (1) (2001) 62–71.
[18] Z.D. Goodman, Grading and staging systems for inflammation and fibrosis in chronic liver diseases, J. Hepatol. 47 (4) (2007) 598–607.
[19] F.K. Chan, K. Moriwaki, M.J. De Rosa, Detection of necrosis by release of lactate dehydrogenase activity, Methods Mol. Biol. 979 (2013) 65–70.
[20] K. Du, A. Ramachandran, H. Jaeschke, Oxidative stress during acetaminophen hepatotoxicity: sources, pathophysiological role and therapeutic potential, Redox Biol. 10 (2016) 148–156.
[21] W.M. Lee, Drug-induced acute liver failure, Clin. Liver Dis. 17 (4) (2013) 575–586 (viii).
[22] F. Jin, C. Wan, W. Li, L. Yao, H. Zhao, Y. Zou, et al., Formononetin protects against acetaminophen-induced hepatotoxicity through enhanced NRF2 activity, PLoS One 12 (2) (2017) e0170900.
[23] J.R. Noh, Y.H. Kim, J.H. Hwang, D.H. Choi, K.S. Kim, W.K. Oh, et al., Sulforaphane protects against acetaminophen-induced hepatotoxicity, Food Chem. Toxicol. 80 (2015) 193–200.
[24] W. Xie, M. Wang, C. Chen, X. Zhang, M.F. Melzig, Hepatoprotective effect of isoquercitrin against acetaminophen-induced liver injury, Life Sci. 152 (2016) 180–189.
[25] G. Gupta, G. Krishna, D.K. Chellappan, K.S. Gubbiyappa, M. Candasamy, K. Dua, Protective effect of pioglitazone, a PPARgamma agonist against acetaminophen-induced hepatotoxicity in rats, Mol. Cell. Biochem. 393 (1–2) (2014) 223–228.
[26] U. Schulze-Topphoff, M. Varrin-Doyer, K. Pekarek, C.M. Spencer, A. Shetty, S.A. Sagan, et al., Dimethyl fumarate treatment induces adaptive and innate immune modulation independent of Nrf2, Proc. Natl. Acad. Sci. U. S. A. 113 (17) (2016) 4777–4782.
[27] P. Seidel, I. Merfort, J.M. Hughes, B.G. Oliver, M. Tamm, M. Roth, Dimethylfumarate inhibits NF-{kappa}B function at multiple levels to limit airway smooth muscle cell cytokine secretion, Am. J. Physiol. Lung Cell. Mol. Physiol. 297 (2) (2009) L326–L339.
[28] S.X. Lin, L. Lisi, C. Dello Russo, P.E. Polak, A. Sharp, G. Weinberg, et al., The anti-inflammatory effects of dimethyl fumarate in astrocytes involve glutathione and haem oxygenase-1, ASN Neuro 3 (2) (2011).
[29] R.H. Scannevin, S. Chollate, Jung M-Y, M. Shackett, H. Patel, P. Bista, et al., Fumarates promote cytoprotection of central nervous system cells against oxidative stress via the nuclear factor (erythroid-derived 2)-like 2 pathway, J. Pharmacol. Exp. Ther. 341 (1) (2012) 274–284.
[30] R.N. Jadeja, N.H. Urrunaga, S. Dash, S. Khurana, N.K. Saxena, Withaferin-a reduces acetaminophen-induced liver injury in mice, Biochem. Pharmacol. 97 (1) (2015) 122–132.
[31] M. Bauer, K. Huse, U. Settmacher, R.A. Claus, The heme oxygenase-carbon monoxide system: regulation and role in stress response and organ failure, Intensive Care Med. 34 (4) (2008) 640–648.
[32] R. Carol, D.E.H. Gardener, Chung S. Yang, Paul E. Thomas, Xu-Jie Zhang, George L. Degoerge, Jeffery D. Laskin ADLL, Role of nitric oxide in acetaminophen-induced hepatotoxicity in the rat, Hepatology 27 (3) (1998) 748–754.
[33] R.D. Curran, F.K. Ferrari, P.H. Kispert, J. Stadler, D.J. Stuehr, R.L. Simmons, et al., Nitric oxide and nitric oxide-generating compounds inhibit hepatocyte protein synthesis, FASEB J. 5 (7) (1991) 2085–2092.
[34] M. Dalmau, M. Joaquin, T. Nakamura, R. Bartrons, J. Gil, Nitric oxide inhibits DNA synthesis and induces activation of poly(ADP-ribose) polymerase in cultured rat hepatocytes, Exp. Cell Res. 228 (1) (1996) 14–18.
[35] H. Wilms, J. Sievers, U. Rickert, M. Rostami-Yazdi, U. Mrowietz, R. Lucius, Dimethylfumarate inhibits microglial and astrocytic inflammation by suppressing the synthesis of nitric oxide, IL-1beta, TNF-alpha and IL-6 in an in-vitro model of brain inflammation, J. Neuroinflammation 7 (2010) 30.
[36] C. Hoffmann, M. Dietrich, A.K. Herrmann, T. Schacht, P. Albrecht, A. Methner, Dimethyl fumarate induces glutathione recycling by upregulation of glutathione reductase, Oxidative Med. Cell. Longev. 2017 (2017) 6093903.
[37] R. Rinaldi, E. Eliasson, S. Swedmark, R. Morgenstern, Reactive intermediates and the dynamics of glutathione transferases, Drug Metab. Dispos. 30 (10) (2002) 1053–1058.
[38] T.P. Dalton, Y. Chen, S.N. Schneider, D.W. Nebert, H.G. Shertzer, Genetically altered mice to evaluate glutathione homeostasis in health and disease, Free Radic. Biol. Med. 37 (10) (2004) 1511–1526.
[39] H. Huang, A. Taraboletti, L.P. Shriver, Dimethyl fumarate modulates antioxidant and lipid metabolism in oligodendrocytes, Redox Biol. 5 (2015) 169–175.
[40] C.Y. Wang, Z.Y. Wang, J.W. Xie, J.H. Cai, T. Wang, Y. Xu, et al., CD36 upregulation mediated by intranasal LV-NRF2 treatment mitigates hypoxia-induced progression of Alzheimer's-like pathogenesis, Antioxid. Redox Signal. 21 (16) (2014) 2208–2230.
[41] L. Gu, W.S. Deng, Y. Liu, C.H. Jiang, L.C. Sun, X.F. Sun, et al., Ellagic acid protects lipopolysaccharide/D-galactosamine-induced acute hepatic injury in mice, Int. Immunopharmacol. 22 (2) (2014) 341–345.
[42] Y. Ishii, K. Itoh, Y. Morishima, T. Kimura, T. Kiwamoto, T. Iizuka, et al., Transcription factor Nrf2 plays a pivotal role in protection against elastase-induced pulmonary inflammation and emphysema, J. Immunol. 175 (10) (2005) 6968–6975.
[43] S. Braun, C. Hanselmann, M.G. Gassmann, U. auf dem Keller, C. Born-Berclaz, K. Chan, et al., Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound, Mol. Cell. Biol. 22 (15) (2002) 5492–5505.
[44] R.K. Thimmulappa, H. Lee, T. Rangasamy, S.P. Reddy, M. Yamamoto, T.W. Kensler,

et al., Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis, J. Clin. Invest. 116 (4) (2006) 984–995.
[45] M.E. Blazka, J.L. Wilmer, S.D. Holladay, R.E. Wilson, M.I. Luster, Role of proinflammatory cytokines in acetaminophen hepatotoxicity, Toxicol. Appl. Pharmacol. 133 (1) (1995) 43–52.
[46] M.E. Blazka, A. Bruccoleri, P.P. Simeonova, D.R. Germolec, K.R. Pennypacker, M.I. Luster, Acetaminophen-induced hepatotoxicity is associated with early changes in AP-1 DNA binding activity, Res. Commun. Mol. Pathol. Pharmacol. 92 (3) (1996) 259–273.
[47] D.M. Dambach, S.K. Durham, J.D. Laskin, D.L. Laskin, Distinct roles of NF-kappaB p50 in the regulation of acetaminophen-induced inflammatory mediator production and hepatotoxicity, Toxicol. Appl. Pharmacol. 211 (2) (2006) 157–165.
[48] A. Wierinckx, J. Breve, D. Mercier, M. Schultzberg, B. Drukarch, A.M. Van Dam, Detoxication enzyme inducers modify cytokine production in rat mixed glial cells, J. Neuroimmunol. 166 (1–2) (2005) 132–143.
[49] P.E. Marques, S.S. Amaral, D.A. Pires, L.L. Nogueira, F.M. Soriani, B.H. Lima, et al., Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure, Hepatology 56 (5) (2012) 1971–1982.
[50] P.A. Ward, J. Varani, Mechanisms of neutrophil-mediated killing of endothelial cells, J. Leukoc. Biol. 48 (1) (1990) 97–102.
[51] I. Bellezza, A. Tucci, F. Galli, S. Grottelli, A.L. Mierla, F. Pilolli, et al., Inhibition of NF-kappaB nuclear translocation via HO-1 activation underlies alpha-tocopheryl succinate toxicity, J. Nutr. Biochem. 23 (12) (2012) 1583–1591.
[52] T. Jiang, F. Tian, H. Zheng, S.A. Whitman, Y. Lin, Z. Zhang, et al., Nrf2 suppresses lupus nephritis through inhibition of oxidative injury and the NF-kappaB-mediated inflammatory response, Kidney Int. 85 (2) (2014) 333–343.
[53] H.Y. Cho, F. Imani, L. Miller-Degraff, D. Walters, G.A. Melendi, M. Yamamoto, et al., Antiviral activity of Nrf2 in a murine model of respiratory syncytial virus disease, Am. J. Respir. Crit. Care Med. 179 (2) (2009) 138–150.
[54] G.E. Kass, P. Macanas-Pirard, P.C. Lee, R.H. Hinton, The role of apoptosis in acetaminophen-induced injury, Ann. N. Y. Acad. Sci. 1010 (2003) 557–559.
[55] M.L. Adams, R.H. Pierce, M.E. Vail, C.C. White, R.P. Tonge, T.J. Kavanagh, et al., Enhanced acetaminophen hepatotoxicity in transgenic mice overexpressing BCL-2, Mol. Pharmacol. 60 (5) (2001) 907–915.
[56] H. El-Hassan, K. Anwar, P. Macanas-Pirard, M. Crabtree, S.C. Chow, V.L. Johnson, et al., Involvement of mitochondria in acetaminophen-induced apoptosis and hepatic injury: roles of cytochrome *c*, Bax, Bid, and caspases, Toxicol. Appl. Pharmacol. 191 (2) (2003) 118–129.
[57] J. Zhang, D. Zhou, L. Zhang, Q. Lin, W. Ren, J. Zhang, et al., Dual effects of *N*,*N*-dimethylformamide on cell proliferation and apoptosis in breast cancer, Dose-Response 15 (4) (2017) 1559325817744450.
[58] F. Treumer, K. Zhu, R. Glaser, U. Mrowietz, Dimethylfumarate is a potent inducer of apoptosis in human T cells, J. Invest. Dermatol. 121 (6) (2003) 1383–1388.
[59] J.P. Nicolay, K. Muller-Decker, A. Schroeder, M. Brechmann, M. Mobs, C. Geraud, et al., Dimethyl fumarate restores apoptosis sensitivity and inhibits tumor growth and metastasis in CTCL by targeting NF-kappaB, Blood 128 (6) (2016) 805–815.
[60] T.M. Stepkowski, M.K. Kruszewski, Molecular cross-talk between the NRF2/KEAP1 signaling pathway, autophagy, and apoptosis, Free Radic. Biol. Med. 50 (9) (2011) 1186–1195.
[61] S.K. Niture, A.K. Jaiswal, Nrf2 protein up-regulates antiapoptotic protein Bcl-2 and prevents cellular apoptosis, J. Biol. Chem. 287 (13) (2012) 9873–9886.