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NRF2 ACTIVATOR DIMETHYL FUMARATE DIMINISHED STEATOSIS, INFLAMMATION AND LIPID PEROXIDATION IN THE LIVER OF BINGE ETHANOL-TREATED RATS

NRF2 AKTİVATÖRÜ DİMETİL FUMARAT, AŞIRI ETANOL UYGULANAN SIÇANLARIN KARACİĞERİNDE YAĞLANMA, ENFLAMASYON VE LİPİD OKSİDASYONUNU AZALTTI

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ABSTRACT

Objective: This study was conducted to explore the impact of dimethyl fumarate (DMF), which has antioxidant and anti-inflammatory effects, on binge ethanol (EtOH)-induced hepatic steatosis, inflammation, and lipid peroxidation in rats.

Material and Method: To examine the potential protective effect of DMF against EtOH-induced hepatic damage, rats were divided into four groups control, DMF, EtOH, and DMF+EtOH. Rats were administered EtOH (4.5 g/kg orally, 3 doses with 12-h intervals). DMF (30 mg/kg; gavage) was applied to rats one hour before each application of EtOH in the DMF+EtOH group. Serum markers of liver damage, triglyceride (TG), tumor necrosis factor-alpha (TNF-α), lipid and protein oxidation products, myeloperoxidase (MPO), and antioxidant enzymes together with histopathological examinations were performed in liver tissue. Protein expressions associated with antioxidant mechanism (nuclear factor erythroid 2-related factor; Nrf2 and heme oxygenase-1; HO-1), lipid metabolism (sterol regulatory element-binding protein-1c; SREBP-1c and peroxisome proliferator-activated receptor-alpha; PPAR-α), oxidative stress (cytochrome P4502E1; CYP2E1), and inflammation (nuclear factor-kappa B; NF-κB) were also investigated in the rats' livers.

Result: DMF reduced elevated levels of serum markers of liver damage and hepatic TG, TNF-α and reactive oxygen species levels, lipid and protein oxidation products, and MPO activity together with the alleviation of histopathological lesions in

ÖZET

Amaç: Bu çalışma, antioksidan ve anti-inflamatuar etkilere sahip dimetil fumaratın (DMF) sıçanlarda binge etanol (EtOH) ile indüklenen karaciğer steatozu, inflamasyon ve lipit peroksidasyonu üzerindeki etkisini araştırmak amacıyla yapıldı.

Gereç ve Yöntem: EtOH ile indüklenen karaciğer hasarına karşı DMF'nin potansiyel koruyucu etkisini incelemek için sıçanlar kontrol, DMF, EtOH ve DMF+EtOH olmak üzere dört gruba ayrıldı. Sıçanlara EtOH (4,5 g/kg oral, 12 saat arayla 3 doz) verildi. DMF+EtOH grubundaki sıçanlara her EtOH uygulamasından bir saat önce DMF (30 mg/kg; gavaj) uygulandı. Karaciğer hasarı serum belirteçleri, trigliserid (TG), tümör nekroz faktörü-alfa (TNF-α), lipid ve protein oksidasyon ürünleri, miyeloperoksidaz (MPO) ve antioksidan enzimler ile birlikte karaciğer dokusunda histopatolojik incelemeler gerçekleştirildi. Karaciğerde antioksidan mekanizma (nükleer faktör eritroid 2 ile ilişkili faktör; Nrf2 ve hem oksijenaz-1; HO-1), lipid metabolizması (sterol düzenleyici element bağlayıcı protein-1c; SREBP-1c ve peroksizom proliferatör ile aktive olan reseptör-alfa; PPAR-α) oksidatif stres (sitokrom P4502E1; CYP2E1) ve inflamasyon (nükleer faktör-kappa B; NF-κB) ile ilişkili protein ekspresyonları da araştırıldı.

Bulgular: DMF, EtOH uygulanan sıçanlarda histopatolojik lezyonların iyileşmesinin yanında artmış serum karaciğer hasarı belirteçlerini, hepatik TG, TNF-α ve reaktif oksijen türleri düzeylerini, lipid ve protein oksidasyon ürünlerini ve MPO aktivitesini de azalttı. DMF+EtOH grubunda Nrf2 ve HO-1ekspresyonlarının

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EtOH-treated rats. Increased Nrf2 and HO-1 and decreased SREBP-1c and CYP2E1 expressions were also detected in the DMF+EtOH group compared with the EtOH group.

Conclusion: Our results demonstrate that DMF may provide a protective effect against EtOH-induced hepatic lesions. These outcomes may be linked to the anti-oxidative, anti-inflammatory, and anti-lipogenic potential of DMF-induced Nrf2 activation.

Keywords: Dimethyl fumarate, nuclear factor erythroid 2-related factor, ethanol, liver damage, oxidative stress

EtOH grubuna göre arttığı ve SREBP-1c ve CYP2E1 ekspresyonlarının ise EtOH grubuna göre azaldığı saptandı.

Sonuç: Sonuçlarımız, DMF'nin EtOH tarafından indüklenen karaciğer lezyonlarının oluşumuna karşı koruyucu bir etki sağlayabileceğini göstermektedir. Bu etkiler, DMF ile indüklenen Nrf2 aktivasyonunun antioksidan, anti-inflamatuar ve anti-lipojenik potansiyeli ile ilişkili olabilir.

Anahtar Kelimeler: Dimetil fumarat, nükleer faktör eritroid2 ilişkili faktör, etanol, karaciğer hasarı, oksidatif stres

INTRODUCTION

Alcoholic liver disease (ALD) develops due to excessive alcohol consumption and has various stages, including steatosis (fatty liver), steatohepatitis (ASH), fibrosis, and cirrhosis. The liver functions as the primary site for alcohol metabolism. The hepatotoxicity of ethanol (EtOH) is attributed to the oxidation byproducts of EtOH rather than EtOH itself. EtOH is converted to acetaldehyde (AA) through reactions catalyzed by alcohol dehydrogenase (ADH), cytochrome P450 (CYP2E1), and catalase enzymes in the liver (1,2). Hepatic lipid accumulation, oxidative stress, inflammation, and toxic AA accumulation are significant factors in the pathogenesis of ALD (1,2).

Steatosis is the first stage of ALD, which is reversible with abstinence and involves fat accumulation along with minimal liver injury. However, according to the 'two hit' hypothesis, steatosis makes the liver sensitive to some insults (as second hit) such as oxidative stress, endotoxins, and cytokines. Thus, the formation of more severe forms of ALD such as ASH is triggered (3).

Nuclear factor-erythroid-2-related factor 2 (Nrf2) acts as a transcription factor. It stimulates the transcription of various antioxidant and cytoprotective genes, including heme oxygenase-1 (HO-1), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) by binding to the antioxidant response element (ARE) by translocating into the nucleus (4). Nrf2 not only controls oxidative stress, but also negatively regulates lipogenesis through sterol regulatory element-binding protein-1c (SREBP-1c) and its target genes, including fatty acid synthase, and ATP-citrate lyase, and positively regulates fatty acid oxidation through PPAR-α and its target genes related to β-oxidation (5). Furthermore, there is an inverse relationship between Nrf2 and "nuclear factor-kappa B" (NF-κB), the primary transcription factor responsible for pro-inflammatory gene expression (6).

Due to the multifunctional efficiency of Nrf2, several antioxidant phytochemicals and some synthetic Nrf2 activators were detected to be useful in several chronic diseases including liver diseases (4,6-8). Moreover, it has been suggested that Nrf2 activation can protect EtOH-treated rodents (9,10). However, contradictory results have also been obtained showing that activation of Nrf2 increases alcoholic liver damage (9,11,12).

Dimethyl fumarate (DMF) is the only Nrf2 activator approved for clinical use. It is used to treat psoriasis and multiple sclerosis and exhibits minimal side effects. DMF exerts immunomodulatory, antioxidative, and anti-inflammatory effects (13). It is metabolized to monomethyl fumarate (MMF) in the small intestine. MMF emerges as an active metabolite of DMF and binds to cysteine 151 of Keap-1. Consequently, Nrf2 dissociates from Keap-1 and migrates to the nucleus to initiate the transcription of antioxidant genes (13). Previous reports have shown that DMF-induced Nrf2 activation has protective potential against tissue/organ damage including the liver (13,14-16). It has also been found to be effective in preventing liver damage induced by ischemia-reperfusion, thioacetamide, and carbon tetrachloride in rodents. Nevertheless, limited studies have investigated the effect of DMF in EtOH-treated rodents (14-19).

This research aimed to examine the influence of DMF treatment on liver injury, steatosis, inflammation, and the balance between oxidant and antioxidant factors induced by binge ETOH consumption. Furthermore, the effect of DMF treatment on protein expressions of proteins/enzymes related to lipid metabolism (SREBP-1c and PPAR-α), oxidative stress (CYP2E1), inflammation (NF-κB), and antioxidant system (Nrf2, HO-1) was investigated in the liver of binge EtOH-treated rats.

MATERIALS and METHODS

Animals

Female Sprague-Dawley rats (180-200 g) were obtained from the Bezmialem Vakif University Experimental Application and Research Center. The rats were housed in a stainless-steel cage (three to four per cage) at 24-26°C temperature 12-hour light/dark cycle and provided with regular feed. All procedures were handled as per the guidelines approved by Bezmialem Vakif University Animal Experiments Local Ethics Committee (Date: 24.01.2019, No: 2019/14).

Experimental procedures

Twenty-four rats were divided into four groups:

a) Control (n=6): Rats were treated with saline in accordance with the experimental protocol.

b) DMF (n=4): DMF was dissolved in dimethyl sulfoxide (DMSO; 0.08%) and applied orally to rats at a dosage of 30 mg/kg three times at 12-hour intervals.

c) EtOH group (n=7): EtOH was diluted with saline (56% v/v; approximately 10 ml/kg) and administered to rats by oral gavage at a concentration of 4.5 g/kg three times at 12-hour intervals (binge model).

d) DMF+EtOH group (n=7); DMF was administered orally to rats at a dosage of 30 mg/kg 1 hour before each EtOH application three times at 12-hour intervals doses.

In this study, the protocol used for ALD induction and DMF doses was based on previous studies (15,17,20,21). DMF and other chemicals were acquired from Sigma Aldrich (Merck KGaA, Darmstadt, Germany).

Blood and tissue samples

At the 12th h after the last EtOH treatments, all animals were injected with xylazine (15 mg/kg) and ketamine (35 mg/kg) intraperitoneally and blood samples were taken from their hearts under anesthesia and collected into dry tubes. Subsequently, they were centrifuged at 2390xg for 15 minutes, and sera were separated. Following excision, the livers were removed and preserved at -20°C till further assessment. The liver index was calculated by dividing the liver weight by the body weight and multiplying the result by 100.

Liver samples were homogenized in ice-cold 0.01 M phosphate-buffered saline (PBS) with a pH of 7.4. Subsequently, the homogenates were subjected to centrifugation at 600xg for 10 minutes to yield supernatant, which was then utilized for biochemical analyses. A portion of this supernatant underwent further centrifugation at 10000xg for 20 minutes at +4°C to yield the postmitochondrial phase, intended for the assessment of antioxidant enzyme activities. The specimens were preserved at -80°C until analyses.

Biochemical analyses in serum

Biochemical evaluations in serum were conducted using a Cobas Integra 800 autoanalyzer manufactured by Roche Diagnostics (Mannheim, Germany), to measure liver function biomarkers including aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities.

Biochemical analyses in the liver

TG and tumor necrosis factor- α (TNF-α) levels and myeloperoxidase (MPO) activity

Hepatic lipids were extracted using chloroform and methanol in a ratio of 2:1, and following the evaporation step, the extracts were reconstituted using a mixture of alcohol:ether (3:1). TG levels were quantified using a kit supplied by Biolabo Biochemistry and Coagulation (Maizy, France). TNF-α levels were assessed using the sandwich ELISA technique (Abbkine, Wuhan, China). Liver homogenates (10%; w/v in 0.01M PBS; pH 7.4) were subjected to sonication for 30 seconds, followed by centrifugation at 5000xg for 5 minutes at +4°C. The supernatants were utilized for the quantification of TNF-α levels. For the determination of MPO activity, pieces of the livers were extensively homogenized in ice-cold 50 mM PBS with a pH: of 6.0, consisting of hexadecyltrimethylammonium bromide (HTAB). Following three cycles of freeze-thawing and subsequent centrifugation at 15000xg, the supernatants were utilized to measure MPO activity (22).

Lipid and protein oxidation products

Lipid peroxidation was carried out by determination of thiobarbituric acid reactive substances (TBARS) and diene conjugate (DC) levels. The Buege-Aust reagent was combined with the homogenates, and the mixture was subjected to incubation in a boiling water bath for 15 minutes, followed by cooling and subsequent centrifugation at 1000xg. The absorbance of supernatants was then recorded at 532 nm for the determination of TBARS levels (23). DC levels were determined following the method described by Buege and Aust. Initially, hepatic lipids were isolated using a mixture of chloroform and methanol in a ratio of 2:1, and subsequently reconstituted in cyclohexane. Absorbance was registered at 233 nm, and DC levels were computed utilizing a molar extinction coefficient of $2.52x10^4 M^{-1} cm^{-1}$ (23). To assess protein oxidation, advanced oxidation protein products (AOPP) were measured spectrophotometrically at 340 nm (24).

Antioxidant parameters

Glutathione (GSH) levels were determined at 412 nm using the Ellman reagent [5,5-dithiobis-(2-nitrobenzoate)] (25). SOD activity was evaluated using a method based on the capability of riboflavin-sensitized o-dianisidine to enhance the rate of photooxidation (26). Spectrophotometric determination of GSH-Px activity was carried out using cumene hydroperoxide as the substrate (27). The enzymatic reaction was monitored at 340 nm and 37°C. The calculation of GSH-Px activity was performed using the NADPH extinction coefficient ($6.22x10^3 M^{-1} cm^{-1}$). Protein levels of supernatants were determined by bicinchoninic acid (28).

Nrf2, HO-1, SREBP-1c, PPAR-α, NF-κB, CYP2E1 protein expressions (Western Blot)

Liver tissues were homogenized using a hand homogenizer in a buffer consisting of 50 mM Tris HCl, 140 μM NaCl, 1% sodium deoxycholate, 1% triton-X100, 2 μM EDTA 8.7% glycerol, 0.1% sodium dodecyl sulfate (SDS) and protease inhibitor cocktail (P8340, Sigma-Aldrich). The homogenates underwent two cycles of freezing and thawing at -80°C, followed by sonication. After that, they were sub-

jected to centrifugation at 13000 xg for 20 minutes at +4°C to collect supernatants. Equal amounts of protein (60 µg protein/well) were loaded onto 10% SDS-polyacrylamide gels within a mini electrophoresis system (Mini-Protean 3 cell) and then transferred to the polyvinylidene fluoride (PVDF) membrane (Millipore Corporation, USA). To prevent non-specific binding, the PVDF membranes were blocked with a PBS-T solution consisting of 0.1% Tween 20 and 5% non-fat dry milk. The membranes were left to incubate overnight at +4°C with primary antibodies: Nrf2 (1:400, #ABP53068, Abbkine, Wuhan, China), HO-1 (1:400, # orb5455, Biorbyt, United Kingdom), SREBP-1c (1:400, #ABP53239, Abbkine, Wuhan, China), PPAR-α (1:400, #ABP55667, Abbkine, Wuhan, China), NF-kB p65 (1:400, #ABP53069, Abbkine, Wuhan, China), CYP2E1 (1:400, #ABP53942, Abbkine, Wuhan, China). Following primary antibody incubation, the membranes were washed with PBS-T and then incubated with a secondary antibody (1:1500, horseradish peroxidase; IgG conjugated with HRP; A21020; goat anti-rabbit secondary antibody, Abbkine, Wuhan, China) for 1 hour and 15 minutes at room temperature. At the end of the incubation, membranes were washed 3x10 min with PBS-T. Immunoreactivity of the protein bands was detected with a chemiluminescence kit (ECL; Abbkine Superluminescence ECL plus) and displayed on the Fusion Fx (Vilber, France). Quantification of protein bands was carried out using ImageJ software (National Institutes of Health, Bethesda, USA). An internal standard (actin; 1:1000, #ABP57457, Abbkine, Wuhan, China) was used to normalize the values of the samples.

Histopathological examination

Tissues were kept in 10% formalin, dehydrated using the rising concentration of EtOH, and subsequently fixed in paraffin wax. Each sample was cut into 4-5 µm slices by using a microtome, and subsequently, the sections were stained using hematoxylin and eosin (H&E) for microscopic analysis.

Statistical analyses

Statistical analysis was conducted using the SPSS software (version 21.0; SPSS Corp., Armonk, NY, USA) program. The data for all variables were presented as mean ± standard deviation (SD). The distribution of data and assessment of normality were conducted using the Shapiro-Wilk test. For variables showing a normal distribution, the one-way ANOVA test was applied, followed by post-hoc Tukey's test. The homogeneity of variances was examined using the Levene test. In cases where the data did not exhibit a normal distribution, the Kruskal-Wallis test was carried out, followed by the post-hoc Mann-Whitney U test. In all cases, levels of $p<0.05$ were considered to be significant.

RESULTS

Final body weight, liver weight, and liver index

No significant alterations were observed in these parameters among the groups when compared to controls (data not shown).

Hepatic damage markers in serum and liver histology

Serum ALT ($p<0.01$), AST ($p<0.05$), and LDH ($p<0.01$) activities exhibited increases in the EtOH group, with no change in ALP activity. Administration of DMF significantly reduced serum ALT, LDH, and ALP levels ($p<0.05$), although AST activity in EtOH-treated rats remained unchanged (Table 1).

Histological examination of the liver revealed macro-microvesicular steatosis and mild inflammation in the EtOH group. Two of the seven rats had decreased steatosis and inflammation and five rats showed normal histology in the DMF+EtOH group (Figure 1).

TG and TNF-α levels and MPO activities in liver

A notable increase (2.2-fold; $p<0.01$) in TG levels was observed in the EtOH group. DMF administration led to a notable reduction (40.4%; $p<0.01$) in TG levels that were elevated due to EtOH. TNF-α levels and MPO activity also exhibited significant (105.0%; $p<0.01$) and (80.2%; $p<0.05$) in the EtOH group, respectively. In the DMF+EtOH group, statistically significant reductions were found in TNF-α levels (53.8%; $p<0.01$) and MPO activity (33.4%; $p<0.05$) compared to the EtOH group (Figure 2).

Hepatic prooxidant and antioxidant parameters

Significant increases were detected in TBARS (42.0%; $p<0.01$), DC (22.2%; $p<0.001$), and AOPP (26.4%; $p<0.05$) levels in the EtOH group. Notably, in rats

Table 1: The effect of dimethyl fumarate (DMF) serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) activities in binge ethanol (EtOH)-treated rats (Means±SD)

	Control (n=6)	DMF (n=4)	EtOH (n=7)	DMF+EtOH (n=7)
ALT (U/L)	53.0±7.63	46.5±10.5	71.8±8.49[a]	56.0±11.7[b]
AST (U/L)	77.7±8.91	77.0±5.95	94.6±8.40[a]	87.0±13.4
LDH (U/L)	370.2±57.3	391.5±60.5	519.4±64.9[a]	401.0±88.8[b]
ALP (U/L)	96.5±11.7	115.0±38.6	107.0±20.5	69.7±22.9[b]

a: $p<0.05$ as compared to the control, b: $p<0.05$ as compared to EtOH

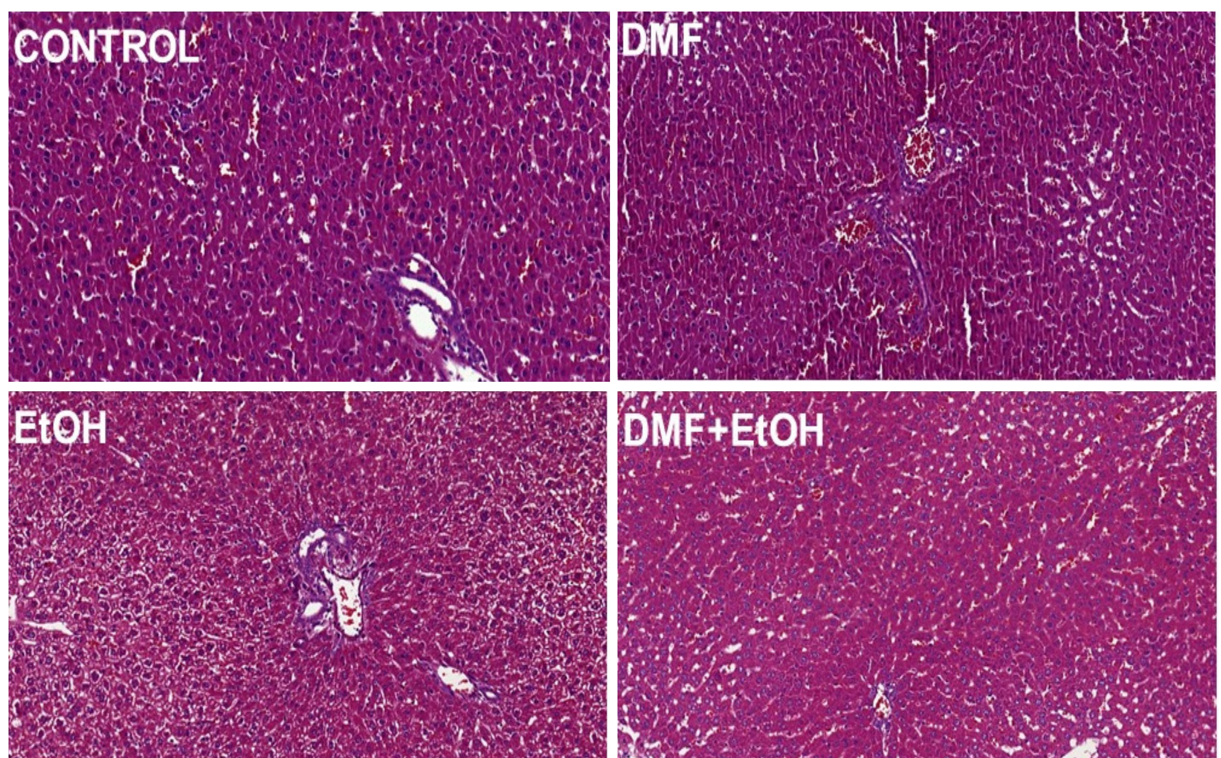


Figure 1: The effect of dimethyl fumarate (DMF) on hepatic histopathology in rats treated with binge ethanol (EtOH) [Hematoxylin and eosin (H&E) staining; x200].

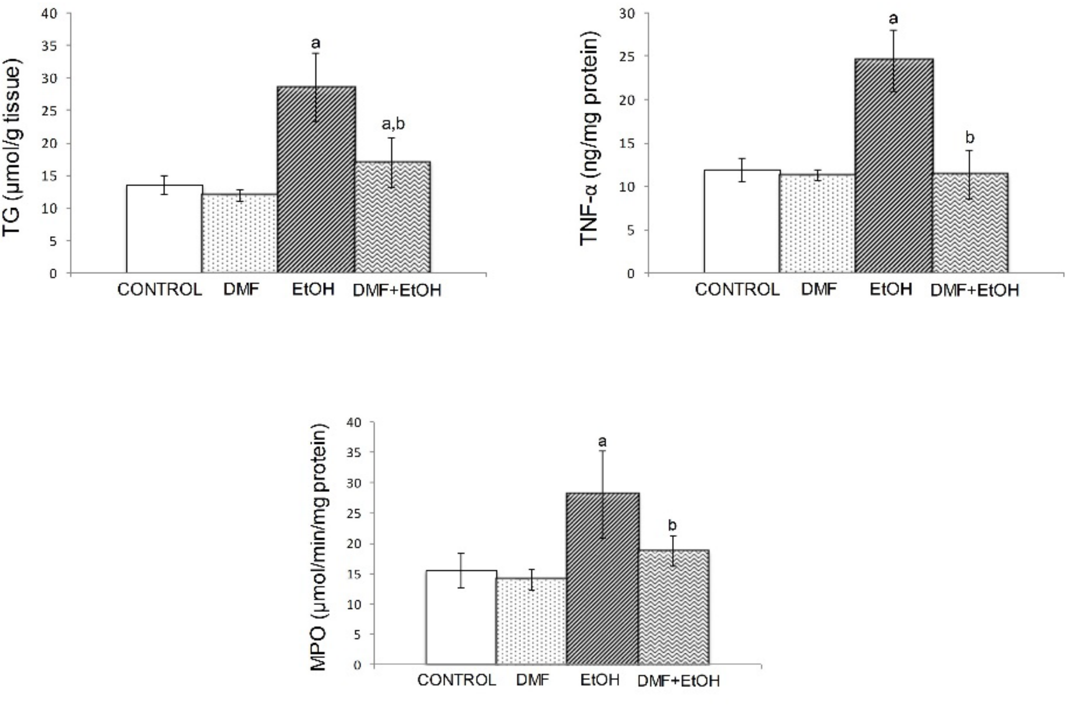
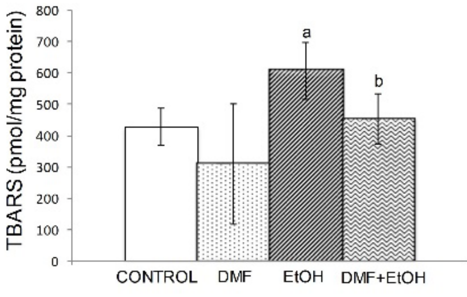
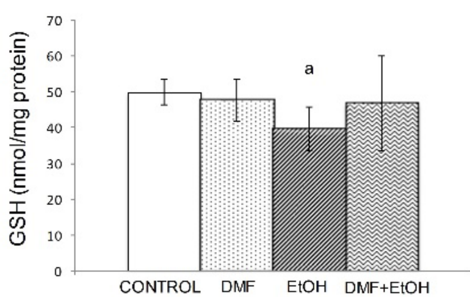
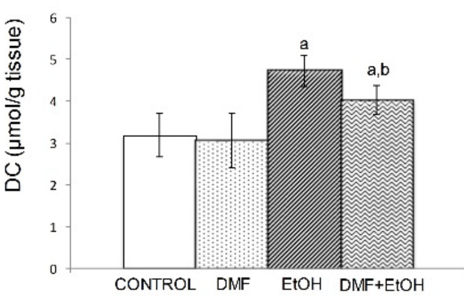
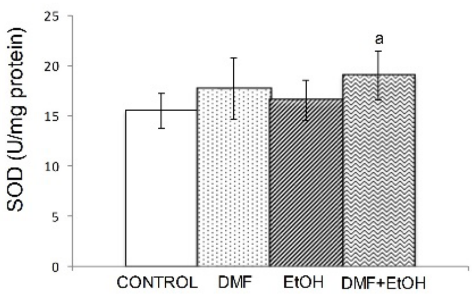


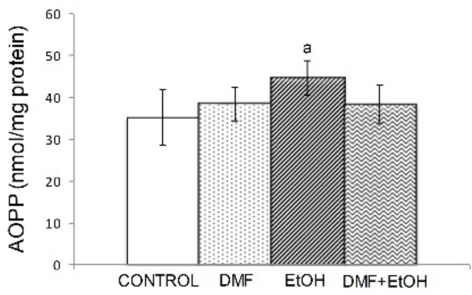
Figure 2: The effect of dimethyl fumarate (DMF) on triglyceride (TG) and tumor necrosis factor-alfa (TNF-α) levels and myeloperoxidase (MPO) activity of the liver in binge ethanol (EtOH)-treated rats (Means ±SD).
a: p<0.05 as compared to the control, b: p<0.05 as compared to EtOH











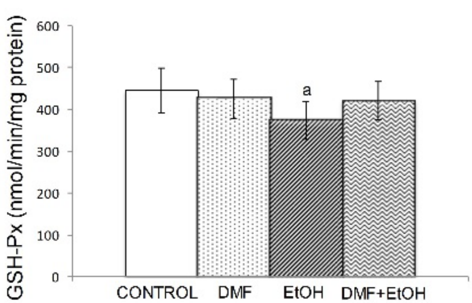


Figure 3: The effect of dimethyl fumarate (DMF) on thiobarbituric acid reactive substances (TBARS), diene conjugates (DC), and advanced oxidized protein products (AOPP) as well as glutathione (GSH) level and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in binge ethanol (EtOH)-treated rats (Means±SD).
[a]p<0.05 as compared to the control, [b]p<0.05 as compared to EtOH.

with steatosis-administered DMF, significant decreases were observed in liver TBARS (25.1%; p<0.05), DC (17.0%; p<0.05), and AOPP levels (14.1%; p<0.05) (Figure 3).

Significant changes were also found in GSH (20.7%; p<0.01) levels and GSH-Px (16.0%; p<0.05) activities in the group. However, the activity of SOD remained unchanged when compared to the control group. In the DMF+EtOH group, GSH levels and GSH-Px activities returned to normal control values.

Protein expressions of Nrf2, HO-1, SREBP-1, PPAR-α, NF-κB and CYP2E1

Significant increases were found in Nrf2 (25.7%; p<0.05) and HO-1 (23%; p<0.01) protein expressions in the DMF-treated control group, whereas other protein expressions remained unchanged. However, an increase in protein expression of Nrf2 (22.7%; p<0.05) was found in the EtOH group as compared to the control. DMF treatment resulted in further increases (24.3%; p<0.05) in Nrf2 protein expression in EtOH-treated rats. Elevations in HO-1 protein expressions (35.7%; p<0.01 and 18.7%;

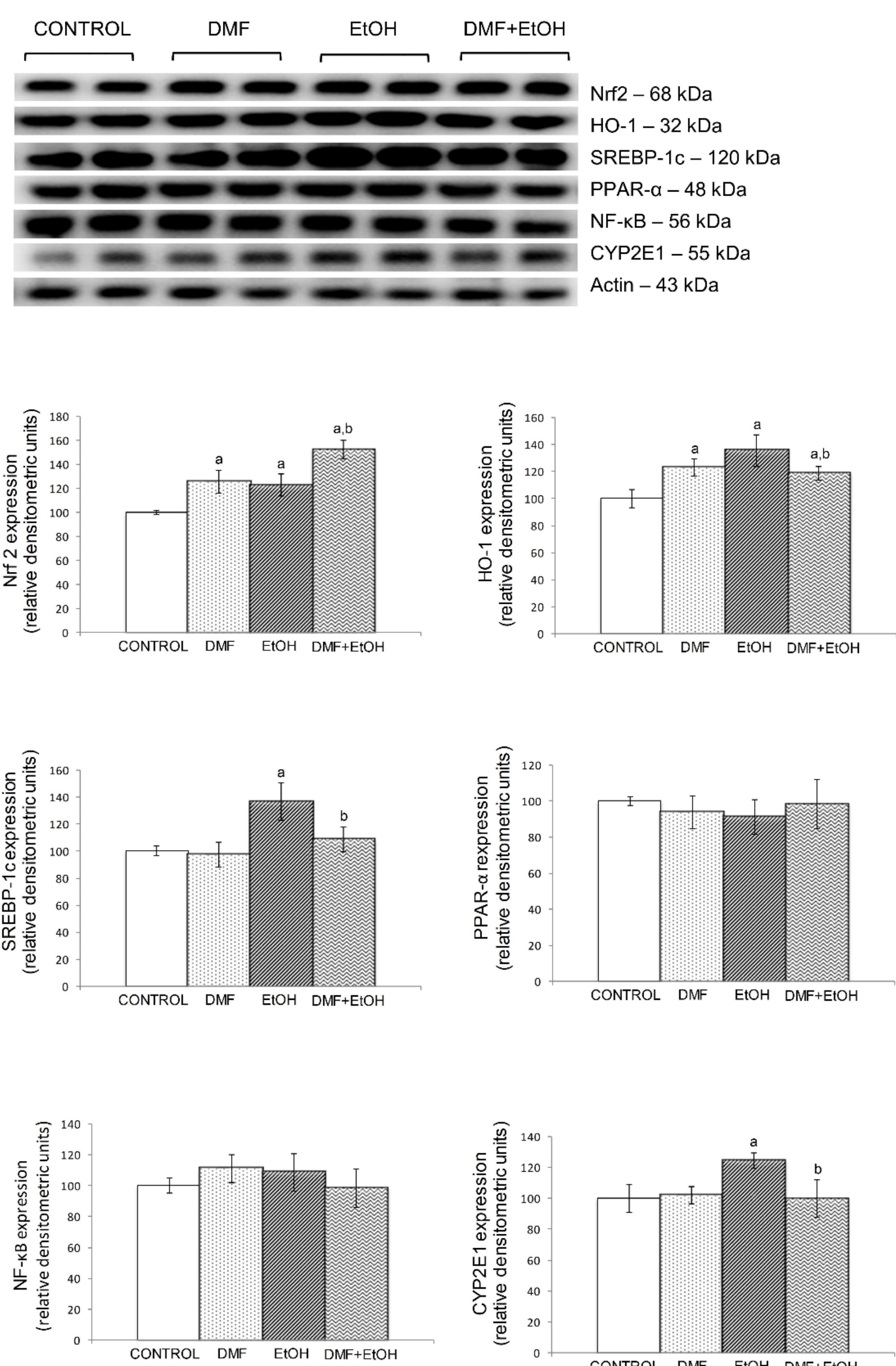


Figure 4: The effect of dimethyl fumarate (DMF) on hepatic nuclear factor erythroid 2-related factor (Nrf2), heme oxygenase-1 (HO-1), sterol regulatory element-binding protein-1c (SREBP-1c), peroxisome proliferator-activated receptor-alpha (PPAR-α), nuclear factor kappa B (NF-κB) and cytochrome P4502E1 (CYP2E1) protein expressions in binge ethanol EtOH-treated rats (Means±SD).
a: p<0.05 as compared to the control, b: p<0.05 as compared to EtOH.

$p<0.05$) were also detected in the EtOH and DMF+EtOH groups, respectively as compared to controls (Figure 4).

SREBP-1c protein expression (36.9%; $p<0.001$) increased significantly in the EtOH group, whereas PPAR-α expression remained unchanged. DMF administration caused a notable reduction (20.4%; $p<0.01$) in SREBP-1c, but PPAR-α expression remained unaffected. Although there were no significant changes in hepatic NF-κB expression in EtOH and DMF+ EtOH groups, CYP2E1 increased significantly (24.2%; $p<0.01$) in the EtOH group. A substantial decrease (19.7%; $p<0.01$) in CYP2E1 expression was found following DMF administration (Figure 4).

DISCUSSION

EtOH was administered orally according to the binge model in our study. This model causes a significant increase in blood EtOH levels. Thus, portal circulation contains high levels of EtOH and the liver is exposed to high EtOH concentrations. Binge EtOH application was reported to induce hepatic steatosis along with a slight elevation of serum transaminase levels. Therefore, this model is useful to investigate steatosis which is an early stage of ALD. Moreover, since female rats were reported to be more sensitive to alcohol than males, female rats were used in this study (29).

Lipogenesis, inflammation, and oxidative stress were reported as significant factors contributing to the development of ALD (1,2). Due to the effectiveness of Nrf2 activation in these events, which are mentioned to be effective in the pathogenesis of ALD, this study explored whether DMF, known as a Nrf2 activator, has a protective impact against EtOH-induced liver injury and uncover the underlying mechanisms. Although some investigators have researched the effect of DMF on cell damage in some tissues including the liver, there is limited information on its efficacy in EtOH-induced hepatotoxicity (17-19). DMF pre-treatment was detected to alleviate lethality induced by acute high doses of EtOH (17). In addition, DMF application was found to protect against hepatic lesions by decreasing increased intestinal permeability and serum LPS levels and oxidative stress in rodents fed on a Lieber De-Carli EtOH liquid diet (18,19).

In this study, statistically substantial increases in serum ALT, AST, LDH, and ALP activities were found in rats treated with EtOH. Histopathological examination of the liver revealed a microvesicular steatosis and mild inflammation. These findings are in accordance with previous studies (21,30,31). DMF treatment resulted in significant decreases in hepatic damage markers in serum and improvement in pathological findings such as steatosis and inflammation in binge EtOH-treated rats.

EtOH stimulates lipogenesis by affecting $NADH/NAD^+$ redox potential in the liver, thus increasing lipid accumulation (1,2). Binge EtOH treatment increased the expression of hepatic SREBP1c and lipogenesis-related genes but it decreased the expression of PPAR-α and β-oxidation-related genes (21,30,32,33). In this study, hepatic TG level and SREBP-1c protein expression increased in ETOH-treated rats and DMF treatment caused significant decreases in these parameters.

Binge EtOH treatment resulted in elevations in inflammatory cytokines such as TNF-α and IL-6 as well as increased NF-κB expression in the liver (30,31,34). NFκB regulates the expression of multiple genes that are effective in inflammatory response by inducing cytokines (6). TNF-α is a major cytokine associated with the inflammatory response and MPO activity is an indicator of neutrophil infiltration in the liver. In this study, the elevated hepatic TNF-α levels and MPO activities induced by EtOH were attenuated following DMF treatment.

Several investigators have reported that there are significant increases in ROS generation, lipid, and protein oxidation products together with changes in antioxidant system parameters in binge-EtOH-treated rodents (20,30,31,33,34). CYP2E1 and NADPH oxidase (NOX) in hepatocytes and Kupffer cells, respectively, are responsible for the formation of ROS due to ethanol (1,2). Hepatic CYP2E1 activity and expression were detected to increase in binge EtOH-treated rats (31,34,35). EtOH-induced oxidative stress augments the translocation of intestinal lipopolysaccharide (LPS) to the liver by increasing intestinal permeability, leading to the activation of NOXs and the formation of proinflammatory cytokines (3). In this study, ROS formation, MDA, DC and AOPP levels along with CYP2E1 protein expression increased, but antioxidant parameters (GSH levels and GSHPx activity) showed statistically insignificant decreases in binge EtOH-treated rats. However, CYP2E1 protein expression, ROS, TBARS, and DC levels were observed to decrease in DMF-treated rats who received binge EtOH drinking. Also, a trend of increasing SOD and GSH-Px activities and GSH levels due to DMF.

Furthermore, increases in protein expressions of Nrf2 and HO-1, a downstream target of Nrf2, were also found in EtOH-treated rats in the current study. Some investigators have also reported that Nrf2 protein and mRNA expressions were detected to increase together with CYP2E1 protein expression in EtOH-treated rats (36). Moreover, EtOH exposure augmented Nrf2-mediated HO-1 transcription in the liver of rats (37). HO-1 is a cytoprotective antioxidant enzyme and is induced by several factors such as Nrf2 translocation, oxidative stress, and inflammatory cytokines (38). Therefore, the Nrf2/HO-1 pathway may protect against further increases in

CYP2E1-induced oxidative stress due to EtOH treatment. However, in this study, this protection appears to be insufficient in binge EtOH-treated rats. It was also observed that CYP2E1 expression was decreased, Nrf2 was further expressed, while HO-1 protein expression remained above normal levels due to DMF application in EtOH-treated rats. These results obtained with DMF against binge EtOH-induced liver damage are in accordance with those previously reported in experimental models of NAFLD induced by a high-fat diet (HFD) (39) and HFD+streptozotocin (40).

CONCLUSION

In conclusion, these findings reveal that DMF exerts a protective impact against binge EtOH-induced liver damage. The multifunctional efficiency of DMF-induced Nrf2 activation such as anti-lipogenic, antioxidant, and anti-inflammatory potential may be effective in the amelioration of EtOH-induced hepatotoxicity. According to this, DMF may be a new therapeutic agent for ALD.

Ethics Committee Approval: This study was approved by Bezmialem Vakif University Animal Experiments Local Ethics Committee (Date: 24.01.2019, No: 2019/14).

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