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Anti-apoptotic and anti-inflammatory effects of *Silybum marianum* in treatment of experimental steatohepatitis

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ABSTRACT

In this study, we were aimed to evaluate the probable effect of the crud extract of *Silybum marianum*, with high polyphenolic content, on experimental nonalcoholic steatohepatitis (NASH). To induce NASH, a methionine and choline deficient (MCD) diet was given to N-Mary rats for 8 weeks. After NASH development, MCD-fed rats were divided into two groups: MCD groups received MCD diet and MCD + S group was fed MCD diet plus crude extract of *S. marianum* orally for 3 weeks. Control group was fed a normal diet for 11 weeks. Finally, all rats were sacrificed. Plasma alanine amino transferase (ALT) and aspartate amino transferase (AST) levels were evaluated. In addition, the following hepatic factors were also evaluated: liver histology, malondialdehyde (MDA) and reduced glutathione (GSH) contents, gene expressions of TNF-α and TGF-β and immunoblot evaluations of caspase-3, ERK/p-ERK, JNK/pJNK and p38/pp38. Histopathological evaluations of the liver samples revealed that treatment with the *S. marianum* extract has abated the severity of NASH among the MCD-fed rats. Also, a significant reduction was observed in the sera ALT and AST activities. In addition, the extract caused dramatic reduction in the elevated hepatic TNF-α and TGF-β mRNA and MDA levels along with an increase in the GSH content. Moreover, the plant extract treatments significantly lowered activation of procaspase-3 to active caspase-3 and also lowered the phosphorylated form of JNK among the same group of rats. These results suggest that the *S. marianum* crude extract beneficial effects on NASH are mainly due to its antioxidant and anti-inflammatory activities.

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a well recognized liver disorder which might progress to various liver diseases as simple as steatosis to the more severe state of nonalcoholic steatohepatitis (NASH) which is commonly characterized by hepatocyte fat accumulation, lobular inflammation, ballooning degeneration with or without fibrosis. The clinical importance of NASH is due to its potential to progress to liver cirrhosis, hepatocarcinoma and liver failure (Angulo, 2002; Harrison et al., 2002). Although the exact molecular mechanism of NASH has not been elucidated yet, but a "two hit" hypothesis has been offered to explain the cause and consequences of NASH development. In the first hit (event), it is believed that fatty acids accumulate in hepatocyte which is followed by oxidative stress and inflammatory responses in the second hit (Day and James, 1998). The high incidence of hepatocyte apoptosis, among NASH patients, has been linked to the severity of hepatic inflammation and fibrosis (Feldstein et al., 2003).

Oxidative stress and tumor necrosis factor-α have been considered as the major players in the incidence of apoptosis among NASH-affected hepatocytes (Tilg and Diehl, 2000). The excess reactive oxygen species (ROS) leads to overexpression of tumor necrosis factor α (TNF-α) in hepatocytes, kupffer cells and adipose tissue. In the liver, TNF-α is involved in pathophysiology of liver diseases including viral hepatitis, alcoholic steatohepatitis and NASH. It is believed that this cytokine plays pivotal roles in apoptosis, mitochondrial dysfunction, insulin resistance and fibrosis throughout NASH developmental stages (Tilg and Diehl, 2000; Copaci et al., 2006).

Exposure to ROS activates some of the signal transduction pathways besides of inducing damages to essential macromolecules such as lipids, proteins and nucleic acids. In that line, mitogen activated protein kinases (MAPKs) have received global attention due to their significant regulatory roles in response to a variety of internal and external stresses (Martindale and Holbrook, 2002). It is believed that the activated c-jun N-terminal kinase (JNK) and p38 induce cell death through regulating the mitochondrial death machinery. Oxidative stress-induced JNK activation has been implicated in the development of insulin resistance, obesity and NASH (Hirosumi et al., 2002). In contrast, some other members of MAPK

family such as extracellular signal-regulated kinase (ERK) execute anti-apoptotic function upon activation (phosphorylation) by an upstream kinase. However, it should be emphasized that the majority of these responses are both cell-type and oxidant-type specific (Martindale and Holbrook, 2002; Ura et al., 2001; Czaja et al., 2003).

Despite the severity of the disease, there are still no appropriate pharmaceutical approaches for NASH treatment. However, following the established role of ROS in NASH incidence, it is speculated that antioxidants, with high free radical scavenging activity, might be beneficial to NASH-affected patients (Bugianesi et al., 2004; Ustundag et al., 2007). In that regard, medicinal plants, with high antioxidant contents, are certainly rich sources for finding new novel antioxidants. *Silybum marianum* has long been used for liver and gastrointestinal disorders (Pradhan and Girish, 2006). Silymarin is a polyphenolic mixture extracted from the seed of *S. marianum*. It contains several flavonolignans including silybin A, Silybin B, isosylibin, silychristin. The *S. marianum* extract has strong antioxidant activity and exhibits anti-inflammatory and cytoprotective effects (Pradhan and Girish, 2006; Kren and Walterová, 2005). In addition, it has been demonstrated that the extract protects hepatocytes against a variety of toxins including ethanol, free fatty acids, carbon tetrachloride (Pradhan and Girish, 2006; Kren and Walterová, 2005; Ramakrishnan et al., 2006).

Regarding the high antioxidant and hepatoprotective activity of *S. marianum*, we decided to evaluate whether *S. marianum* extract is capable of attenuating the incidence of methionine and choline deficient (MCD) diet-induced NASH and in that case, to what extent the activities of MAPKs would be modulated.

2. Methods

2.1. Animals and diets

Male N-Mary rats, weighing 175–220 g, were housed in cages with a 12 h/12 h light/dark cycle, and were allowed free access to food and water *ad libitum*. All animal experiments were in accordance with the animal ethics committee of University of Tehran. Animals were divided into two groups: group 1 (control, $n = 5$) was fed a normal diet for 11 weeks. Group 2 (MCD) received a MCD diet for 8 weeks. After confirmation of NASH development, MCD group was divided into two groups: MCD group ($n = 5$) continued to get MCD diet and group 3 (MCD + S, $n = 5$) was fed MCD diet plus the *S. marianum* extract with a dose equivalent to 1.0 g seeds powder/kg body weight/day by gavages for 3 weeks. The diet composition has been described previously (Ustundag et al., 2007).

Finally, all rats were sacrificed under diethyl ether anesthesia. Plasma and liver samples, after collections, were individually snap-frozen in liquid nitrogen and kept at −80 °C for biochemical, histopathological and molecular examinations.

2.2. Plant extract preparation

The seeds of *S. marianum* were collected from Hamadan province (Iran) during summer. Seeds were air-dried, protected from direct sunlight, and then finely powdered. The powdered was kept in a closed container at 4 °C. Fifty grams of the seeds was initially defatted with 200 ml petroleum ether at room temperature for overnight, then it was extracted four times with ethanol (EtOH 96%), at room temperature. The accumulated extract was concentrated under reduced pressure on a rotary evaporator to a volume of 50 ml. The aliquoted samples were stored at −20 °C.

2.3. Histopathological examinations

A part of each fresh liver tissue was fixed in 10% formalin and then their paraffin blocks were prepared. Sections from the blocks were stained with hematoxylin–eosin (HE) and masson trichrom. The histopathological evaluations were performed blindly by an expert pathologist using a scoring system proposed by Kleiner et al. (2005): steatosis (0–3), lobular inflammatory changes (0–3) and hepatocyte ballooning (0–2). Fibrosis was evaluated as absent/present.

2.4. Preparation of liver homogenate

The liver samples were individually cut into small pieces and homogenized in Tris–HCl buffer (25 mM, pH 7.5) with a homogenizer to give a 10% (w/v) liver homogenate. The homogenates were then centrifuged at 12,000 rpm for 15 min at 4 °C (Beckman). Aliquots of each supernatant were then used for malondialdehyde (MDA) and reduced glutathione (GSH) analyses. The protein concentration of each extract was determined by the method of Lowry et al. (1951) using bovine serum albomin (BSA) as the standard.

2.5. Biochemical analyses

The plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using enzymatic kits according to the manufacture's instructions (Pars Azmun, Tehran, Iran).

2.6. Measurement of hepatic lipid peroxidation

Hepatic MDA level of each sample was determined by the double heating method (Draper and Hadley, 1990). The method is based on spectrophotometeric measurement of the purple color generated by the reaction of thiobarbituric acid (TBA) with MDA. Briefly, 0.5 ml of each liver homogenate was mixed with 2.5 ml of trichloroacetic acid (TCA) (10%, w/v) solution followed by boiling in a water bath for 15 min. After cooling to room temperature, the sample was centrifuged at 3000 rpm for 10 min and 2 ml of each sample supernatant was transferred to a test tube containing 1 ml of TBA solution (0.67%, w/v). Each tube was then placed in a boiling water bath for 15 min. After cooling to room temperature, the absorbance was measured at 532 nm with respect to the blank solution. The concentration of MDA was calculated based on the absorbance coefficient of the TBA–MDA complex ($\varepsilon = 1.56 \times 10^5 \text{ cm}^{-1}\text{ M}^{-1}$) and it was expressed as nmol/mg protein.

2.7. Determination of reduced glutathione

Hepatic GSH content was determined by the method of Jollow et al. (1974). An aliquot (0.5 ml) of each tissue homogenate was precipitated with 1 ml of sulphosalicylic acid (4%, w/v). The precipitate was removed by centrifugation. The filtrate (0.5 ml) was mixed with 0.1 ml 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) (4 mg/ml) and 0.9 ml phosphate buffer (0.1 M, pH 7.4). The developed yellow color was read at 412 nm. Reduced glutathione was expressed as μg/mg of protein.

2.8. Determination of TNF-α and TGF-β mRNA levels using real-time PCR

Total RNA was isolated from frozen liver tissues using the TRIzol reagent according to the manufacturer's protocol (Invitrogen, Carlsbard, CA). The RNA concentration and the extent of its purity were determined spectrophotometrically at 260 nm and by the $A260/A280$ ratio, respectively. Total RNA (4 μg) was then reversed transcribed into cDNA using 200 U of M-MuLV reverse transcriptase (Fermentas, Lithuania) and 0.2 μg of random hexamer (Fermentas, Lithuania) as the primer. The amplification reactions were performed on a Roche light cycler instrument (Roche diagnostics

GmbH, Mannheim, GER) applying the following thermal cycling conditions: an initial activation step for 3 min at 95 °C followed by 45 cycles including a denaturation step for 10 s at 95 °C, annealing step for 15 s at 55 °C and extension step for 20 s at 72 °C. β-Actin was used as a normalizer and the fold change in expression of each target mRNA relative to β-actin was calculated based on $2^{-\Delta\Delta ct}$ comparative expression method. The primers and probes used are listed in Table 1 (Rioja et al., 2005; Nagata et al., 2002; Gold et al., 2006).

2.9. Immunoblot analyses

Each frozen liver tissue (10–20 mg) was homogenized in 0.2 ml lysis buffer containing 10 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1 mM NaF, 20 mM sodium pyrophosphate, 2 mM Na_3VO_4, 1% Triton X-100, 10% glycerol, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulphonyl fluoride (PMSF), 10 μg/ml leupeptin, 1 μg/ml pepstatin and 60 μg/ml aprotinin. After 30 min, each liver homogenate was centrifuged at 14,000 rpm for 15 min at 4 °C. Protein concentration of each sample was determined using Lowry's procedure. Equal quantities of protein (50 μg/lane) were separated on a 15% SDS-PAGE and electroblotted to a polyvinylidene difluoride (PVDF) membrane (Amersham, Bioscience, UK). Membranes were blocked in Tris-buffered saline pH 7.6 containing 0.1% Tween-20, 0.05% sodium azide and 4% non-fat dry milk overnight at 4 °C. Blocked blots were incubated with primary antibodies for 2 h at room temperature using diluted antibody in Tris-buffered saline pH 7.6, 0.05% Tween-20 and 1% non-fat dry milk as recommended by the manufacturer. Primary antibodies were: anti caspase-3, anti ERK, anti phospho ERK, anti JNK, anti phospho-JNK, anti p38, anti phospho p38 (1:1000, Biosource Intl, Camarillo, CA); anti β-tubulin (1:3000, Sigma, St. Louis, Missouri, USA). After 1 h incubation at room temperature with anti-rabbit or anti-mouse horseradish peroxidase-conjugated secondary antibodies (Biosource Intl, Camarillo, CA), the proteins were detected by an enhanced chemiluminescence (ECL) detection system (Amersham-Pharmacia, Piscataway, NJ) according to the manufacture's instructions. The specific signal was revealed by autoradiography. In all experiments, equal protein loadings have been confirmed by the β-tubulin content.

2.10. Statistical analyses

Data, except those of immunoblots, were expressed as mean ± SD of five independent triplicate measurements and statistically analyzed using Student's t-test. Values of $p < 0.05$ were considered significant.

3. Results

3.1. Food consumption and body weight

We monitored the food consumption and the body weight of each group throughout the experimental period. Food consumption was not affected by S. marianum extract treatment. Overall, the mean body weight was not significantly changed among different groups (data not shown).

3.2. Effect of S. marianum extract on histopathological lesions and liver injuries

Fig. 1A clearly indicates that the livers of control rats histologically look normal. In contrast, feeding MCD diet for 11 weeks caused grade 1 steatosis associated with inflammation and ballooning degeneration of hepatocytes (Fig. 1B). As shown in Fig. 1C, treatment with the extract improved these factors to grade 0 in

Table 1
Sequences of primers and probes used for real-time PCR.

Gene	Primers/probes	Sequence
TNF-α	Forward	CCAGGTTCTCTTCAAGGGACAA
	Reverse	CTCCTGGTATGAAATGGCAAATC
	Probe	Fam-CCCGACTATGTGCTCCTCACCCACA-Tamra
TGF-β	Forward	TAGCAACAATTCCTGGCGTTAC
	Reverse	GGACTGATCCCATTGATTTCC
	Probe	Fam-CAGTGGCTGAACCAAGGAGACGGAATACAG-Tamra
β-ACTIN	Forward	CGTGAAAAGATGACCCAGATCA
	Reverse	CACAGCCTGGATGGCTACGT
	Probe	Fam-TTTGAGACCTTCAACACCCCAGCCA-Tamra

80% of the rats, whereas grade 1 steatosis was found in the remaining 20% of NASH-affected rats. Masson trichrom staining did not confirm the incidence of fibrosis in either group. The ALT and AST sera levels were determined to assess the liver function. As shown in Fig. 2, NASH-affected rats had elevated sera ALT and AST levels to the extent of 125% and 35%, respectively, compared to the control group ($p < 0.05$). However, treatment with the S. marianum extract markedly reduced these levels to 45% and 24%, respectively compared to the extract-untreated MCD-fed rats, implying that the plant extract had executed a protective effect against the MCD diet-induced liver injuries.

3.3. Effect of S. marianum extract on hepatic oxidative status

In order to explore the effect of the S. marianum extract on the liver oxidative stress status, the liver GSH and MDA contents were assessed. The rat liver MDA level is regarded as a marker of oxidative stress-induced lipid peroxidation (de Zwart et al., 1999; Draper and Hadley, 1990). After 11 weeks of MCD diet feeding, the MDA level was greatly increased compared to control group (0.50 nmol/mg protein in control group versus 1.05 nmol/mg protein in NASH-affected rats). The enhanced MDA level was associated with a reduction in the GSH content by 54%, relative to control group. However, as shown in Fig. 3, administration of the extract significantly reduced the MDA level by 40% and increased the GSH content by 65%, relative to the extract-untreated MCD-fed rats.

3.4. Effect of S. marianum extract on TNF-α and TGF-β gene transcription

Higher than normal level of TNF-α gene expression has been documented in NASH patient (Copaci et al., 2006). Our data, based on real-time RT-PCR analyses (Fig. 4) also showed that TNF-α and TGF-β mRNA levels have increased by 1.9 and 1.5-fold among MCD group compared to normal healthy rats. However, treatment with the extract greatly decreased the levels by 47% and 26%, respectively, relative to the relevant control rats.

3.5. Effect of S. marianum extract on caspase-3 level

Procaspase-3 is proteolytically processed into active p17 fragment (active caspase-3) during caspase-dependent apoptosis. To evaluate the influence of MCD diet on production of caspase-3, we assessed the level of caspase-3 by immunoblot analyses. According to Fig. 5A, MCD feeding led to significant elevation in the level of caspase-3 relative to the control diet-fed rats. However, as shown in Fig. 5A, treatment by the S. marianum extract markedly attenuated MCD-induced production of caspase-3.
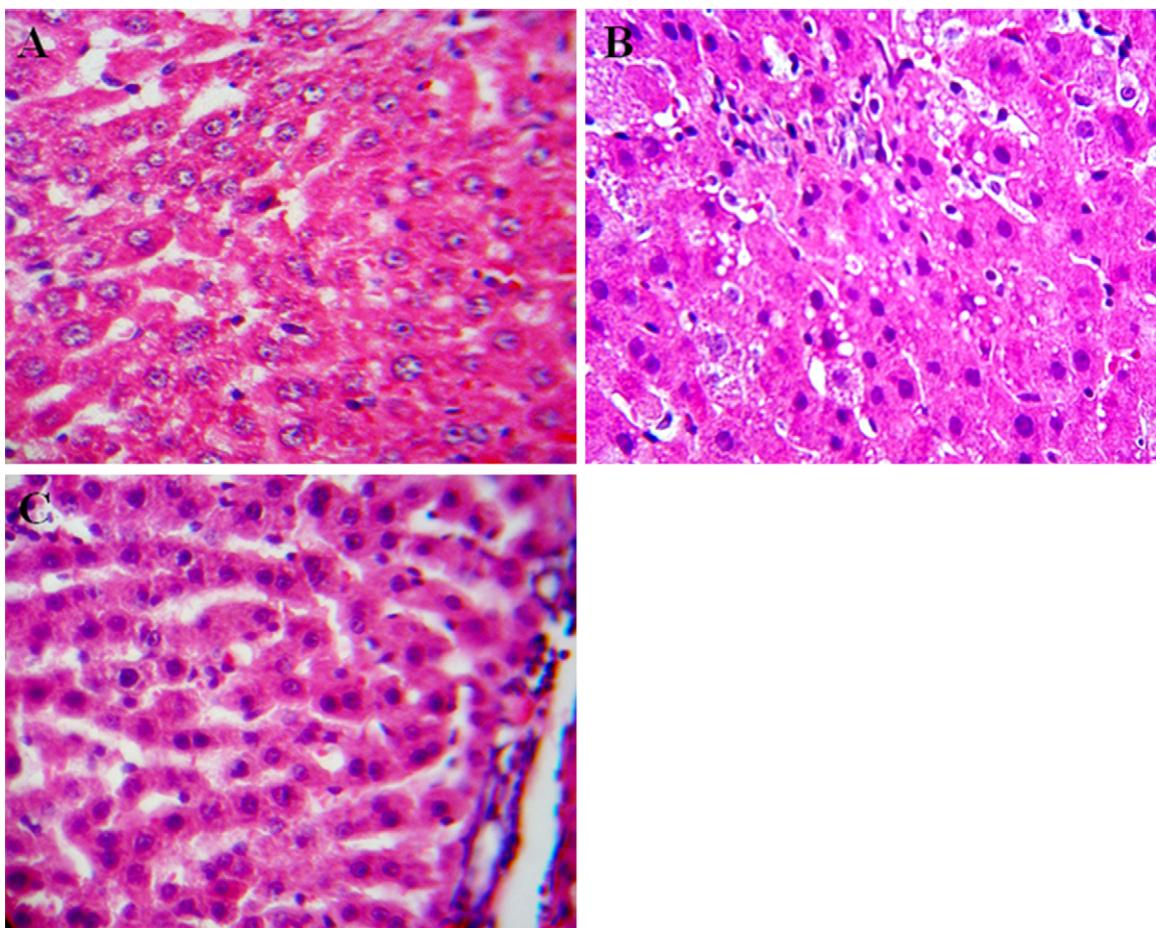
Fig. 1. Histological analyses of the liver samples of rats fed diets (normal, MCD and/or MCD + S). Liver sections were stained with hematoxylin–eosin (HE) and masson trichrom. (A) Control group had normal liver (HE×400), (B) MCD group showed micro- and macro-vesicular steatosis, ballooning degeneration and lobular inflammation around central vein (HE×400) and (C) MCD + S group had no evidence of histological lesions of NASH (HE×400).

3.6. Effect of S. marianum extract on MAPKs activation

The activation/deactivation of some members of MAPKs in the incidence of oxidative stress has been reported by many investigators (Martindale and Holbrook, 2002; Ura et al., 2001; Czaja et al., 2003). Following enormous increase in MDA level among the MCD-fed rats, we became interested to evaluate the possibility and the pattern of MAPKs involvement among the rats which have been fed either MCD or MCD + S diets.

As shown in Fig. 5B, the MCD diet feeding has led to significant phosphorylation of JNK relative to rats fed normal diet. Treatment with *S. marianum* extract has resulted in significant decrease in the
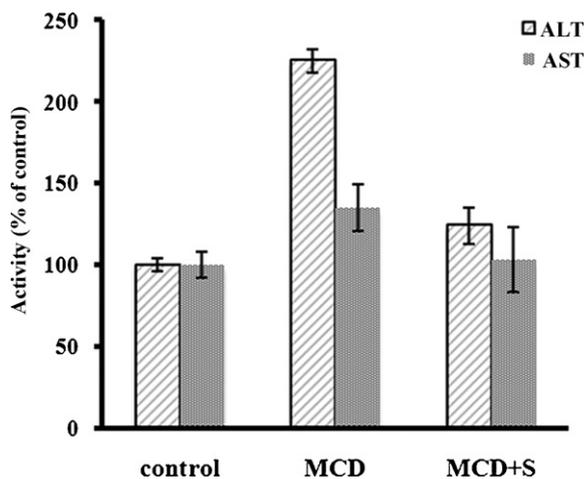
Fig. 2. Alteration of the sera liver enzymes of rats fed diets (normal, MCD and/or MCD + S). Data represent means ± SD of five independent triplicate measurements of each sample. *Significantly different from control group ($p < 0.05$). **Significantly different from MCD group ($p < 0.05$).
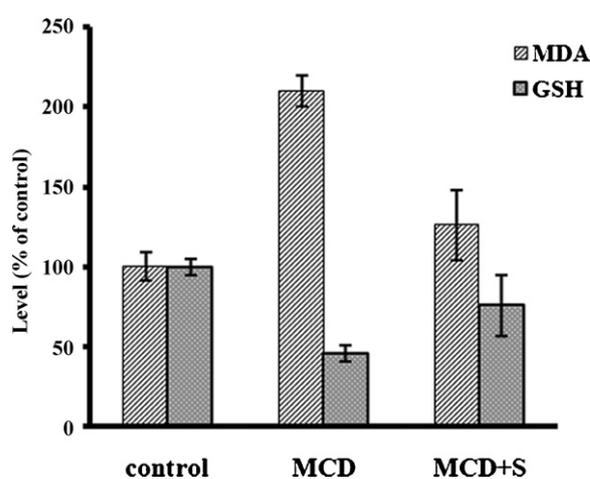
Fig. 3. Hepatic levels of MDA and GSH in rats fed diets (normal, MCD and/or MCD + S). Data represent means ± SD of five independent triplicate measurements of each sample. *Significantly different from control group ($p < 0.05$). **Significantly different from MCD group ($p < 0.05$).
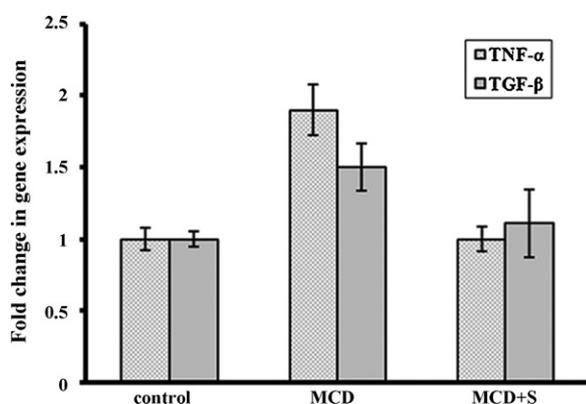
Fig. 4. TNF-α and TGF-β gene expression in rats fed diets (normal, MCD and/or MCD + S). The relative mRNA expression of TNF-α was measured using real-time PCR after normalizing the cycle thresholds (Ct) of each sample against their corresponding β-actin. Data represent means ± SD of five independent triplicate measurements of each sample. *Significantly different from control group ($p < 0.05$). **Significantly different from MCD group ($p < 0.05$).

extent of JNK phosphorylation. Unlike JNK, phosphorylation of ERK and p38 were not affected by MCD diet and/or MCD + S. In addition, as it is evident in Fig. 5B, no considerable alteration in total hepatic JNK, ERK and p38 have occurred among different groups.

4. Discussion

Oxidative stress is a well-established pathogenetic factor in the incidence of many diseases including NASH (Oliveira et al., 2002). A number of studies have confirmed the elevated levels of oxidative stress markers among NASH patients while the levels of some endogenous antioxidants such as vitamin E and glutathione are suppressed (Grattagliano et al., 2000). In the present study, we showed that *S. marianum* crude extract, containing various polyphenolic compounds (Pradhan and Girish, 2006; Kren and Walterová, 2005; Ramakrishnan et al., 2006), effectively attenuated the histopathological features of NASH along with beneficial effects on the liver functions of NASH-affected rats. These results suggest that the crude extract of *S. marianum* has improved the liver injuries among MCD-fed rats relative to respective untreated group.

The extract of *S. marianum* has high polyphenolic content. It has been demonstrated that *S. marianum* exerts its hepatoprotective effects against the prooxidant products mainly through free radical scavenging (Kren and Walterová, 2005). The extract also increased the hepatic GSH content, which acts as the first line of defense against intermediate reactive oxygen products of lipid peroxidation

and hence, its content is an index of tissue susceptibility to oxidative stress (Kren and Walterová, 2005; Valenzuela and Garrido, 1994). A number of studies have indicated that antioxidants such as plant-derived flavonoids and isoflavonoids can prevent or attenuate the extent of lipid peroxidation among various experimental NASH model systems mainly through free radical scavenging activity (Laurent et al., 2004). Our results similarly confirmed that the *S. marianum* extract reduced the hepatic level of MDA, a byproduct of free radical reactions, and it also attenuated the hepatic glutathione depletion. Based on these results, it can be concluded that the *S. marianum* crude extract might exert its action against NASH complications mainly through its antioxidant activity.

Apoptosis has been recognized as an indispensible event in many liver injuries including inflammation and cirrhosis (Canbay et al., 2004). Induction of apoptosis and the subsequent activation of procaspase-3 has been documented as a prominent pathological feature among NASH patients and the animal model of NASH (Feldstein et al., 2003; Wieckowska et al., 2006). Our work, while supporting the elevated level of caspase-3 among NASH-affected rats, clearly indicated that the extract treatment significantly suppressed the activation of procaspase-3 to caspase-3.

In addition, our results demonstrated that the extract treatment attenuated the elevated TNF-α level among NASH-affected rats probably through inhibition of oxidative stress. It has been shown that enhanced intrahepatocyte ROS content leads to production of proinflammatory cytokines such as TNF-α which plays a significant etiological role in pathogenesis of NASH (Fernández-Escalante and Pons-Romero, 2001; Tilg and Diehl, 2000; Sluss et al., 1994). Tumor necrosis factor-α, along with other cytokines, mediates inflammatory responses and cell death. Yalniz et al. (2007) have shown that TNF-α level among the high fat-fed rats, declined following administration of ginestein, an isoflavone with strong antioxidant property.

Activation of hepatic stellate cells along with fibrogenesis reflects the clinical severity of NASH (Li et al., 2008). Although the underlying mechanisms remain unknown, a large body of evidence supports the role of oxidative stress in the onset of fibrosis. Excess ROS and lipid peroxidation products play a major mediatory role in induction of extracellular fibrillar matrix synthesis. Transforming growth factor β (TGF-β) is one of the critical cytokines involved in fibrogenesis (Bissell et al., 2001). Although no evidence of fibrosis was found among NASH-affected rats, the hepatic TGF-β level was high among the MCD-fed rats. This observation probably demonstrated the early activation of fibrotic process in rats and it seems that longer (>11 weeks) exposure to high level of oxidants, produced by MCD feeding, is required for full development of fibrosis.

Several studies have regarded JNK/MAPKs as the mediators of many hepatocyte injuries such as steatosis, insulin resistance and
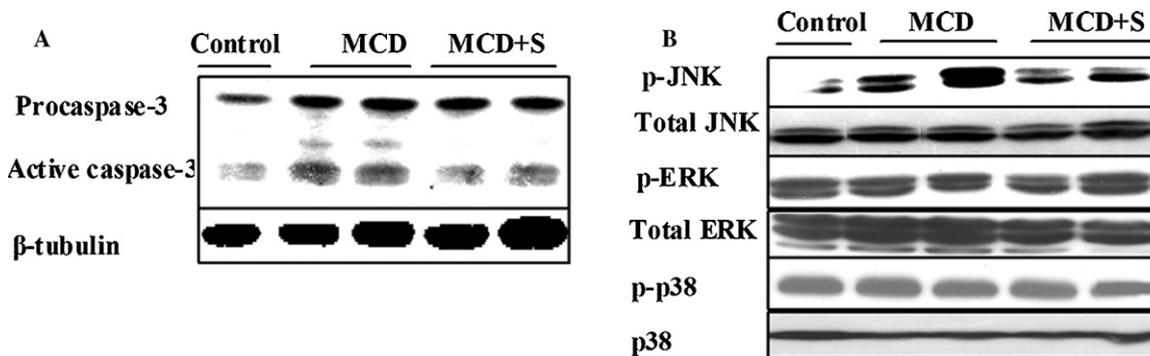
Fig. 5. A representative western blot pattern of the rats' livers fed diets (normal, MCD and/or MCD + S) for at least 8 weeks. (A) Effect of *S. marianum* extract on procaspase-3 activation in hepatocyte. (B) Effect of *S. marianum* extract on hepatic MAPKs pathway. Following the process described in Section 2, equal amount of protein of each liver sample was subjected to western blot analysis using specific antibodies for the respective caspase-3, JNK1/2/pJNK1/2 and ERK1/2/p-ERK1/2 and p38/pp38. Equal protein loading in each gel well was confirmed by the β-tubulin content.

apoptosis (Hirosumi et al., 2002; Schattenberg et al., 2006; Weston and Davis, 2007). Based on these reports, it seems that enhanced levels of oxidative stress and TNF-α are crucial for sustained JNK activation which then mediates ROS and TNF-α induced cell death in the late stage of NASH (Schattenberg et al., 2006). This has been confirmed by inhibition of JNK activity by genetic knock-out among the MCD-fed rats (Hirosumi et al., 2002). It is believed that JNK promotes cell death by inducing an imbalance between Bcl-2 family members by up-regulating the pro-apoptotic (e.g. Bax) or down-regulating the anti-apoptotic (e.g. Bcl-2 or Bcl-xl) proteins. The JNK-induced phosphorylation of Bcl-2 appears to suppress the pro-survival function of Bcl-2 protein (Martindale and Holbrook, 2002; Weston and Davis, 2007; Ura et al., 2001). Our data, parallel to the present literature, demonstrated that MCD diet has led to higher phosphorylation of JNK, but not p38 and/or ERK, and this activation markedly decreased upon *S. marianum* extract administration. In addition, our results showed that activation of ERK and p38 were affected by neither MCD diet nor the *S. marianum* extract administration. These results are in full contrast to our previous report on the effect of ethyl acetate extract of *Teucrium polium* (TPE) on ERK phosphorylation (Aghazadeh and Yazdanparast, 2009). The TPE extract enhanced the phosphorylation (activation) of ERK. The difference between the function of these two medicinal plants with antioxidant activity might be due to the presence of different antioxidant constituents in each extract with different mode of action.

In conclusion, the hepatoprotective effect of *S. marianum* extract might be attributed to its antioxidant activity which leads to suppression of intrahepatocyte ROS level followed by suppression of TNF-α expression and JNK phosphorylation. Further details on the mode of action of the extract, awaits full purification and structure elucidation of the active components.

Acknowledgment

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