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Synergistic effects of deuterium depleted water and *Mentha longifolia* L. essential oils on sepsis-induced liver injuries through regulation of cyclooxygenase-2

Azadeh Rasooli[a], Faezeh Fatemi[b], Reza Hajihosseini[a], Atoosa Vaziri[a], Kambiz Akbarzadeh[c], Mohammad Reza Mohammadi Malayeri[d], Salome Dini[e] and Maria Foroutanrad[a]

[a]Department of Biochemistry, Faculty of Sciences, Payame-e-Noor University, Tehran, Iran; [b]Materials and Nuclear Fuel Research School, Nuclear Science and Technology Research Institute, Tehran, Iran; [c]Faculty of Medicine, Mashhad University of Medical Science, Mashhad, Iran; [d]Department of Pathobiology, Faculty of Veterinary Medicine, Garmsar Branch, Islamic Azad University, Garmsar-Iran; [e]Young Researchers and Elite Club, Karaj Branch, Islamic Azad University, Karaj, Iran

ABSTRACT

Context: *Mentha longifolia* L. (Lamiaceae), a traditional medicinal herb, has been highly valued for exhibiting antimicrobial, antioxidant and antispasmodic properties.

Objective: For the first time, the synergetic anti-inflammatory effects of deuterium depleted water (DDW) and *M. longifolia* essential oils (ML) were investigated in experimental sepsis.

Materials and methods: Fifty Wistar rats were divided into 5 groups ($n = 10$): negative control (laparotomy), CLP, treatment groups including the combination of DDWs (15 and 30 ppm) and ML (100 mg/kg b.w) and indomethacin. At 24 h after CLP induction, lipid peroxidation (LP), glutathione (GSH), glutathione in *S*-transferases (GST), ferric reducing ability of plasma (FRAP), myeloperoxidase (MPO), prostaglandin E2 (PGE2), and COX-2 expression were determined in the plasma and liver tissues.

Results: Compared with the CLP group, the administration of DDWs and ML significantly ($p < 0.05$) prevented the increase of LP, COX-2 and PGE2 levels and liver enzymes. Additionally, the decreased levels of FRAP and GSH induced by sepsis were remarkably ($p < 0.05$) risen by the administration of DDWs and ML in comparison to the CLP group. However, no significant ($p > 0.05$) differences were observed regarding GST, ALP and bilirubin levels. Our results also proved the synergistic anti-inflammatory activities of the DDWs and ML. The anti-inflammatory effects of the DDWs and ML were confirmed by histopathological studies.

Discussion and conclusions: The combination of DDWs and ML exerted synergistic anti-inflammatory activity against CLP-induced sepsis possibly through modulating oxidative stress/antioxidant parameters.

Introduction

Natural products have been a valuable source of therapeutic agents, and nowadays still represent an important tool for the identification of novel drugs (Kinghorn et al. 2011; Newman and Cragg 2012). Deuterium depleted water (DDW) is water which has a lower concentration of naturally occurring deuterium (20–25 ppm vs. 150 ppm) (Bowen et al. 2005). In living organisms, the deuterium concentration is above the range of calcium, magnesium or potassium (Somlyai et al. 2010), and concentrations of deuterium in body water correlate well with the deuterium level of the environment (Hobson et al. 2004). The studies showed that a decrease quantity of deuterium in drinking water has beneficial effects on the organism such as anticancer and anti-hepatic oxidative injuries properties (Olariu et al. 2007; Somlyai et al. 2010; Rasooli et al. 2016).

Mentha longifolia L. (Lamiaceae), a native Iranian plant, has traditional uses mainly against respiratory and digestive diseases, recent research focused on the antimicrobial and antioxidant activities of its essential oils (Džamiae et al. 2010; Hafedh et al. 2010; Ahmad et al. 2011; Niksic et al. 2012; Iqbal et al. 2013; Stanisavljevic et al. 2014). Regarding the beneficial therapeutic

properties of these natural products, their consideration as probable effective anti-inflammatory and antioxidant agents in diseases such as sepsis would be a great potential goal.

The cecal ligation and puncture (CLP) model is a stable, repetitive and applicable model that leads to the pollution of the abdominal cavity by bacteria-carrying intestinal contents and induces a wide range of systemic inflammatory responses (Ritter et al. 2003; Liu et al. 2014) leading to sepsis. Sepsis is a complex inflammatory response to infections and remains the major cause of morbidity and mortality in the most intensive care units (ICU), it affects 27 million people each year worldwide (Maslove and Wong 2014; Mayr et al. 2014). Deregulation of the immunoinflammatory response as seen in sepsis may culminate in the host cell and consequently organ damage and ultimately death. The composition of Gram-negative bacteria cell walls, such as lipopolysaccharide induces gene activation and subsequent inflammatory mediator expression (Macdonald et al. 2003). Regulation and coordination of the immunoinflammatory response by cytokines and other mediators are essential for host defense. The involved molecular events are complex and culminate in altered gene expression. Deregulation of this response may occur in sepsis, leading to the excessive or inappropriate release of mediators and ultimately host cell and organ damage

(Macdonald et al. 2003; Hotchkiss et al. 2013a, 2013b; Angus and Van der Poll 2013).

Our previous studies showed that *M. longifolia* essential oil had antioxidant and anti-inflammatory activities in septic rats (Dadkhah et al. 2018). The protective activity of deuterium depleted water was also reported on hepatic oxidative injuries induced by acetaminophen (Rasooli et al. 2016).

In following of previous researches, the aim of this study was to investigate the anti-inflammatory and antioxidant activities of the natural compounds such as DDWs and ML with the evaluation of COX-2 gene expression and oxidative stress/antioxidant parameters in sepsis induced by CLP model.

Materials and methods

Plant materials and DDW preparations

DDWs (15 and 30 ppm) was prepared from Atomic Energy Organization of Iran was applied for our study. Also, the essential oils from *M. longifolia* (ML) were prepared from Barij Essence Pharmaceutical Co, Kashan, Iran (Batch No: 3138-031-93/8 (707051); Sample Serial No: BE930347).

Animals

Male Wistar rats $(250 \pm 20 \, g)$ were used for all experiments. The animal studies had been approved by the Medical Ethics Committee of Tarbiat Modares University based on the World Medical Association Declaration of Helsinki. The animals were divided into five groups $(n = 10)$ as shown in Table 1. The *M. longifolia* essential oils (100 mg/kg b.w) and also indomethacin (2 mg/kg b.w) were dissolved in dimethyl sulfoxide (DMSO). Sepsis was induced in rats by CLP as described by Hubbard et al. (2005). After 24 h of CLP surgery, the heparinized blood samples were collected and the liver samples were removed and then homogenized. The homogenates were used to measure the biochemical parameters.

Assessment of prostaglandin E2 (PGE2)

Plasma prostaglandin E2 level was measured using the Enzyme-linked Immunosorbent assay kit (ELISA Kit; BioAssay System Co, USA) according to the producer's instructions.

COX-2 gene expression

Total RNA from the liver tissues was prepared with the RNA total kit (BioBasic Inc, Canada). cDNA was synthesized with PrimeScript™ RT reagent kit (Takara Bio Inc, Japan) and Oligo (dT) primers (Takara Bio Inc, Japan), according to the manufacturer's protocol.

Then the primers for PCR were designed with the Gene Runner software Version 3.05 and primer 3 servers (COX-2 forward: 5′-ACCTCTGCGATGCTCTTC-3′; COX-2 reverse: 5′-AGG AATCTCGGCGTAGTAC-3′; GAPDH forward: 5′-TGCCAGCC TCGTCTCATAG-3′; GAPDH reverse: 5′-ACTGTGCCGTTGAA CTTGC-3′). Blast N searches were used to check primer specificity. The cDNA samples were amplified by PCR amplification and then checked by 2.5% agarose gel electrophoresis to ensure whether PCRs contained a product with the expected size.

The relative-expression of selected gene was carried out with real-time PCR System (Rotor-Gene Q-QIAGEN Co, USA). The reaction mixture contained 5 μL SYBR Green real-time PCR Master Mix (QIAGEN) which encloses Taq DNA polymerase, dNTP, $MgCl_2$ and SYBR Green I dye, 0.2 μL of a 10 mM solution of sense/anti-sense primer, 0.5 μL of template cDNA added with H_2O to a total of 10 μL. The negative controls were also designed as above excluded cDNA. Thermal cycling conditions were carried out by an initial denaturation stage at 95 °C for 2 min, followed by 40 cycles at 95 °C for 15 s, 60 °C for 20 s and 72 °C for 20 s. At the completion of each run, melting curves for the amplicons were measured by raising the temperature by 0.3 °C from 57 °C to 95 °C while monitoring fluorescence. All expression data were normalized using GAPDH expression as the internal standard and the fold change in the COX-2 gene was calculated by the formula $2^{-\Delta\Delta Ct}$.

### Assessment of antioxidant and liver parameters

The concentration of thiobarbituric acid reactive substances (TBARS) as an indicator for lipid peroxidation (LP) production was measured spectrophotometrically using TBA reagent based on the procedure described by Buege and Aust (1978). Glutathione (GSH) was estimated in liver homogenates according to the procedure of Sedlak and Lindsay (1968). CDNB (a general substrate) was used to measure liver cytosolic glutathione S-transferase (GST) activity spectrophotometrically (Habig et al. 1974). The specific activity was calculated based on the nmol/min/mg protein in samples measured by Bradford assay (Bradford 1976). Ferric reducing ability of plasma (FRAP) assay was performed using TPTZ reagent as described by Benzie and Strain (1996). Tissue myeloperoxidase (MPO) activity was measured with minor modification, according to the procedure of Hillegass et al. (1990). MPO activity was expressed as the amount of enzyme that reduces 1 μmol peroxide/min. Serum alanine transaminase (ALT), aspartate aminotransferase (AST) (Pars Azmoon Co, Iran), alkaline phosphatase (ALP) (Ziest Chem Diagnostics Co, Iran) and total bilirubin (BILI) (Darman Faraz Kave Co, Iran) were measured spectrophotometrically in according to the procedure described in the kit purchased.

Histopathological evaluation

The biopsy of liver tissue for histopathological examination was removed. For this purpose, small portion of the tissues

Table 1. Treatment groups Lap group, animals received DMSO as vehicles for 2 weeks then laparotomy was done; in CLP group, animals received vehicles only; In DDW15 + ML and DDW30 + ML groups, essential oil (100 mg/kg) and DDWs at two doses of 15 and 30 ppm were orally used together before CLP operation, a group of CLP rats treated with indomethacin (2 mg/kg b.w) as positive control.

Groups	DMSO (essential oil solution)	DDW 15	DDW 30	ML100	Indomethacin
LAP (as negative contol group)	+	−	−	−	−
CLP	+	−	−	−	−
ML100 + DDW15 + CLP	−	+	−	+	−
ML100 + DDW30 + CLP	−	−	+	+	−
Indomethacin (as positive control group)+CLP	−	−	−	−	+
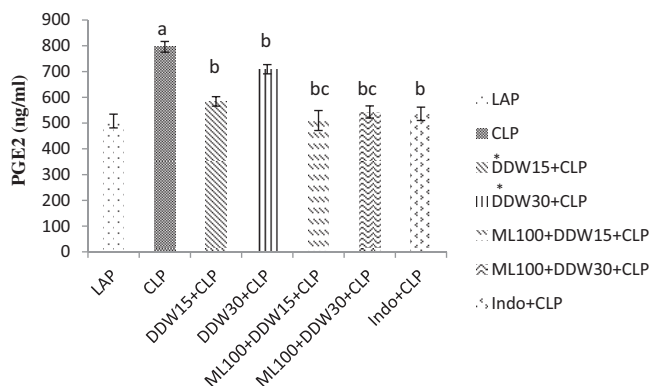
Figure 1. Effect of DDWs and ML on PGE2 in sepsis [a]$p < 0.05$ is considered significantly between LAP group and CLP group. [b]$p < 0.05$ is considered significantly between CLP group and treated groups. [c]$p < 0.05$ is considered significantly between *DDWs treatment groups with DDWs + ML groups. Data are presented as mean ± SD. *These data are presented here in order to make a new compression among treatment groups (Fatemi et al. 2019).

Figure 2. Effect of DDWs and ML on COX-2 gene expression in sepsis. [a]$p < 0.05$ is considered significantly between LAP group and CLP group. [b]$p < 0.05$ is considered significantly between CLP group and treated groups. [c]$p < 0.05$ is considered significantly between *DDWs treatment groups with DDWs + ML groups. Data are presented as mean ± SD. *These data are presented here in order to make a new compression among treatment groups (Fatemi et al. 2019).

(maximum 0.5 cm) were fixed in 10% formalin buffer solution. Forty-eight hours later, the formalin solution was replaced and then the samples were kept at room temperature until the preparation of microscopic sections (Dadkhah et al. 2018).

Statistical analysis

Results are presented as mean ± SD. Statistical procedures were performed by one-way ANOVA followed by Tukey's HSD (Honestly Significant Differences) using SPSS 22.0 software (SPSS Inc., Chicago, IL). The significance was considered as p value <0.05.
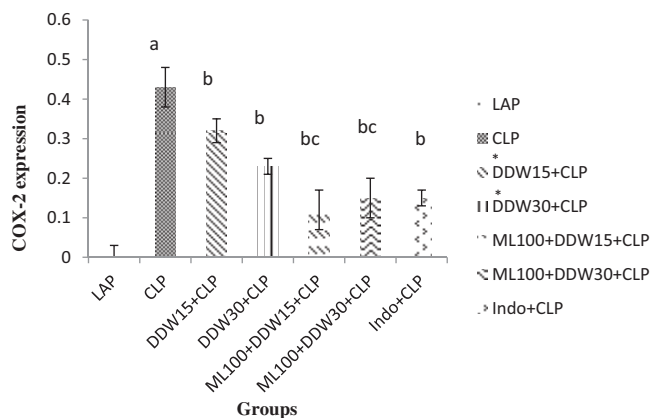
Results

Effect of DDWs and ML on PGE2 in sepsis

CLP surgery showed a significant effect on the activity of PGE2 ($p < 0.05$) (Figure 1). PGE2 level in the CLP-induced sepsis rats pretreated with DDWs + ML reduced as compared with the CLP group (Figure 1). It is pointed that the DDWs + ML treatment is more effective than DDWs alone in reducing the anti-inflammatory PGE2 level (Figure 1) ($p < 0.05$).

Effect of DDWs and ML on the COX-2 gene expression in sepsis

As shown in Figure 2, in comparison to the LAP operated animals, the CLP animals expressed higher levels of COX-2 ($p < 0.05$). Furthermore, the treatment of rats with DDWs + ML significantly inhibited the CLP-induced production of serum COX-2 levels ($p < 0.05$) (Figure 2). DDWs + ML were more effective than DDWs in decreasing of gene expression. Similarly, indomethacin decreased hepatic COX-2 gene expression when compared to the CLP group ($p < 0.05$) (Figure 2).

Effect of DDWs and ML on the oxidative stress/antioxidant parameters in sepsis

CLP-induced sepsis had a significant effect on the levels of antioxidant enzymes. There was a significant increase in the level of LP in the CLP induced sepsis rats as compared to the LAP control group. Administration of DDWs + ML at both doses had an inhibitory effect on the levels of LP in the comparison to the CLP group ($p < 0.05$) (Figure 3(A)). On the other hand, the induction of sepsis with CLP decreased the level of GSH as compared to the LAP group. Pre-treatment with DDWs and ML (100 mg/kg b.w) could increase the GSH level to the LAP group ($p < 0.05$) (Figure 3(B)). As shown in Figure 3(C), CLP surgery, DDWs and ML treatments did not alter GST activity ($p > 0.05$) (Figure 3(C)). Also, indomethacin had partly the same effect as ML and DDWs ($p < 0.05$). Furthermore, CLP could diminish FRAP level in compression to the LAP group. Although the administration of DDWs + ML changed the level of FRAP to the LAP group ($p < 0.05$), indomethacin did not influence on the FRAP level ($p > 0.05$) (Figure 3(D)).

Liver MPO activity as a marker of neutrophil sequestration increased significantly in the CLP group compared to the CLP-induced sepsis group ($p < 0.05$) (Figure 3(E)). However, MPO activity in the CLP-induced sepsis rats pretreated with DDWs and ML markedly reduced compared with the CLP group (Figure 3(E)). Surprisingly, the combination of DDWs and ML treatment showed a statistically significant better result when compared with DDWs treatment individual on the antioxidant parameters (LP, GSH, FRAP and MPO) except GST (Figure 3).

Effect of DDWs and ML on the liver enzymes in sepsis

DDWs (15 and 30 ppm) and ML (100 mg/kg b.w) treatment significantly decreased the liver injuries as indicated by the lower levels of the liver enzymes ($p < 0.05$). The serum levels of AST and ALT were drastically increased 24 h after sepsis ($p < 0.05$) (Table 2). Similar results also observed in the group of indomethacin. The administration of DDWs and ML diminished the AST and ALT enzymes to the negative control group ($p < 0.05$) (Table 2). DDWs + ML had better effects on decreasing AST enzyme than DDWs individual, whereas no difference was found between the DDWs and DDWs + ML groups in ALT level. But plasma ALP and total bilirubin were not changed in all treated animals ($p > 0.05$) (Table 2).
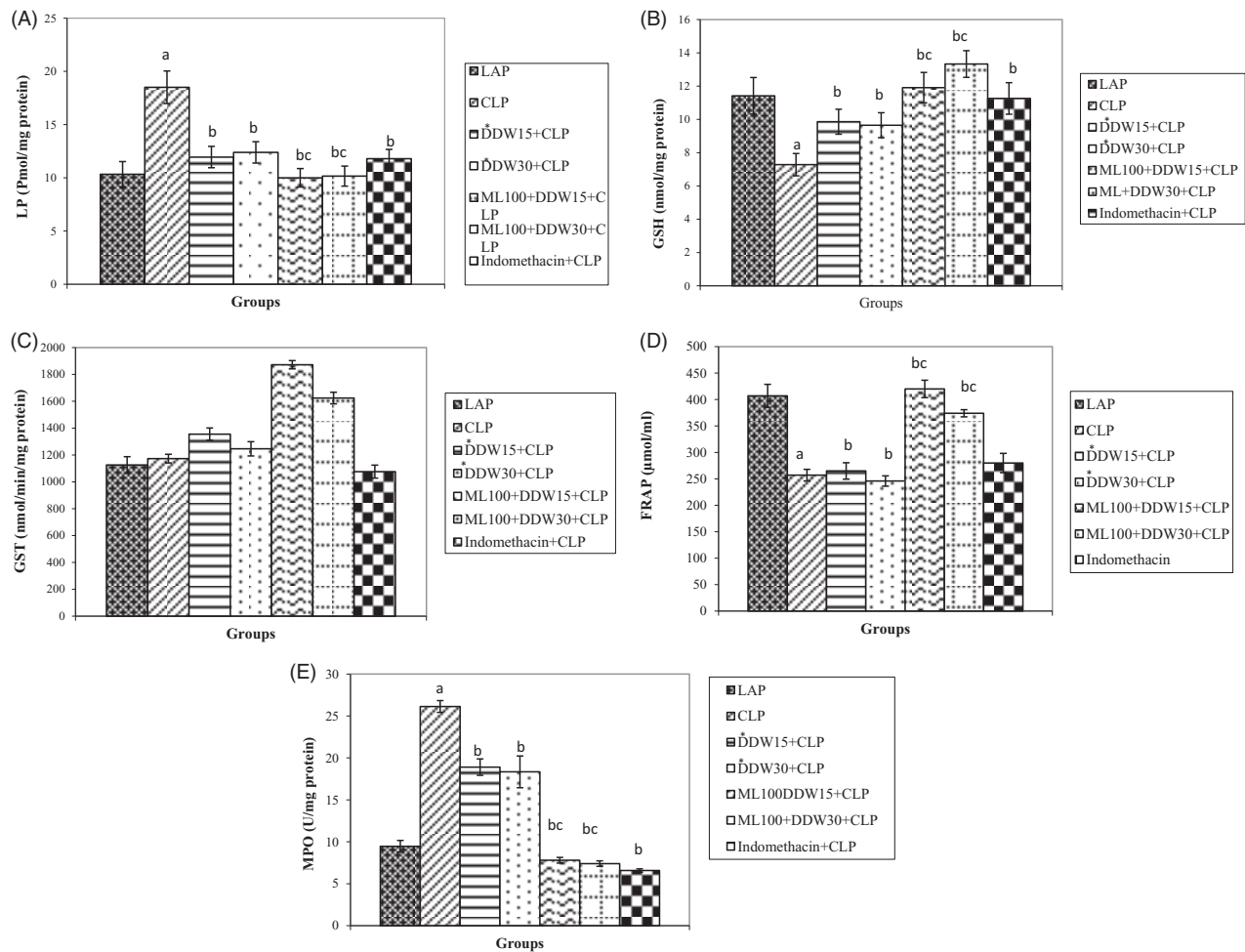
Figure 3. Effect of DDWs and ML on oxidative stress/antioxidant parameters in sepsis; (A) LP, (B) GSH, (C) GST, (D) FRAP and (E) MPO. [a]$p < 0.05$ is considered significantly between LAP group and CLP group. [b]$p < 0.05$ is considered significantly between CLP group and treated groups. [c]$p < 0.05$ is considered significantly between DDWs treatment groups with DDW+ML groups. Data are presented as mean ± SD. *These data are presented here in order to make a new compression among treatment groups (Fatemi et al. 2019).

Histopathological finding

The histopathological assessment revealed that there were some mild changes consisting of congestion and granular degeneration of the hepatocytes in LAP group (Figure 4(A)). Severe congestion, interstitial oedema and also margination of neutrophils in the venules and sinusoids were observed in the CLP group. Infiltration of neutrophils and mononuclear cells in the portal tracts and sinusoids, Kupffer cell hyperplasia and granular degeneration were the other observed changes in the CLP group. There were no any signs of necrosis in hepatocytes. All the changes in the CLP group revealed a kind of hepatitis called non-specific reactive hepatitis (Figure 4(B1,B2)). However, the treated groups improved the histopathological lesions. There was a mild neutrophil infiltration in the portal tract and the parenchyma in DDW15 + ML treated group (Figure 4(C)). Also, there was no any infiltrated neutrophil in the DDW30 + ML treated group. Kupffer cells were also in the normal range in this group (Figure 4(D)). In addition, reduced neutrophil infiltration and a few Kupffer cells were observed in the indomethacin-treated group (Figure 4(G)).

The CLP group obviously showed the neutrophil margination and infiltration, mononuclear cell infiltration and Kupffer cell hyperplasia as compared with the LAP group ($p \leq 0.05$)

(Table 3). Concerning portal inflammation, it was also meaningful in the CLP group in comparison with the LAP group ($p \leq 0.05$). However, there were no obvious difference regarding granular degeneration and inflammatory foci between all study groups ($p > 0.05$). To confirm the results seen in Figure 4, all the treatment groups prominently reduced neutrophil margination and infiltration, mononuclear cells infiltration, Kupffer cell hyperplasia and portal inflammation in comparison with the CLP group ($p \leq 0.05$).

Discussion

Our previous studies proved that *M. longifolia* essential oil and DDWs had antioxidative and hepatoprotective activities against CLP-induced sepsis, caused by reactive species. The positive effects of the essential oil and DDWs were mainly due to reverse the oxidative stress/antioxidant parameters and to restore the ideal concentration of inflammatory parameters (Dadkhah et al. 2018; Fatemi et al. 2019). There is also an evidence to show the hepatoprotective activity of DDW against acetaminophen toxicity in *in vivo* system (Rasooli et al. 2016). Following these, the present study was aimed to assess the synergetic anti-inflammatory effects of DDWs plus ML against the liver damages induced by

Table 2. Effect of DDWs and ML on liver enzymes in sepsis.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	BILI (mg/dL)
LAP	132 ± 9.58	61 ± 5.35	364 ± 33.8	0.54 ± 0.05
CLP	317 ± 13.58[a]	136 ± 8.76[a]	400 ± 25.8	0.6 ± 0.05
*DDW15 + CLP	168 ± 11.76[b]	74 ± 7.63[b]	394 ± 33	0.59 ± 0.04
*DDW30 + CLP	171 ± 9.91[b]	78 ± 8.01[b]	377 ± 30.8	0.58 ± 0.04
ML100 + DDW15 + CLP	151 ± 10.84[bc]	75 ± 6.42[b]	387 ± 28.7	0.59 ± 0.06
ML100 + DDW30 + CLP	150 ± 15.42[bc]	74 ± 8.41[b]	365 ± 27.7	0.56 ± 0.05
Indomethacin + CLP	150 ± 11.72[b]	73 ± 4.48[b]	371 ± 30	0.54 ± 0.04

[a] $p < 0.05$ is considered significantly between LAP group and CLP group.
[b] $p < 0.05$ is considered significantly between CLP group and treated groups.
[c] $p < 0.05$ is considered significantly between *DDWs treatment groups with DDWs + ML groups. Data are presented as mean ± SD. *These data are presented here in order to make a new compression among treatment groups (Fatemi et al. 2019).

CLP in rats along with comparing the current data with our previous researches on the anti-inflammatory activities of the DDWs individual.

Our results showed significant differences between the LAP and CLP groups. CLP surgery resulted in the significant increase in the LP, MPO, and PGE2 levels and COX-2 expression as well as AST and ALT activities with a concomitant decrease in the GSH and FRAP levels ($p < 0.05$) (Table 2) (Figures 1, 2 and 3). In contrast, no difference in the GST, ALP and bilirubin levels was observed among any experimental groups ($p > 0.05$) (Table 2) (Figure 3(C)). These results were further confirmed by the pathological examinations of the liver tissues (Figure 4).

Sepsis is defined as immune and inflammatory responses that is capable of inducing multiple organ failures and death (Singer et al. 2016). A liver dysfunction has exhibited as an early event in sepsis in recent studies (Marshall 2012). The liver plays an important role in protective responses to scavenge bacteria and produce inflammatory mediators during the sepsis (Marshall 2012). Some data showed that liver dysfunction in septic patients occurs about 34.7% (Kobashi et al. 2013). Hepatic dysfunction may be a result of undesirable side effects of the treatment provided (Wang et al. 2014). Parallel with increased plasma AST level (Table 2), the changes in the hepatic oxidative stress parameters (Figure 3) indicated that the liver function was damaged by sepsis. Elevated activities of the serum AST, ALT are a common sign of liver diseases (Arkkila et al. 2001). The maintenance of the plasma ALP and bilirubin levels (Table 2) indicated that the hepatic damage did not affect the bile ducts.

On the other hand, an increase in the MDA level (Figure 3(A)) had the main role of LP in the initiation of oxidative stress (Dadkhah et al. 2015). Also, GSH plays an important role in preventing the cells from oxidative damage (Villa et al. 2002). The reduction observed in the hepatic GSH levels in the septic groups (Figure 3(B)) as compared with the LAP group demonstrated that the sepsis promoted a disturbance in balancing redox reactions. Myeloperoxidase (MPO) is a protein in neutrophils that participates in early inflammatory process in patients with sepsis (Metzler et al. 2011; Kothari et al. 2011), its elevation in the septic animals (Figure 3) led to the hepatic dysfunction.

Moreover, COX-2 is an early expressed gene and is not detected in most normal tissues, but it is induced by stimuli such as proinflammatory cytokines (Konturek et al. 2005) leading to PGE2 production which acts on neurons and contributes to the systemic responses to inflammation (Samad et al. 2002). In our study, an increase of PGE2 level in the CLP group (Figure 1), can be due to COX-2 overexpression (Figure 2) and considered as the most important downstream effectors of COX-2.

Naturally, synthetic and recombinant products are important sources on developing new anti-inflammatory agents (Harvey 2008). Medicinal plants are also used as an alternative therapy in developing countries, due to its accessibility, low cost and popular culture (Silva et al. 2012) in many diseases such as sepsis. Deuterium depleted water (DDW) is water with a low deuterium content. Many studies proved that DDW has a special influence on the cells and tissues development with antioxidant properties that influence the whole organism such as the liver (Somlyai et al. 2010; Petcu et al. 2012; Gyongyi et al. 2013; Fatemi et al. 2015). *Mentha longifolia* is a well-known remedy in Iranian traditional medicine for treatment of various ailments (Jalilzadeh-Amin and Maham 2015). In the current study, we demonstrated that the treatment of septic animals with DDWs and ML could return the antioxidant parameters (LP, GSH and MPO) and the liver biomarkers (AST and ALT) to the LAP group level (Figure 3 and Table 2). It means that DDWs and ML have hepatoprotective activity against sepsis induced by CLP which was confirmed by histopathological studies (Table 3, Figure 4). Furthermore, PGE2 level and COX-2 expression were decreased by DDWs + ML (Figures 1 and 2) indicated the anti-inflammatory effects of DDWs and ML. It is noteworthy to indicate that the DDWs + ML treatment had more efficient than DDWs alone in hepatoprotection.

The antioxidant activity of DDW could be responsible for protecting the liver from toxicity (Rasooli et al. 2016) which can be more successful in adjuvant with ML which indicated earlier potent antioxidant/anti-inflammatory property (Dadkhah et al. 2018).

The consumption of deuterium-depleted water leads to the reduction of deuterium concentration in liquids and tissues of organisms. Moreover, DDW may affect cell cycle and cell proliferation by isotope exchange reactions (Sergeevich et al. 2018).

A previous study demonstrated that carvone and limonene as major compounds of *M. longifolia* essential oil had a great potential in *in vitro* antioxidative properties (Dadkhah et al. 2018). Also, other studies proved that carvone and limonene in *Mentha* species have antimicrobial, antifungal and antioxidant activities (Zare Bidaki et al. 2014; Kamkar et al. 2009; Singh et al. 2011).

Additionally, non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used therapeutic drugs. In various inflammatory diseases, through their anti-inflammatory, antipyretic and analgesic activities, they represent a choice treatment (Fiorucci et al. 2001). Our results revealed that the anti-inflammatory effect of indomethacin was comparable with the DDWs + ML groups (Table 2 and Figures 1, 2 and 3).

Conclusions

In conclusion, the present study demonstrated that the mechanism of the protective action of DDWs and ML seemed to involve its ability to reduce inflammatory response concomitant with hepatic injury inhibition by decreasing oxidative stress parameters and also restoring the ideal concentration of inflammatory parameters. The results also confirmed that the strong synergistic protective activities of the combination of DDWs and ML against liver injuries induced by CLP model by modulating the various oxidative stress/antioxidant parameters as well as histopathological assessment.
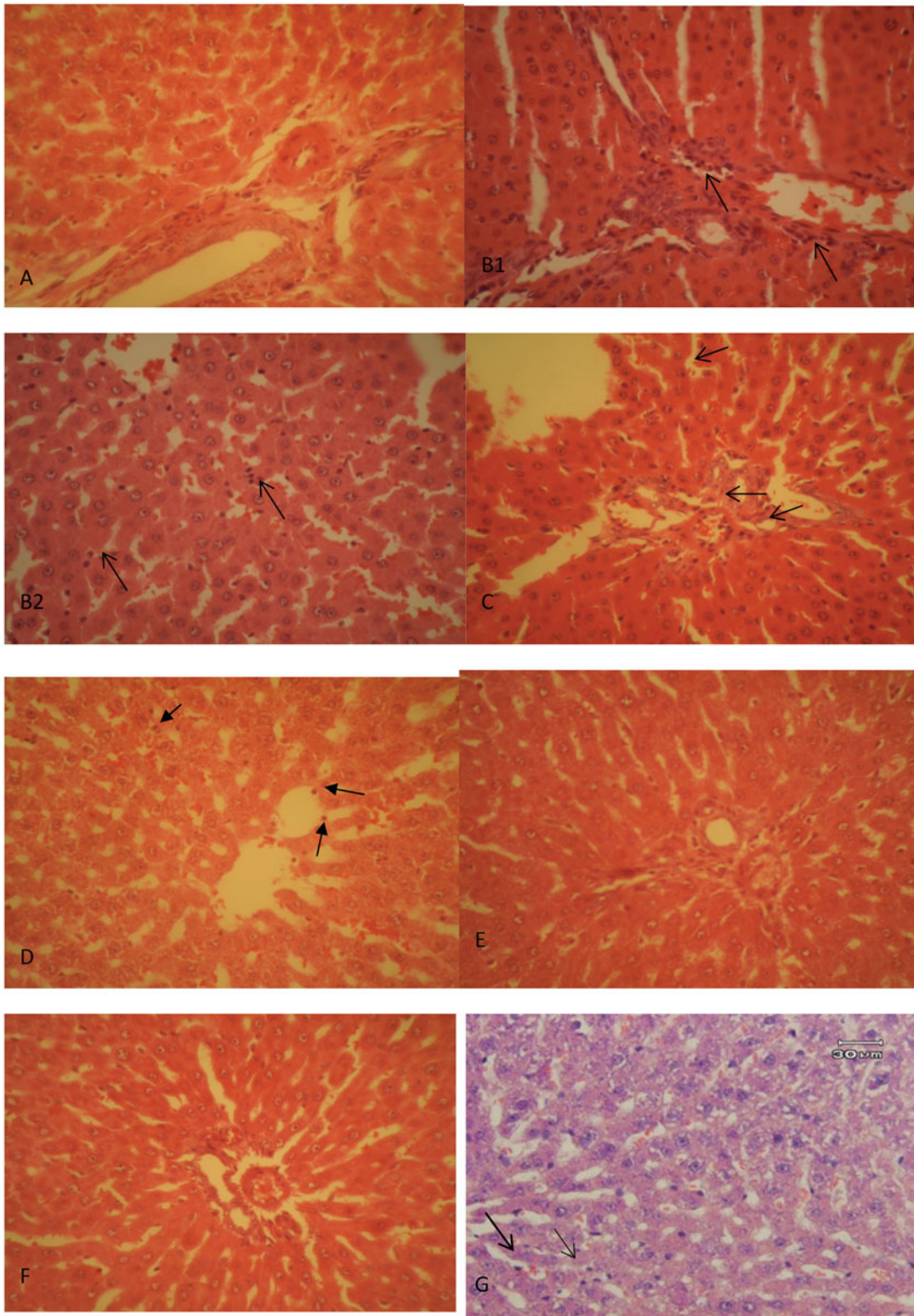
Figure 4. Histopathological studies. (A) LAP group, the portal tract and the hepatocytes in normal condition. (B1) CLP group, neutrophil infiltration in the portal tract (arrows). (B2) CLP group, neutrophil infiltration in the sinusoids which can be seen easily with their dark nuclei (arrows). (C) DDW15 + ML, mild neutrophil infiltration in the portal tract and the parenchyma (thin arrows), H&E, 400*. (D) DDW30 + ML group, there is not any infiltrated neutrophil in the picture. Kupffer cells also could be seen (arrows), H&E, 400*. (E) *DDW15 group, the portal tract and the parenchyma in normal condition. H&E, 400*. (F) *DDW30 group, the portal tract and the parenchyma in normal condition. H&E, 400*. (G) Indomethacin group, a few infiltrated neutrophils (arrows) could be seen in the picture. H&E, 400*. *These data are presented here in order to make a new compression among treatment groups (Fatemi et al. 2019).

Table 3. Mean values and standard error of histopathologic variables of the liver specimens in the study groups.

Study groups	Neutrophil margination and infiltration	Granular degeneration	Inflammatory foci	Mononuclear cells infiltration and kupffer cell hyperplasia	Portal inflammation
LAP	0 ± 0	0.4 ± 0.24	0 ± 0	0 ± 0	0 ± 0
CLP	2.75 ± 0.25^{a}	0.75 ± 0.75	1.5 ± 0.86	3 ± 0.4^{a}	2.25 ± 0.25^{a}
*DDW15 + CLP	0.4 ± 0.24^{b}	0.4 ± 0.24	0 ± 0	1.4 ± 0.4^{b}	0.4 ± 0.24^{b}
*DDW30 + CLP	1 ± 0^{b}	0 ± 0	0.8 ± 0.8	0.8 ± 0.2^{b}	0.2 ± 0.2^{b}
ML100 + DDW15 + CLP	0.8 ± 0.2^{b}	0 ± 0	0 ± 0	1 ± 0^{b}	0.8 ± 0.2^{b}
ML100 + DDW30 + CLP	1.4 ± 0.24^{b}	0 ± 0	0 ± 0	1.4 ± 0.24^{b}	0.6 ± 0.24^{b}
Indomethacin + CLP	1.6 ± 0.16^{b}	0.29 ± 0.19	0.45 ± 0.21	1.3 ± 0.21^{b}	0.5 ± 0.21^{b}

[a]$p < 0.05$ is considered significantly between LAP group and CLP group. [b]$p < 0.05$ is considered significantly between CLP group and treated groups. [c]$p < 0.05$ is considered significantly between *DDWs treatment groups with DDW+ML groups *These data are presented here in order to make a new comparison among treatment groups (Fatemi et al. 2019).

Disclosure statement

No potential conflict of interest was reported by the authors.

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