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Full Length Article

LPS-induced oxidative inflammation and hyperlipidemia in male rats: The protective role of *Origanum majorana* extract

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ABSTRACT

The antimicrobicidal activity of the phenolic compounds in the methanolic extract of *Origanum majorana* was recommended. The present study aimed to investigate the protective effect of *Origanum majorana* against LPS-induced toxicity in rats. Forty-eight male Sprague-Dawley rats were randomly divided into four equal groups, with 12 rats each group. Group C was used as control, while group E was treated with plant extract orally for 10 days (0.5 mg/kg/day). Group I was given LPS at a single i.p. dose (10 mg/kg BW) and group E + I was treated with plant extract (0.5 mg/kg/day) for 10 days, followed by a single i.p. dose of LPS (10 mg/kg BW). The WBC count and the number of macrophages in addition to the nitric oxide level in the peritoneal fluid were determined. Also, the lipids profile and the levels of urea and creatinine were detected. In addition, the MDA, glutathione and total proteins, as well as AST and ALT activities, were measured in all groups. The results indicated that the LPS injection caused significant decrease in the WBC count, hepatic glutathione and the total proteins, as well as serum HDL-c. On the other hand, LPS injection showed significant increase in the number of peritoneal macrophages, the levels of nitric oxide and MDA. Moreover, the total lipids, total cholesterol, triglycerides, urea, and creatinine concentrations, as well as AST and ALT activities, were significantly elevated. The pretreatment with *Origanum majorana* extract prior to LPS antagonized and alleviated its toxic effects in the treated animals. The results indicated that the treatment with *Origanum majorana* extract alone did not affect the tested parameters, except the number of peritoneal macrophages, which were significantly decreased.

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1. Introduction

Lipopolysaccharide (LPS) is viewed by the host as an alarm molecule indicating microbial invasion by gram-negative bacteria pathogen (Opal, 2007). LPS triggers a global activation of inflammatory responses leading to liver injury in humans. Furthermore, it was established that the inflammatory response to LPS administration is frequently associated with overproduction of nitric oxide, tissue injury and organ failure

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(Feihl et al., 2001; Weigand et al., 2004). The release of large quantities of LPS into the blood stream induces a severe form of host systemic inflammatory reactions implicated in pulmonary, hepatic and renal failures (Wang et al., 2009).

The world nowadays has given special attention to the medicinal and aromatic plants as an excellent source for bioactive agents. *Origanum majorana* is a herbaceous and perennial plant native to southern Europe and the Mediterranean (Vera and Chane-Ming, 1999; Wang, 2014). *Majorana* is employed to flavor sausages, meats, salads, and soups. Traditionally, it is used as a folk remedy against asthma, indigestion, headache and rheumatism (Banchio et al., 2008; Baranauskienė et al., 2006). The methanol extract of *Origanum majorana* was found to have an *in vitro* microbicidal activity due to the presence of 1,8-cineol, borneol, terpinen-4-ol, p-cymene, α and β-pinene and γ-terpinene (Hayouni et al., 2009; Muñoz et al., 2009). Monoterpenes of marjoram as carvacrol has antibacterial, antifungal, antispasmodic, acetylcholine esterase inhibition, radical scavenging effect, white blood cell macrophage stimulant and cardiac depressant activity (Hayouni et al., 2009). It was reported that *Origanum majorana* has potent antioxidant, antimicrobial and anti-inflammatory effects, which are attributed to its high content of phenolic acids and flavonoids (Banchio et al., 2008; Qari, 2008). Ursolic acid isolated from the ethanol extract of marjoram was reported to produce antitumor activities. It has been found that both oleanolic acid and ursolic acid isolated from marjoram are effective in protecting against chemically induced liver injury in laboratory animals (Liu, 1995).

Therefore, the present study was carried out to investigate (1) the alterations in the biochemical parameters, free radicals, including NO, the number of peritoneal macrophages, and the total white blood cells count induced by LPS in the male rats, and (2) the role of *Origanum majorana* extract in alleviating the oxidative-inflammatory and hyperlipidemic effects of LPS on the assayed parameters.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (LPS) from *Escherichia coli* serotype O127:B8, 1,1,3,3-tetramethoxypropan (TMP), thiobarbituric acid (TBA), N-(1-naphthyl)-ethylenediamine dihydrochloride (NEDD) and vanadium III chloride (VCl_3) reduced glutathione (GSH), sulfanilamide (SULF), pyrogallol and 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich Company, Germany. High density lipoprotein (HDL-c), cholesterol, total lipids, triglycerides (TG), urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total protein kits were purchased from Biodiagnostic, Dokki, Giza, Egypt.

2.2. Plant extract

Origanum majorana (sweet marjoram) was purchased from a local herb store. The row material was a grayish-brown fine leaves with a characteristic scent. The plant extract was prepared according to the method of Vági et al. (2005). Dried leaves of marjoram were extracted with 95% ethanol (1:20 w/v) using a Soxhlet extraction method. The obtained extract was evaporated under vacuum using rotary evaporator (BUCHI rotavapor, Swiss) at 40 °C. The obtained concentrated extract was dried over anhydrous sodium sulfate and the phenolic content was determined using HPLC.

2.2.1. Experimental animals

Adult male albino rats of the Sprague-Dawley strain weighing 250–290 g were purchased from the National Research Center, Dokki, Giza, Egypt. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH). Animals were fed a rodent laboratory chow and water *ad libitum*, kept on a 12 h light–dark cycle periods and acclimatized for at least one week prior to the experiment.

2.2.2. Experimental design

After two weeks of acclimation, animals were divided into four equal groups, with 12 animals each group. Group C was used as control, while group E was treated with plant extract orally for 10 days (0.5 mg/kg/day). Group I was given LPS at a single i.p. dose (10 mg/kg BW) and group E + I was treated with plant extract (0.5 mg/kg/day) for 10 days, followed by a single i.p. dose of LPS (10 mg/kg BW). Rats were starved for 12 h and then sacrificed by decapitation, and the liver tissues were immediately removed and kept at −20 °C until analysis.

2.3. Blood sampling

Blood samples were collected in tubes containing EDTA for white blood cells count. Serum samples were prepared and placed in a pyrogen-free Eppendorf and stored at −20 °C for analysis.

2.3.1. Isolation of peritoneal macrophages and cell count

Immediately after decapitation of rats, cells of peritoneal macrophages were isolated (Kolaczkowska et al., 2008). Cells were centrifuged off for 10 minutes at 1200 ×*g*, then washed twice with saline, and resuspended in lysis buffer (NH4Cl) for 5 minutes to lyse red blood cells. This suspension was centrifuged for 10 minutes at 1200 ×*g* and resuspended in 1.0 ml of saline. Cell suspension counting was estimated using a hemocytometer. The number of viable cells was calculated and kept constant at 1 × 106 cell/ml.

2.3.2. Determination of nitric oxide production in the peritoneal macrophages

Diluted cell suspensions were activated according to the method of Kim et al. (2004).The nitric oxide concentrations were determined in the isolated peritoneal macrophages after being activated using Griess mixture (N-(1-naphthyl)-ethylenediamine dihydrochloride and sulfanilamide; 1:1) according to the method of Miranda et al. (2001). The developed color was measured spectrophotometrically at 540 nm.

2.4. Determination of nitric oxide production in the liver homogenate

Rat liver (1 g) was homogenized in 9 ml ice cold saline using Teflon glass homogenizer to get 10% w/v homogenate. Half

milliliter of tissue homogenate was added to 0.5 ml of cold absolute ethanol, then centrifuged at 4000 rpm for 10 minutes. Of the supernatant, 0.3 ml was added to 0.3 ml of VCl_3, followed immediately by 0.3 ml of Griess mixture and 0.1 ml of deionized water. The mixture was left at room temperature for 35 minutes, and then the developed color was measured spectrophotometrically at 540 nm. The concentration of NO was determined using standard curve of sodium nitrite.

2.5. *Determination of hepatic MDA and GSH*

Lipid hydroperoxides and aldehydes in the liver homogenate are expressed in terms of MDA equivalents, which were determined according to the method of Ohkawa et al. (1979). Also, the content of reduced glutathione in liver homogenate was determined by Ellman's method (Ellman, 1959).

2.6. *Lipid profile*

Serum total lipids, cholesterol and triglycerides (TG) were determined by the methods of Zollner and Kirsch (1962), Allain et al. (1974), and Fossati and Prencipe (1982), respectively. High density lipoproteins (HDL) were determined according to the method of Lopez-Virella et al. (1977).

2.7. *Liver and kidney functions tests*

Serum aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) were assayed by the method of Reitman and Frankel (1957). Hepatic total proteins were measured according to Gornall et al. (1949). Also, serum urea and creatinine concentrations were determined by the methods of Fawcett and Scott (1960) and Bartels et al. (1972), respectively.

2.8. *Statistical analysis*

Data were analyzed as a completely randomized design, and the obtained data were subjected to one-way analysis of variance (ANOVA) using the statistical software SPSS, version 11.5. Differences between groups were significant at $P < 0.05$.

3. Results

3.1. *HPLC analysis of phenolic compounds*

HPLC analysis revealed the presence of 3,4-dicaffeoylquinic acid (1.37 mg/g), 4,5-dicaffeoylquinic acid (0.81 mg/g), gallic acid (0.78 mg/g), chlorogenic acid (0.33 mg/g), rutin (0.21g/g), tannic acid (0.45 μg/g), quercetin (0.42 μg/g), cinnamic acid (0.33 μg/g) and phloridzin (0.05 μg/g) in the ethanolic plant extract (Fig. 1).

3.2. *White blood cells count*

The injection with LPS (Group I) caused a highly significant ($P < 0.01$) decrease in the white blood cells count ($2.6 \pm 0.24 \times 10^3$) compared to the control group ($5.2 \pm 0.29 \times 10^3$). On the other hand, there was a highly significant ($P < 0.01$) increase in the white blood cells in the combination group (E + I) compared to the LPS-treated rats, while there was a non-significant ($P > 0.05$) change in the white blood cells count in rats treated

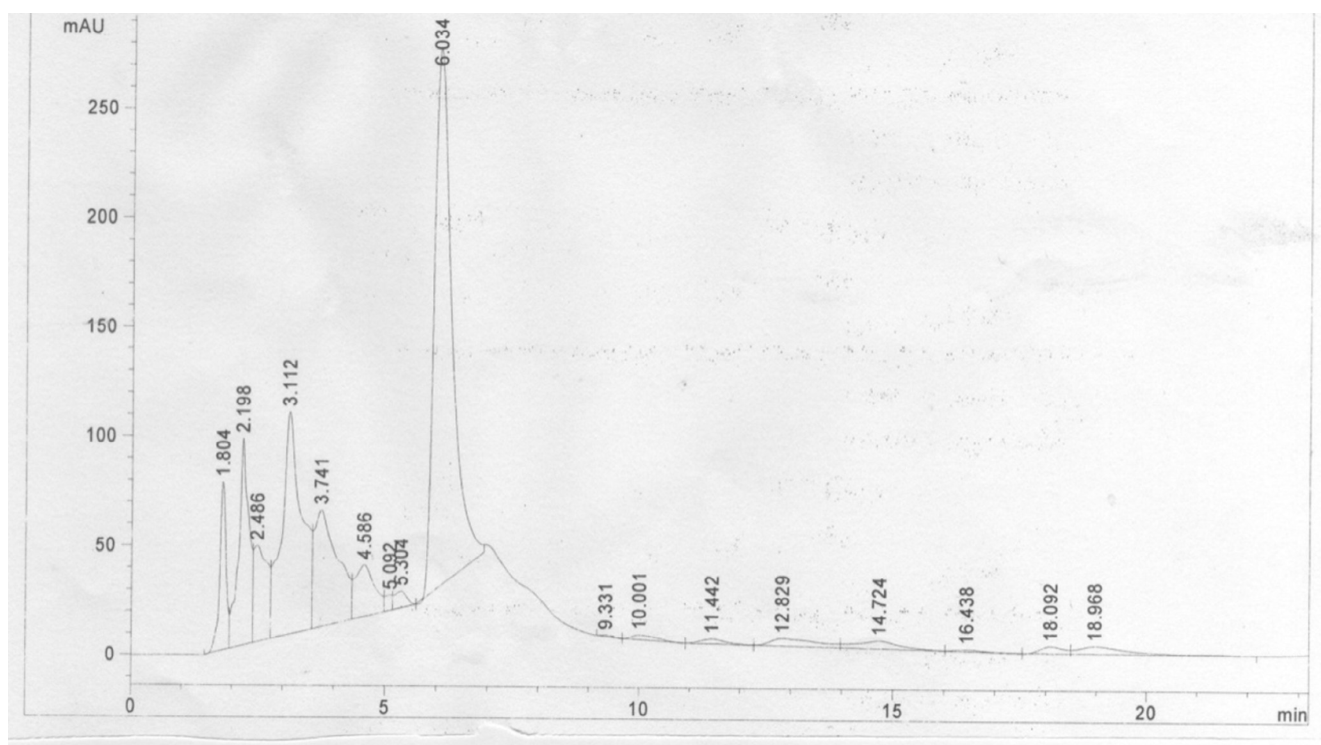

Fig. 1 – HPLC analysis of phenolic compounds in *Origanum majorana extract* at wavelength = 320 nm.

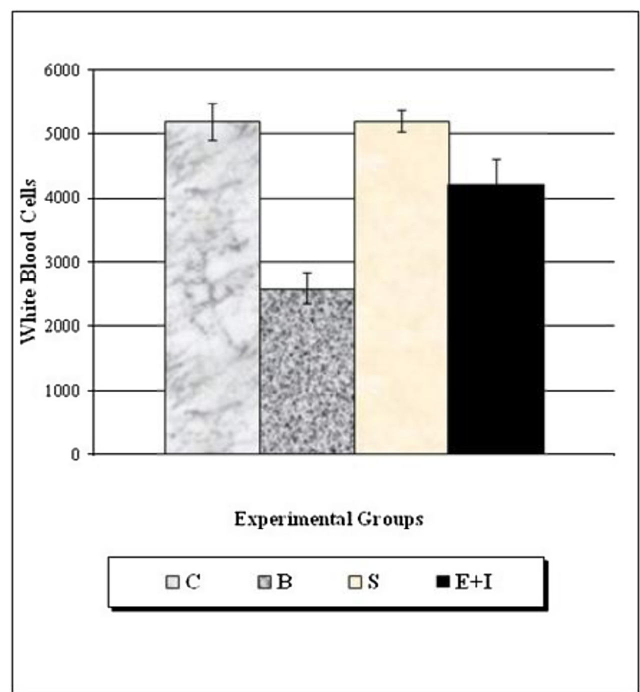

Fig. 2 – The effect of lipopolysaccharide (I), *Origanum majorana* extract (E) and their combination (E + I) on the number of white blood cells compared to a control group (C) of male rats.

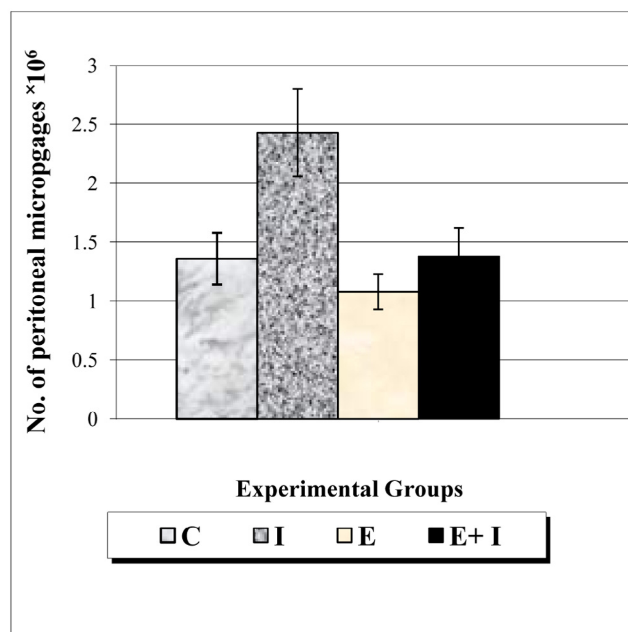

Fig. 3 – The effect of lipopolysaccharide (I), Origanum *majorana* extract (E) and their combination (E + I) on the number of peritoneal macrophages compared to a control group (C) in male rats.

with the plant extract (E group) ($5.2 \pm 0.17 \times 10^3$) compared to the control group (Fig. 2).

3.3. *Number of peritoneal macrophages*

Injection of rats with LPS caused a highly significant ($P < 0.01$) increase in the number of peritoneal macrophages ($2.43 \pm 0.37 \times 10^6$), whereas there was a significant ($P < 0.05$) decrease in the peritoneal macrophages in the extract-treated rats ($1.08 \pm 0.15 \times 10^6$) compared to the control ones ($1.36 \pm 0.22 \times 10^6$). Also, there was a significant ($P < 0.05$) decrease in the peritoneal macrophages of the protected group (group E + I) compared to the LPS-treated rats (Group I), as shown in Fig. 3.

3.4. *NO levels*

Our results indicated that there was a highly significant ($P < 0.01$) increase in the hepatic NO level (3.68 ± 0.24 μmol/g tissue) of the LPS-treated rats (Group I) compared to the control group (0.67 ± 0.09 μmol/g tissue). Nevertheless, there was a highly significant ($P < 0.01$) decrease in hepatic NO level of the combination group (E + I) (1.41 ± 0.10 μmol/g tissue) compared to the LPS-injected one (Group I), as illustrated in Fig. 3. Furthermore, LPS-injected rats had a highly significant ($P < 0.01$) increase in NO level of the peritoneal macrophages (5.55 ± 1.07 μmol/l) compared to the control rats (3.38 ± 0.39 μmol/l). It is noteworthy that there was a highly significant ($P < 0.01$) decrease in NO level in the combination group (E + I) (2.82 ± 0.44 μmol/l) compared to the LPS-treated rats (Group I), as shown in Fig. 4.

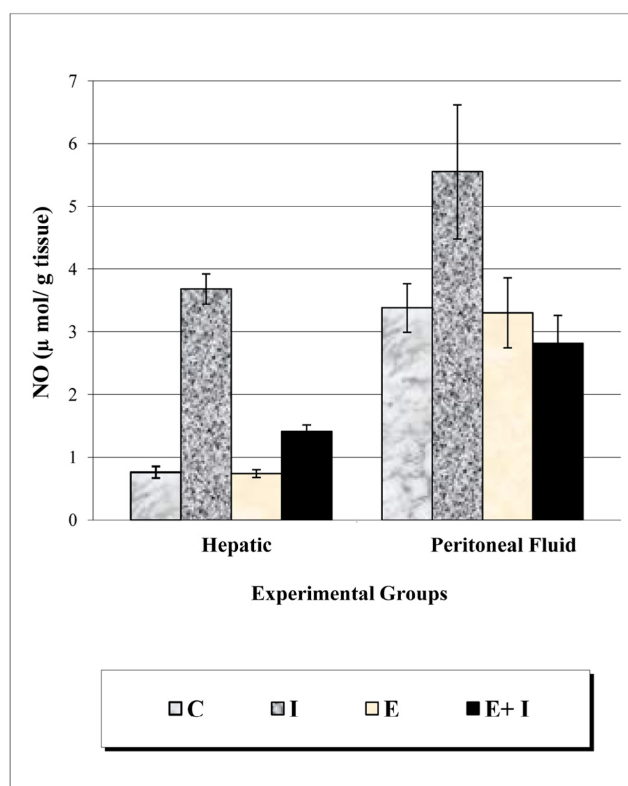

Fig. 4 – The effect of lipopolysaccharide (I), *Origanum majorana* extract (E) and their combination (E + I) on the concentration of NO (μ mol/ g tissue) in the hepatic extract and the peritoneal macrophages compared to a control group (C) of male rats.

Table 1 – The effect of lipopolysaccharide (I), *Origanum majorana* extract (E) and their combination (E + I) on liver biochemical parameters compared to a control group (C) of male rats.

Parameters	Experimental groups			
	C	I	E	E + I
MDA (n mol/g tissue)	188.17 ± 17.70	238.83 ± 15.79[a]	207.33 ± 10.99[a]	210.17 ± 11.81[a,b]
GSH (μ mol/ g tissue)	0.651 ± 0.064	0.411 ± 0.077[a]	0.636 ± 0.114	0.641 ± 0.086[b]
Total proteins (g/dl)	1.45 ± 0.06	1.09 ± 0.10[a]	1.51 ± 0.10	1.31 ± 0.03[a,b]

The values are expressed as mean ± SE.
[a] The mean values are significantly different in comparison with control (p ≤ 0.01).
[b] The mean values are significantly different in comparison with the infected rats (p ≤ 0.01).

3.5. *Hepatic MDA and GSH*

Injection of rats with LPS caused a highly significant ($P < 0.01$) increase in the MDA level (238.83 ± 15.79 nmol/g tissue), while the rats that received plant extract had a non-significant elevation in the MDA level (207.33 ± 10.99 nmol/g tissue) compared to the control ones (188.17 ± 17.70 nmol/g tissue). Additionally, there was a highly significant ($P < 0.01$) decrease in MDA level of the combination group (E + I) (210.17 ± 11.81 nmol/g tissue) compared to the LPS-treated one. On the other hand, hepatic GSH level showed a highly significant ($P < 0.01$) decrease (0.411 ± 0.077 μmol/ g tissue) in the LPS-treated rats compared to those of control ones (0.651 ± 0.064 μmol/g tissue). Pretreatment of rats with plant extract prior to LPS injection led to a highly significant($P < 0.01$) increase in GSH level (0.641 ± 0.086 μmol/g tissue) compared to the LPS-treated group, reaching that of the control levels (0.651 ± 0.064 μmol/g tissue), as shown in Table 1.

3.6. *Lipid profile*

Our results showed that there was a highly significant ($P < 0.01$) increase in the serum total lipids of the LPS-treated rats (0.290 ± 0.007 g/dl), while there was a non-significant ($P > 0.05$) increase in serum total lipids in rats pretreated with plant extract (0.276 ± 0.009 g/dl) compared to that of the control (0.272 ± 0.008 g/dl). It is noteworthy that the pretreatment of rats with plant extract prior to LPS injection resulted in a highly significant ($P < 0.01$) decrease in the total lipids (0.257 ± 0.007 g/dl) compared to the LPS-treated group (0.290 ± 0.007 g/dl). Injection of LPS caused a highly significant ($P < 0.01$) increase in serum TG (129.30 ± 7.34 mg/dl), while treatment of rats with the plant extract resulted in a highly significant ($P < 0.01$) decrease in serum TG (95.53 ± 6.03 mg/dl) compared to control ones (111.97 ± 7.48 mg/dl). Also, there was a highly significant ($P < 0.01$) decrease in serum TG in the combination group (E + I, 86.40 ± 4.52 mg/dl) compared to the LPS-treated one (Table 2).

The LPS-treated rats showed a highly significant ($P < 0.01$) elevation in the total cholesterol level (110.22 ± 7.19 mg/dl), while there was a non-significant ($P > 0.05$) increase in total cholesterol in rats pretreated with plant extract (88.23 ± 3.80 mg/dl) compared to that of control (84.32 ± 6.76 mg/dl). Interestingly, there was a highly significant ($P < 0.01$) reduction in the total cholesterol level of the combination (E + I) group (84.20 ± 6.21 mg/dl) compared to the LPS-treated one. Differently, LPS treatment resulted in a highly significant ($P < 0.01$) decrease in the HDL-c level (22.48 ± 4.65 mg/dl) and a non-significant ($P > 0.05$) decrease in HDL-cholesterol in rats pretreated with plant extract (42.92 ± 4.53 mg/dl) compared to controls (49.32 ± 5.57 mg/dl), whereas pretreatment with plant extract prior to LPS led to a highly significant ($P < 0.01$) elevation in HDL-c level (49.53 ± 4.58 mg/dl) compared to the LPS-treated rats, normalizing its level (Table 2).

3.7. *Liver and kidney functions tests*

The LPS-treated rats had a highly significant ($P < 0.01$) increase in the serum AST and ALT activities (154.22 ± 8.22 U/ml

Table 2 – The effect of lipopolysaccharide (I), *Origanum majorana* extract (E) and their combination (E + I) on serum biochemical parameters of male rats compared to the controls.

Parameter	Experimental groups			
	C	I	E	E + I
Total lipids (g/dl)	0.272 ± 0.008	0.290 ± 0.007[a]	0.276 ± 0.009	0.257 ± 0.007[a,b]
Total cholesterol (mg/dl)	84.32 ± 6.76	110.22 ± 7.19[a]	88.23 ± 3.80	84.20 ± 6.21[b]
HDL_c (mg/dl)	49.32 ± 5.57	22.48 ± 4.65[a]	42.92 ± 4.53	49.53 ± 4.58[b]
Triglycerides (mg/dl)	111.97 ± 7.48	129.30 ± 7.34[a]	95.53 ± 6.03[a]	86.40 ± 4.52[a,b]
AST activity (U/ml)	83.72 ± 8.86	154.22 ± 8.22[a]	73.87 ± 6.75	59.20 ± 7.45[a,b]
ALT activity (U/ml)	12.72 ± 3.38	148.03 ± 4.12[a]	15.32 ± 6.46	15.00 ± 4.72[b]
Urea (mg/dl)	33.03 ± 3.71	74.50 ± 6.67[a]	26.60 ± 3.41[a]	30.22 ± 6.79[b]
Creatinine (mg/dl)	0.34 ± 0.03	0.72 ± 0.05[a]	0.43 ± 0.05[a]	0.57 ± 0.02[a,b]

The values are expressed as mean ± SE.
[a] The mean values are significantly different in comparison with control (p ≤ 0.01).
[b] The mean values are significantly different in comparison with the infected rats (p ≤ 0.01).

and 148.03 ± 4.12 U/ml, respectively) compared to the control (83.72 ± 8.86 U/ml and 12.72 ± 3.38 U/ml, respectively), while plant extract treatment did not change the activities of AST and ALT compared to the control. Our results showed a highly significant ($P < 0.01$) decrease in the activity of serum AST in the E + I group (59.20 ± 7.45 U/ml) compared to the LPS-treated one (154.22 ± 8.22 U/ml). Moreover, there was a significant decrease in serum ALT activity in the (E + I) group (15.00 ± 4.72 U/ml) compared to the LPS-treated one (154.22 ± 8.22 U/ml), as shown in Table 2.

The results in Table 1 indicated a highly significant ($P < 0.01$) decrease (1.09 ± 0.10 g/dl) in the hepatic total proteins of LPS-treated rats, while there was a non-significant ($P > 0.05$) increase in liver total proteins in rats pretreated with plant extract (1.51 ± 0.10 g/dl) compared to that of control (1.45 ± 0.06 g/dl). Meanwhile, there was a highly significant ($P < 0.01$) increase in the hepatic total proteins in (E + I) group (1.31 ± 0.03 g/dl) compared to the LPS-treated rats (1.09 ± 0.10 g/dl).

The results in Table 2 showed a highly significant ($P < 0.01$) elevation in the serum urea (74.50 ± 6.67 mg/dl) and creatinine (0.72 ± 0.05 mg/dl) in the LPS-treated rats compared to the control levels, whereas treatment of rats with the plant extract prior to LPS injection significantly decreased serum urea (30.22 ± 6.79 mg/dl) and creatinine (0.57 ± 0.02 mg/dl) compared to LPS-treated group (74.50 ± 6.67 mg/dl and 0.72 ± 0.05 mg/dl, respectively).

4. Discussion

Our results revealed a significant decrease in the white blood cells (Fig. 2) in the LPS-treated rats in response to the induced inflammation, whereas Fig. 3 showed a significant increase in the peritoneal macrophages in the LPS-treated rats compared to the control group. These results are in coincidence with Shin et al. (2007), who reported that the activated macrophages play a central role in the regulation of the immune and inflammatory activities in which neutrophils and macrophages rapidly migrate from the blood to the sites of infection under the effect of chemotactic factors produced by the innate humeral immune system. This migration is evidenced by the significant decrease in the white blood cells and the significant increase in the peritoneal macrophages. Meanwhile, our data confirmed a significant improvement in both white blood cells count and peritoneal macrophages in the *Origanum majorana*-treated rats. This effect may be attributed to the antimicrobial effect of *Origanum majorana* extract (Ayari et al., 2013; Qari, 2008).

In our investigation, LPS caused highly significant increase in the hepatic nitrite of the LPS-treated rats compared to the control (Fig. 4). Abd Allah (2006) reported that the injection of LPS to rats produced a marked induction of NO in the liver tissue. This increase in the nitrite levels was explained by the activation of iNOS expression in the Kupffer cells and the hepatocytes in response to LPS, which could impair hepatic function by direct injury to hepatocytes (Kaur et al., 2006). Likewise, Fig. 4 showed a high significant increase in NO level in the peritoneal macrophages of the LPS-treated rats compared to control. While NO generated by the endothelial cells is mostly considered responsible for inflammation-induced injury, the migration of the inflammatory cells might be the potential source of NO at the site of inflammation (Sethi and Dikshit, 2000). Previous study stated that the concentration of nitrate in the circulation was proposed as a reliable marker for the morbidity and mortality of the patients, as well as experimental animals (Hwang et al., 2007).

In our study, the alcoholic extract of *Origanum majorana* was found to protect rats from LPS-induced inflammation. These anti-inflammatory properties are evidenced by the significant decrease in NO in the liver and peritoneal fluid of group (E + I) compared to group I. These results were found to be coinciding with Tunçtan et al. (2003), who found that of cyclooxygenase inhibitors significantly decreased the serum levels of nitric oxide and the survival in a mice model of sepsis.

HPLC analysis data indicated the presence of certain bioactive components of *Origanum majorana*, such as quercetin, 3, 4-dicaffeoylquinic acid, 4, 5-dicaffeoylquinic acid, gallic acid, chlorogenic acid, rutin, tannic acid, cinnamic acid and phloridzin in the ethanolic plant extract (Fig. 1). Another working group has affirmed that *Origanum majorana* contains phenolic terpenoids, flavonoids, tannins, hydroquinone and phenolic glycosides as a plausible explanation for its antitoxic and the protective action (Yazdanparast and Shahriyary, 2008). These constituents have inhibitory effect on the activities of lipoxygenase, cyclooxygenase, inducible nitric oxide synthase and monooxygenase (Moon et al., 2006). Quercetin and a number of other flavonoids were found to have a free radical scavenging and anti-inflammatory suppressing NO production in macrophages activated by LPS in vitro (Abd El-Gawad and Khalifa, 2001). Recently, Ayari et al. (2013) also correlated the antioxidant and antimicrobial activities of marjoram methanolic extract to its phenolic content. Moreover, LPS administration significantly increased the circulating levels of MDA, a marker of oxidative stress, which is in agreement with other studies (Kaur et al., 2006; Minutoli et al., 2008). NO reacts with superoxide anion ($O2^-$) producing peroxynitrite ($ONOO^-$), a powerful oxidant, which damages the biological molecules, increases lipid peroxidation, and leads to oxidative damage in lungs, kidneys and liver tissue (Çağlıkülekci et al., 2004). The response to such oxidative stress is achieved by defense mechanisms, including GSH which is responsible for maintaining the redox state of the cells. We evidenced that the hepatic GSH level was significantly decreased in the LPS-treated rats compared to the control (Table 1). This result was supported by the data of Kaur et al. (2006), who found significant decrease in the liver GSH level after LPS injection. Previous studies suggested that NO itself may negatively regulate the antioxidative GSH system by inhibiting GSH reductase through the formation of S-nitrosoglutathione (Eum et al., 2006). It was observed that there was a highly significant decrease in MDA level of the combination group (E + I) compared to the LPS-treated one. On the other hand, pretreatment of rats with plant extract prior to LPS injection led to a highly significant increase in GSH level compared to the LPS-treated group, reaching that of the control levels. Plant constituents such as caffeic acid derivatives exhibit various properties, such as antioxidative, antimutagenic, anti-inflammatory and hepatoprotective effects (Fecka and Turek, 2006). Our results indicated that the ethanolic extract of *Origanum majorana* contain 3,4-dicaffeoylquinic acid,

4,5-dicaffeoylquinic acid, gallic acid, chlorogenic acid, rutin, tannic acid, quercetin, cinnamic acid and phloridzin as the main polyphenols content. Lima et al. (2006) reported that these polyphenols attenuated the decrease in GSH level induced by tetra butyl hydroperoxide. This explains our finding that pretreatment with *Origanum majorana* in rats resulted in decreased MDA level and elevated GSH level. It was reported that the administration of *marjoram* could reverse the side effects of potassium bromate by scavenging the free radicals by the plant bioactive antioxidant constituents (Bulbul et al., 2012).

Habibi et al. (2015) revealed that *O. vulgare* with high amount of flavonoids and phenolic compounds induces potent hepatoprotective mechanisms. Therefore, *O. vulgare* might help defend the body against side effects, particularly hepatic damages induced by chemotherapeutic agents. Afifi et al. (2014) indicated that oral administration of *Origanum majorana* plant increased the antioxidant enzymes and significantly decreased liver enzyme levels when given in repeated doses. Thus, methanolic extract of *Origanum majorana* possesses a significant antioxidant activity.

Furthermore, data represented in Table 2 showed an altered lipid metabolism represented by a significant increase in the serum total lipids, total cholesterol and triglycerides in LPS-treated rats compared to the control. Meanwhile, LPS treatment caused a significant decrease in HDL-c compared to the control. Our data are supported by the work of Crespo et al. (1999), who found that the administration of LPS to rats was associated with a significant rise in the serum levels of cholesterol, triglycerides, aspartate and alanine aminotransferases, creatinine, and urea. Van Oosten et al. (2001) suggested that the increased level of serum lipoproteins may be attributed to the physiological defense mechanism of the rat body to combat the toxic effects of circulating LPS. They also reported that serum lipoproteins may play an important role in the protection against infection and inflammation, binding to LPS and reducing its toxic properties. Our study showed that *Origanum majorana* extract has a lowering effect on LPS-induced hyperlipidemia and hypercholesterolemia; there was a significant decrease in the cholesterol and triglycerides levels in group E + I compared to LPS-treated rats (Table 2). Our data are in agreement with the published data by El Ashmawy and Salama (2005), who suggested that the chlorogenic acid and caffeic acid in *Origanum majorana* extract may act as hypocholesterolemic agent, reducing the LDL-c levels, when administrated orally to humans.

Our data confirmed a highly significant increase in the serum activities of AST and ALT and significant decrease in the total protein content of the LPS-treated rats compared to that of control ones (Table 2). Pradeep et al. (2007) established that the high serum levels of transaminases are taken as an index of hepatic injury. Mittal et al. (2006) and Kaur et al. (2006) suggested that the generation of superoxide anion (O_2^-), NO and other ROS and reactive nitrogen species (RNS) accelerate peroxidation of native membrane lipids, leading to a loss of membrane integrity and subsequent release of the cytosolic contents. Serum AST and ALT activities were significantly decreased in E + I group compared to LPS-treated group. However, significant increase in the total proteins was noticed in both the plant extract-treated rats and E + I group, demonstrating the role of active ingredients in *Origanum majorana* in liver treatment. Furthermore, Table 2 revealed a significant increase in the levels of serum urea and creatinine in the LPS-treated rats compared to control. The protective effect of *Origanum majorana* was proven by the significant decrease in urea and creatinine in group E + I compared to LPS-treated rats. It could be concluded that *Origanum majorana* plays an important role in ameliorating liver and kidney functions. Shati (2011) found that *Origanum majorana*-treatment alleviated all the Cd-disrupted levels of liver biomarkers, urea, creatinine, total protein levels, and the activities of liver and kidney antioxidants.

5. Conclusion

Ethanolic extract of *Origanum majorana* has been shown to possess anti-inflammatory, antioxidative, hypolipidemic and hepatoprotective effects against LPS-induced endotoxemia. So it can be used as an effective and safe alternative complementary treatment.

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