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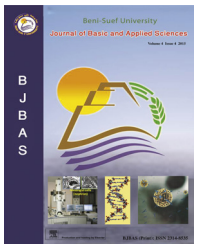

Full Length Article

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.

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