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The Role of CD14 and TNF-α During Liver Endotoxemia with Emphasis on the Immunomodulatory Effect of Wheat Germ Oil

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Abstract: Septic shock is a serious medical condition caused due to the infection with bacterial endotoxin. It is the prime causes of multiple organ dysfunction syndrome and death. Bacterial endotoxin lipopolysaccharide (LPS) induces liver toxicity due to release of reactive oxygen species that destroy normal cells, leading to the release of TNF-α and increase CD14 expression in septic conditions. Accordingly, an attempt was made to discover the immunomodulatory effect of wheat germ oil (WGO) against liver damage induced by LPS. Male rats were divided into four groups: where group I was considered as control. Rats in group II received WGO. Rats in group III were injected with LPS. Animals in group IV received WGO and then injected with LPS. The histopathological and immunological changes in liver tissue and blood were reported in treated groups. CD14 expression, TNF-α and IL-10 were measured. Data produced from this study indicate the positive role of WGO administration appeared in the histological picture viz: regular arrangement of the hepatic cords with intact nuclei of the hepatic cells and decrease in the lipid droplets. The immunomodulatory effect of WGO appeared obviously by the inhibition of CD14 expression, as well as preserve cytokine activities by suppressing the levels of TNF-α and IL-10.

Key words: CD14 · TNFα · IL10 · Wheat germ oil · Lipopolysaccharide

INTRODUCTION

Gram-negative sepsis remains an important cause of morbidity and mortality in intensive care units despite recent advances in critical care [1]. The arrays of pathophysiologic features that accompany gram-negative bacterial sepsis appear to be qualitatively similar to those encountered after lipopolysaccharide (LPS) insult [2,3]. Rose *et al.* [4] reported that LPS play a crucial role in triggering the inflammatory cascade and the haemodynamic events in sepsis. Over the last decade, accumulating evidence has shown that oxidative stress, defined as the imbalance between oxidants and antioxidants in favour of the former, is involved in the sepsis-induced multiple organs dysfunction [5]. Its most common victims are children and the elderly, as their immune systems cannot deal with the infection as effectively as those of healthy adults.

Volk *et al.* [6] show that during stages of sepsis, massive deterioration of the immune response occurs, which is characterized by suppression of both macrophage and lymphocyte immune function. Indeed, a significant percentage of survivors of sepsis have an elevated risk of succumbing to bacterial superinfection. The endothelium, with its diversity of physiological functions is the main target of bacterial toxins, resulting in endothelial dysfunction which believed to contribute to the underlying patho-mechanisms and the collapse of homeostasis of organ function [7].

Bautista [8] and Limaye *et al.* [9] delineated that LPS in the blood is bound by lipopolysaccharide binding protein, which interacts with CD14 receptors on hepatic Kupffer cells, setting off a series of pro-inflammatory events. Evidence of a physiological role for membrane CD14 in response to LPS has been shown using models of endotoxemia or infection. Thus, CD14 is required for the induction of endotoxemia and/or shock by LPS [10,11].

In septic conditions, TNF-α is the initial and most important cytokine, because it not only causes the production of IFN-γ, IL-6 and IL-12, but it also directly activates caspase 8–dependent apoptotic signals by binding to the TNF receptor on the surface of

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hepatocytes, after which caspase 8 triggers the activation of caspase 3, a downstream cysteine proteinase, in multiple apoptosis signal pathways and is critical for the programming of cells for apoptosis [12].

Wheat is an important source of vitamins, minerals, dietary fibers and phytochemicals. The oil is a rich source of tocopherols, B complex vitamins. These components may have significant implications in chemoprevention [13]. Recent research confirmed that wheat germ oil can help reduce oxidative stress due to its favourable fatty acid pattern [14,15]. Wheat germ appears thus very effective to improve antioxidant defence status, especially in tissues, irrespective of modifications of lipids status [16].

It was hypothesized that the administration of wheat germ oil (WGO) as a natural antioxidant rich with vitamin E in the form of á-tocopherol, should attenuate such tissue injury. Therefore, the aim of this study was to elucidate the benefits of WGO and to clarify its capability of minimizing the histopathological and immunological changes in experimental endotoxemic rats.

MATERIALS AND METHODS

Reagents: Lipopolysaccharide (LPS) (*E. coli*, 055:B5) was purchased from Sigma (St. Louis, MO, USA). LPS was dissolved in normal saline. The extract of wheat germ oil was purchased from Cap Pharm for Extracting Natural Oils & Herbs, Cairo, Egypt. Each 100 ml of wheat germ oil contains 89.393 mg of vitamin E. Colorimetric kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were purchased from Biolabo SA, France. Elisa colorimetric kits for TNF-α and IL-10 were purchased from R&D System, USA.

Experimental Animals and Induction of Acute Endotoxin: Pathogen-free male albino rats of the *Rattus rattus* strain (200±10 g) were obtained from the Serum & Antigen Laboratories at Helwan, Egypt. They were housed in a controlled environmental room, with a light-controlled (12L:12D) cycle. Animals were randomly divided into four groups (10 rats/group): *Control* (injected i.p. with normal saline); *wheat germ oil* (receiving 12 ml per 200g rat to be equivalent to the dose of vitamin E [17]); this dose divided into 2 small doses (6 ml each) through the day, *lipopolysaccharide* (10 mg kg^{-1} i.p.) according to Yerer *et al.* [18]. While, rats belonging to the fourth group received both WGO every 24 h for 7 consecutive days then injected with a single dose of LPS. Groups were studied after 24 h of LPS injection.

In survival rate experiments, extra 45 rats were divided into 3 groups each consisting of 15 rats. The animals in the 1st control group received i.p. injection of saline, animals of the 2nd group received LPS (10 mg/kg, i.p.), while those of the 3rd one were given WGO (12 ml/rat, i.p.), then injected with LPS, 24 hr later. The behaviour and survival of the animals was monitored over 24 hr after LPS injection.

Histological and Ultrastructure Examinations: Liver specimens were collected from all dissected animals; immersed in saline then put in 10% neutral buffered formalin for histopathological studies. For electron microscopic studies, fresh small pieces of liver were fixed in 3% glutaraldhyde in 0.1M sodium cacodylate buffer (pH 7.4) at room temperature for 4h. Subsequently, the liver specimens were osmicated in 1% osmic acid. After dehydration with series of ethanol and propylene oxide the samples were embedded in an Araldite-dodecenyl succinic anhydride mixture. Ultrathin sections were obtained with diamond knife, after which they were double stained with uranyl acetate and lead citrate. Samples were finally examined with JOEL 1200 EXII microscope at the Central Lab., Faculty of Science, Ain Shams University.

Biochemical Investigation: Blood was collected by heart puncture when sacrificed. Sera were collected and stored at 20°C for spectrophotometrically measurement of serum markers of liver injury. Activities of AST, ALT and ALP were determined according to Reitman and Frankel [19] and Bowers and McComb [20]. The values were expressed as units/liter (U/l).

Cytokines Activity: Cytokine activities of TNF-α and IL-10 levels in serum were measured via a highly sensitive commercially available rat DuoSet ELISA kit assay from R&D Systems (Minneapolis, MN, USA). Briefly, 96-well microplates were coated with TNF-α and IL-10 antibodies and incubated overnight at room temperature. The plates were washed with PBS containing 0.05% Tween 20 and then blocked by PBS with 1% bovine serum albumin and 5% sucrose. After the addition of diluted samples and standard TNF-α and IL-10 dilutions, plates were incubated for 2 h at room temperature. Biotinylated goat anti-rat TNF-α was used as the detection antibody and streptavidin-HRP was added as the conjugate to each well. Equal proportions of hydrogen peroxide and tetramethylbenzidine were used as the substrate solution and the reaction was stopped by adding 2N sulfuric acid. All samples and standards were run in duplicate and

optical density was determined with a microplate reader at a wavelength of 450 nm. The values of plasma cytokines concentration were expressed as pg/ml.

Flow Cytometry Studies: Expression of (CD14) was determined by flow cytometric analysis in blood samples. Blood samples were collected in EDTA tubes. 50 µl of monoclonal antibody (CD14) added to each test tube and 50 µl of the appropriate isotypic control to each control tube. Add 50 µl of the test sample to all tubes, then vortex gently. Incubate for 15 to 20 min. at (18-25°C), protected from light. Then perform lysis of the red blood cells by following the recommendations of the lysis reagents used. Vortex immediately for one second and incubate for 10 min. at (18-25°C), protected from light. Centrifuge for 5 min. at (18-25°C). Remove the supernatant by aspiration. Resuspend the cell pellet using 3 mL of PBS, then centrifuge for 5 min. Remove the supernatant by aspiration and resuspend the cell pellet using PBS. The complete blood count was performed within 2 hours after the blood was drawn. After this the cells were analyzed by flow cytometry (Coulter EPICS XL), Faculty of Medicine, Ain Shams University and the percentage of CD14 positive cells were taken as the indexes.

Statistical Analysis: To identify the significant differences among groups, the data analysed by analysis of variance (ANOVA). Once a significant F test was obtained, LSD comparisons were performed to assess the significance of the difference among various treated groups, with the significance level of $P<0.05$. Statistical processor system support "SPSS" for Windows software, release 10 (SPSS, Chicago, IL) was used.

RESULTS

All animals were alive after 24 h of administration of saline and WGO. However, following the administration of LPS (10 mg/kg) the survival rate of the animals was reduced to 40% (6 out of 15, $P<0.01$ vs corresponding control group) after 24 h. The animals showed signs of sepsis such as diarrhoea and apathy. The pre-treatment of WGO before LPS injection exhibited a significant prolongation in survival rate reached 73.33% (11/15) as reported in Table 1.

Liver sections from control rats illustrated preserved architecture with hexagonal lobules consisting of central veins and polygonal hepatocytes spread out with their rounded nuclei (Fig. 1). Sections from liver tissue of rats treated with WGO showed no histopathological changes when compared with control animals (Fig. 2). Within 24 h, rats treated with LPS revealed abnormal histological alterations viz: degenerated hepatocytes with pleomorphic nuclei, dilated sinusoids and many Kupffer cells; some of which appeared with pyknotic nuclei (Fig. 3). Mononuclear cell infiltrate extending through hepatic tissue, Kupffer cell appeared engulfing debris (Fig. 4), with disarrangement of the hepatic cords, lipid vacuoles of different sizes and distinct Kupffer cells (Fig. 5). Figure (6) showed dilated portal vein with blood congestion and hypertrophied muscle layer, hepatocytes lost their cellular outlines and billary epithelium hyperplasia. Liver sections from WGO pre-treatment to endotoxemic-rats, delineated an improved histological picture that were visualized in the well organization of the hepatic cords, regeneration in the hepatocytes and the appearance of many binucleated liver cells. Hepatocytes appeared with prominent nuclei, preserved cytoplam (Fig. 7).

The ultrastructural examination of normal control rat liver showed normal hepatocytes with oval nucleus prominent nucleoli; containing mitochondria, rough endoplasmic reticulum and glycogen particles scattered in the hepatocytes (Fig. 8). After the injection of LPS, liver sections revealed many histopathological disturbances such as hepatocyte with pyknotic nuclei, fragmented endoplasmic reticulum and mitochondria lost their cristae (Fig. 9). Hepatocyte appeared with pyknotic nucleus, lipid droplets of different sizes and disappearance of most of the cell organelles (Fig. 10) and Kupffer cell showed with pyknotic nucleus, disappearance of its cellular organelles and containing degenerated mitochondria (Fig. 11). The hepatocytes regained their normal appearance in the group of rats treated with WGO before LPS. Electron micrograph from a rat treated with WGO and LPS showing well defined rounded nucleus with prominent nucleolus, mitochondria, rough endoplasmic reticulum and glycogen particles (Fig. 12).

Table 1: Survival rates of the different experimental groups

Experimental groups Duration (24 h)	Number of rats	% of survival
C	15/15	100
WGO	15/15	100
LPS	6/15	40
WGO+LPS	11/15	73.33

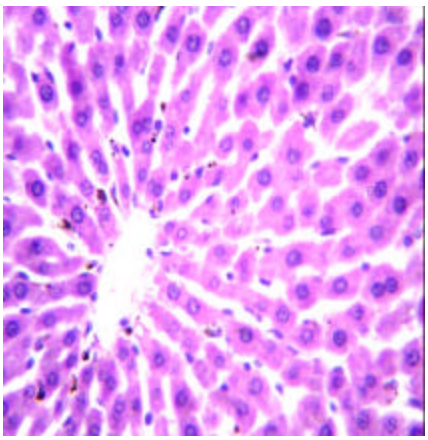

Fig. 1: Photomicrograph of the control presented normal liver sections. A preserved architecture with hexagonal lobules consisting of central veins (CV) and polygonal hepatocytes spread out with their rounded nuclei. The sinusoids (S) lined with endothelial cells were also present (H-E, X400)

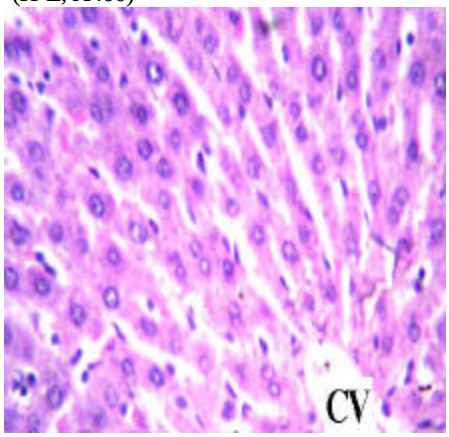

Fig. 2: Photomicrograph of liver section from a rat treated with WGO showing normal histological profile of the hepatic tissue (H-E, X400)

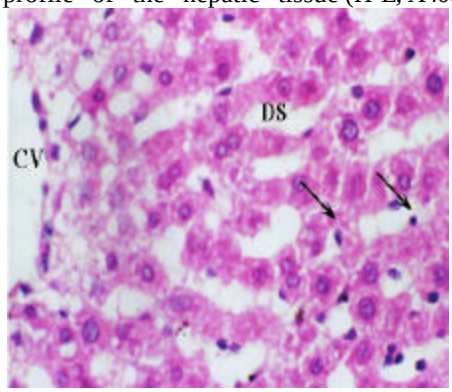

Fig. 3: Photomicrograph of liver section from a rat injected with LPS showing degenerated hepatocytes with pleomorphic nuclei, dilated sinusoids (DS) and many Kupffer cells; some of which appeared with pyknotic nuclei (arrow) (HE, X400)

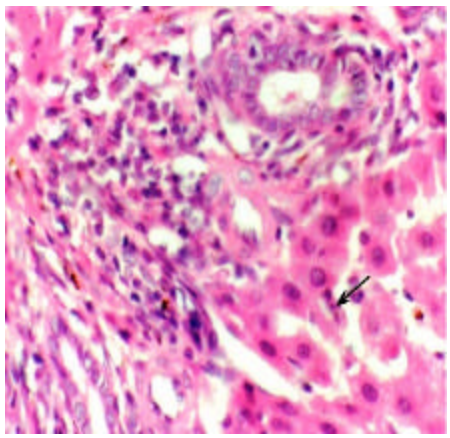

Fig. 4: Photomicrograph of liver section from LPS injected rat showing mononuclear cell infiltrate extending through hepatic tissue, Kupffer cell appeared engulfing debris (arrow) and hyperplasia of bile duct (H-E, X400).

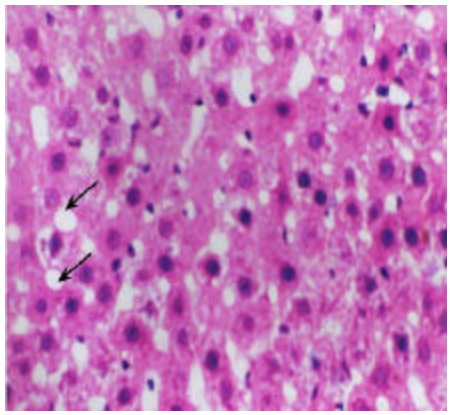

Fig. 5: Photomicrograph of liver section from a rat treated with LPS showing disarrangement of the hepatic cords, lipid vacuoles of different sizes (arrows) and distinct Kupffer cells (H-E, X400).

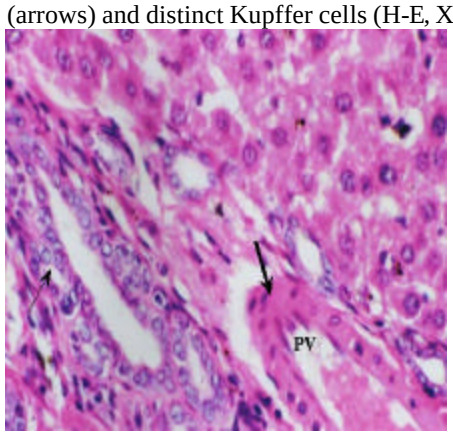

Fig. 6: Photomicrograph of liver section from LPS injected rat showing dilated portal vein (PV) with blood congestion and hypertrophied muscle layer (thick arrow), hepatocytes lost their cellular outlines and hyperplasia (thin arrow) of the bile duct (H-E, X400).

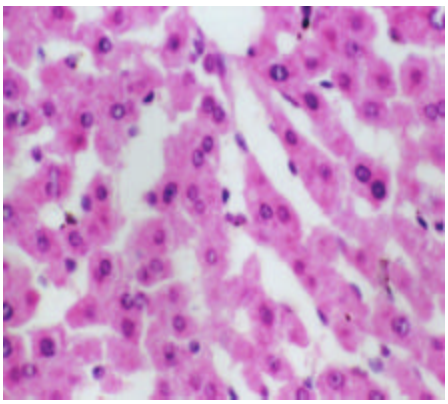

Fig. 7: Photomicrograph of liver section from a rat treated with WGO and LPS showing near to normal arrangement of the hepatic cords, regeneration in the hepatocytes and many binucleated liver cells (arrows) (H-E, X400).

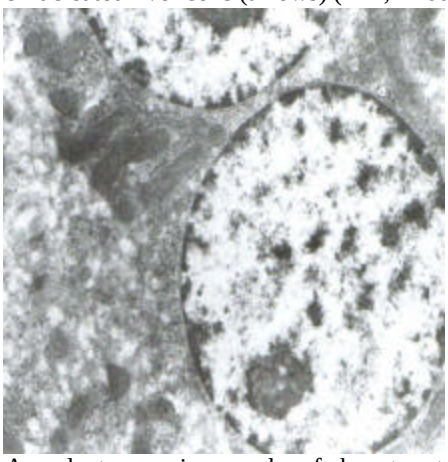

Fig. 8: An electron micrograph of hepatocyte from control rat showing oval nucleus (N), mitochondria (arrows) and glycogen particles (G) (X7500).

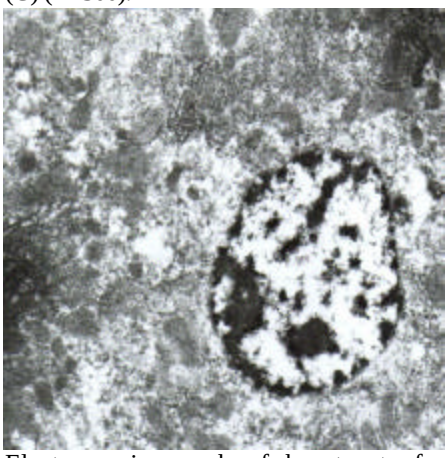

Fig. 9: Electron micrograph of hepatocyte from LPS injected rat showing pyknotic nuclei (PN), fragmented endoplasmic reticulum (thick arrows) and mitochondria lost their cristae (thin arrow); (X 6000).

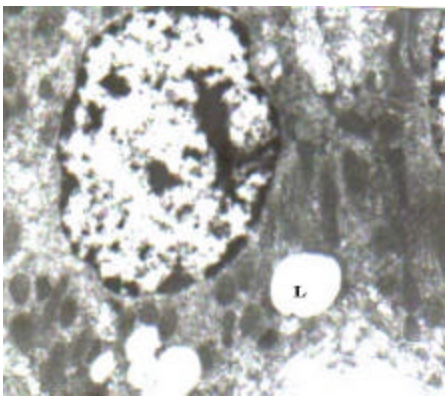

Fig. 10: Electron micrograph from a rat treated with LPS showing a hepatocyte with pyknotic nucleus, lipid droplets (L) of different sizes and disappearance of most of the cell organelles (X7500).

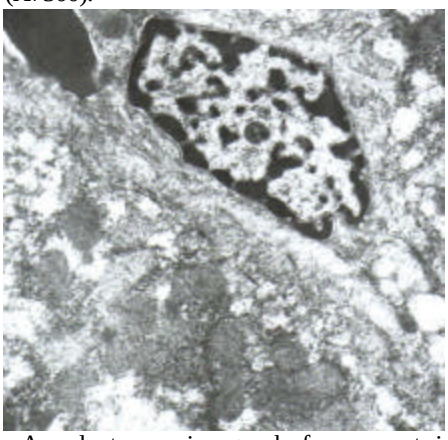

Fig. 11: An electron micrograph from a rat injected with LPS showing Kupffer cell appeared with pyknotic nucleus, disappearance of its cellular organelles and containing degenerated mitochondria (arrow) (X7500)

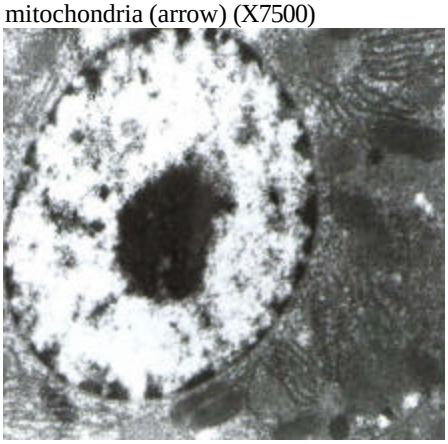

Fig. 12: Electron micrograph from a rat treated with WGO and LPS showing well defined rounded nucleus with prominent nucleolus, mitochondria (thin arrow), rough endoplasmic reticulum (thick arrows) and glycogen (G) particles (X1000).

Table 2: Liver Function Tests in Control and Experimental Groups (U/l)

Groups (n=10)	C	WGO	LPS	WGO+LPS
AST	62.3±1.30	61.7±1.35	333.0±1.65	161.6±1.380
ALT	46.8±0.89	41.5±1.40	143.4±1.24	51.5±0.710
ALP	52.7±0.94	50.2±1.34	197.5±2.50	66.0±1.630

Values are mean±SE. Superscribt letters denote the significant difference at (P<0.05)

a: values are significantly different from control group

b: values are significantly different from WGO group

c: values are significantly different from LPS group

Table 3: Serum Levels of TNF-α and IL-10 in Control and Experimental Groups (pg/ml)

Groups (n=10)	C	WGO	LPS	WGO+LPS
TNF-α	144.1±1.47	138.8±1.48	688.5±1.88	303.5±1.71
IL-10	37.9±0.94	33.8±1.30	435.3±1.42	205.0±1.76

Values are mean±SE. Superscribt letters denote the significant difference at (P<0.05)

a: values are significantly different from control group

b: values are significantly different from WGO group

c: values are significantly different from LPS group

Table 4: Percentage of CD14 Positive Cells in Blood Samples of Control and Experimental Groups.

Groups (n=10)	C	WGO	LPS	WGO+LPS
Mean±SE	3.8±0.13	4.0±0.11	39.02±0.21^{ab}	10.78±0.15^{abc}

Values are mean±SE. Superscribt letters denote the significant difference at (P<0.05)

a: values are significantly different from control group

b: values are significantly different from WGO group

c: values are significantly different from LPS group

Biochemical data for all the studied parameters showed non-significant differences between the control group and WGO treated group. Consistent with histological findings, the activity of the injury marker AST increased significantly (333 U/l) after endotoxic-induced rats. The liver-specific marker ALT and ALP were increased and reached 143.4 and 197.5 U/l respectively, as compared with normal control. It could be concluded from this results, that injection with LPS abolished the liver injury and dysfunction. On the other hand, pre-njection of WGO diminishes endotoxin shock by preventing the augmentation of AST, ALT and APL as reported in Table 2.

Detection of the cytokine TNF-α T and IL-10 by ELISA, revealed that LPS exposure elevated their concentrations relative to control group within 24 h after its injection; reached 688.5 pg/ml and 435.3 pg/ml respectively as recorded in Table 3. WGO pre-treatment counteracted LPS effect on cytokine production where these levels recorded 303.5 and 205 pg/ml for TNF-á and IL-10 respectively.

To confirm the role of CD14 expression in endotoxemia, the binding of FITC to the cells was delineated. The percentage of CD14 positive cells were 3.8% in rats of normal group. But in rats with endotoxemia, CD14 positive cells was 39.02% 24 h after stimulation of LPS, which were significant different when compared with normal group of animals (P<0.05) (Table 4). On the other hand, Wheat germ oil pre-treatment improving the levels of CD14 expression reached 10.78 %.

DISCUSSION

Survival experiment reported that 40% of animals were survived within 24 h after LPS injection, indicating that the mechanisms leading to death operated soon after LPS administration in endotoxic shock model. This was in harmony with data obtained by Bernard *et al.* [21] which showed that 80% of male SHR rats and 20% of male Wistar rats were survived at 10 mg/kg of LPS injected intravenously at 24 h. Also rats injected with LPS showed signs of sepsis such as laziness, piloerection, diarrhea, polypnea and conjunctivitis [21]. WGO administered 24 h before LPS injection significantly protected against endotoxin induced lethality. The survival rate increased up to 73.33% as compared with LPS injected rats and this is in consistence with the study of Altavilla and coworkers [22] which reported that the administration of

IRFI 042, a novel dual vitamin E-like antioxidant, improved the survival rates in endotoxemic rats. Again, the pretreated male BALB/c mice with extracted green tea prior to an intraperitoneal injection of 40 mg LPS. At 24 h, there were no deaths in the polyphenol-treated groups compared to 60% lethality in the control group receiving LPS alone as reported by Yang *et al.* [23].

The current study demonstrated that within 24 of LPS injection, several histopathological disturbances occurred in the hepatic tissues. These findings were in accordance with many authors who reported that fat accumulation was found consistently in the cytoplasm of hepatocytes of rats during LPS injection and caused increased in triglyceride concentration [1,24,25]. On the other hand, Kok *et al.* [26] and Feingold *et al.* [27] delineated that the administration of LPS at a high dose, mimicking infections, produces hypertriglyceridemia by decreasing the activity of lipoprotein lipase, thus slowing the clearance of triglyceride-rich lipoproteins.

Sepsis is a serious medical condition that is characterized by a whole-body inflammatory state [28]. Bacterial endotoxin/lipopolysaccharide elicits inflammatory responses and also elevates circulating LPS that shown to induce the cascade of mediators leading to septic shock and their overproduction which is associated with elevated body temperature, hypotension, tachycardia, tachypnea, leukopenia, end-organ dysfunction and death [29].

Septic shock is more often caused by bacterial endotoxoin LPS and usually occurs in immuno-compromised patients and those with chronic diseases. Endotoxins are not primarily cytotoxic but exert their effects by "abusing" physiological target cell functions to elicit an inappropriate host response. Examples of these are the excessive release of cytokines, platelet activating factor, leukotriens, nitric oxide (NO) and reactive oxygen species "ROS" or the abundant expression of adhesion molecules by endothelial cells under attack by endotoxins. This abuse of host cell functions may contribute greatly to the pathogenesis of sepsis and septic shock [7].

The present study delineated an increase in liver function tests as a result of LPS injection. These data are in accordance with many investigations [30,31] which reported that serum AST, ALT and ALP activities increased after LPS administration [1,32].

LPS induced extensive immune responses that can lead to fatal septic shock syndrome and it is recognized by both innate and adaptive immune systems. LPS is composed of O-antigen repeats, the core region and lipid A. The O-antigen structures vary by strain and are recognized by the adaptive immune system, resulting in the production of specific antibodies. The target structure for the innate immune system is lipid A, which has conserved structures Raetz [33]. TNF is an important proinflammatory cytokine involved in normal physiological immune and inflammatory processes. Hewett *et al.* [34] and Yee *et al.* [35] showed that there is a causative role for TNF-α not only in promoting liver injury has been identified for inflammation interaction with a number of xenobiotic agents, but also because TNF-α can take part in extrahepatic tissue alterations after LPS. TNF induces the expression of several cytokines including IL-1ß, IL-6, IL-8, MIP-2, as well as other gene products capable of damaging the liver directly or in concert with TNF-α as reported in some studieds [36,37]. Ji *et al.* [38] also reported that TNF-α and IL-1ß activate polymorphonuclear leukocytes, induce their own production, stimulate production of other inflammatory mediators and antagonize anti-inflammatory cytokines such as IL-10.

Several mediators are secreted by macrophages upon LPS stimulation [39,40]. Some of them are cytokines that contribute to multiple organ failure, notably, TNF-α, which is an initiator of the coagulation cascade in sepsis; other inflammatory mediators can elevate levels of plasminogen activator inhibitor, thus suppressing the fibrinolytic system [41].

The flow cytometric investigation reported an increase in CD14 expression as a result of LPS injection. Results obtained by Suzuki and his colleagues [30] indicated that i.p. injection of LPS at the high dose of 10 mg/kg into rats induced a condition compatible with septic multiple organ dysfunction syndrome. LPS specifically binds the macrophage cell surface receptor, CD14, which subsequently interacts with the Toll-like receptor 4 (TLR4) as showed by Beutler *et al.* [42]. TLR4 next recruits the Toll-adaptor protein, myeloid differentiation factor 88 (MyD88), which activate NF-κB and thereby induce the upregulation of proinflammatory cytokines in a study designated by Weighardt *et al.* [43]. Again, Conte *et al.* [44] showed that that the activation by LPS of a macrophage results in enhanced phagocytosis of bacteria and the release of cytokines, prompting other macrophages, phagocytes and T cells to the site of infection. This initiates a proinflammatory response and thereby influences the nature of the adaptive immune response. Macrophages are now well recognized to be the primary mediators for the lethal effects caused by LPS-induced septic shock [45].

Antioxidants are able to reduce the effect of free radicals formed in the body either due to exposure to environmental pollutants or because the bodies own defence mechanisms are reduced in dealing with natural production of these compounds. Wheat germ is considered as a free radical scavenger, has been reported to be protective in various models of oxidative stress, both through its free radical scavenging effect as well as by directly increasing antioxidant activity [13]. While, another study designated by Kirimlioglua *et al.* [46] reported that when rats treated with vitamin E, glutathione levels were increased and content of malondialdehyde, nitric oxide and superoxide dismutase were decreased as compared with control levels. Although WGO has been proposed to be useful for the prevention of oxidative stress during liver injury, to our knowledge, there has been no available report studying the effects of WGO therapy on serum concentrations of CD14, IL-10 and TNF-α in the endotoxemic-hepatotoxic rats.

Pre-trearment of WGO to endotoxemic rats revealed an improvement in the histological profile. The hepatic tissue revealed decrease in lipid droplets in endotoxemic rats treated with WGO this may be due to decreased hepatic lipogenesis and triglyceride-VLDL secretion by the liver [26,47]. The average number of colon tumors per individual animal has been significantly decreased upon the effect of the administration of WGO as mentioned by Zalatnai *et al.* [14]. This may explained the protective effect of WGO against endotoexemic effect of LPS.

The current study reported decreasing in the biochemical hepatic functions, inhibition in the cytokines assay and in the expression of CD14 levels. In this concern, Altavilla *et al.* [22] showed that the treatment with IRFI 042 (a novel dual vitamin E-like antioxidant) decreased serum TNF-α and hepatic liver mRNA for TNF-α and reversed the endotoxic shock. Again, in patients with hypercholesterolemia, WGO supplementation was associated with parallel reduction of oxidative stress and CD40L expression suggesting that n-3 fatty acids downregulated CD40L via an oxidative stress-mediated mechanism [15].

It could be concluded in the light of the abovementioned data that, WGO pre-treatment delineated decreasing in serum levels of AST, ALT and ALP in endotoxemic animals along with suppression of TNF-α, IL-10 levels in addition to the inhibition of CD14 expression thus support a hepatoprotective role of WGO in septic conditions. Since WGO was shown to be a putative biological antioxidant by its radical scavenging activity and immunomodulatory action which stimulating functions of cytokines, so it was of interest to introduce WGO as a food additive.

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