Middle East Journal of Applied Sciences
ISSN 2077-4613

Volume : 06 | Issue :03 | July-Sept.| 2016
Pages: 460-467

Immunomodulatory and Anti- oxidant Effects of Alpha-Lipoic Acid and Vitamin E on Lipopolysaccharide-induced Liver Injury in Rats

El-Feki, M. A., Amin, H. M., Abdalla, A. A. and Fesal, M.

Department of Zoology, Faculty of Science, El-Minia University, El- Minia, Egypt.

Received: 01 June 2016 / Accepted: 02 July 2016 / Publication date: 30 July 2016

ABSTRACT

Lipopolysaccharide (LPS) is a key inflammatory component of Gram-negative bacteria, which after entering the systemic circulation contributes to the development of septic hepatic failure. The present study aimed to investigate the protective effects of vitamin E and Alpha lipoic acid (ALA) on hepatotoxicity induced by LPS. Male albino rats were divided into four groups each of ten rats. Group I, animals were injected intraperitoneally (i.p) with saline solution (0.9%NaCl), 1ml for each rat. Group II, animals were injected (i.p) with a single dose of LPS (1mg/kg b. w). Group III, animals pre-treated orally with (60 mg ALA /kg b. w) daily for 4 weeks, then intoxicated with LPS (1mg/kg b. w). Group IV, animals were treated orally with (40 mg Vitamin E /kg b. w) twice a week for 4 weeks, then, intoxicated with LPS (1mg/kg b. w). Serum and liver samples were collected at 2 and 6 hours post injection of LPS. The results indicated that hepatotoxicity was induced by administering LPS, by a significant ($p<0.001$) increase in blood serum ALT, AST and ALP. Liver lipid peroxidation (MDA) was significantly ($p<0.001$) elevated in LPS challenged rats, compared with those obtained in the control group. The levels of pro and anti-inflammatory cytokines (IL-6 & TNF-α and IL-10) were significantly ($p<0.001$) increased in LPS group. On the other hand, LPS caused a significant ($p<0.001$) decrease in liver antioxidant enzymes (reduced glutathione & catalase). Pre-treatment with ALA and vitamin E ameliorated the all previous studied parameters compared with LPS group.

Key words: Lipopolysaccharide, Alpha-lipoic acid, Vitamin E, Oxidative stress, Liver enzymes, Cytokines.

Introduction

Administration of lipopolysaccharide (LPS) to animals is widely used to study responses to in vivo-induced acute systemic inflammation (Yongke *et al.,* 2005). LPS is one component of gram-negative bacterial cell wall, which is released by destruction of cell wall and acts as a potent bacterial product used in the induction of host inflammatory responses and tissue injury (Kheir-Eldin *et al.,* 2001). Massive release of endotoxin or LPS into systemic circulation after bacterial killing results in severe or acute hepatotoxicity (Pilkhwal *et al.,* 2010).LPS directly causes liver injury by certain mechanisms involving activation of inflammatory cells such as Kupffer cells. Most of the toxicities observed in LPS-induced injury has been attributed to toxic mediators produced by activated macrophages, including cytokines, such as tumor necrosis factor-α (TNF-α), interleukins (IL-1, IL-6, IL-8, and IL-12), other pro-inflammatory molecules, including platelet-activating factor, prostaglandins, as well as reactive oxygen and nitrogen species (RONS), such as nitric oxide (Wang *et al.,* 2005). Several studies have shown that endotoxins result in a pro-oxidant state (Giriwono *et al.,* 2011) and cause tissue damage by inducing apoptotic and necrotic events in liver (Aoshiba *et al.,* 2009). Moreover, LPS induces elevation in lipid peroxidation which is an index of oxidative stress that depends on both time and dose (James *et al.,* 2002). Detoxification of endotoxin is mainly mediated by the reticuloendothelial system, particularly Kupffer cells of the liver (Roberts *et al.,* 2007).

The defense mechanism against Reactive oxygen species (ROS), mediators of liver damage, includes enzymatic antioxidants, such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), and glutathione S-transferase (GST), and non-enzymatic compounds, such as glutathione (GSH), vitamins A, E, C and Lipoic acid (Crimi *et al.,* 2006).

Therefore, antioxidants are vital substances which possess the ability to protect the body from damage caused by free radicals which induce oxidative stress (Ozsoy *et al.,* 2008). Vitamins are ideal antioxidants by increasing tissue protection from oxidative stress due to their easy, effective and safe dietary administration in large range of concentrations (Kanter *et al.,* 2005). One of the most important vitamins for the body is vitamin E which is a liposoluble molecule and therefore, after dietary intake, vitamin E is not only absorbed easily from the intestinal lumen but is also dispersed between lipids and proteins in cell membranes. Vitamin E molecules can also interrupt free radical chain reactions by capturing the free radicals (Kaya, 2009). Vitamin E has been reported to be beneficial in preventing formaldehyde-induced tissue damage in rats (Gurel *et al.,* 2005). In

Corresponding Author: Mostafa Fesal, Department of Zoology, Faculty of Science, El-Minia University, El- Minia, Egypt.
E-mail: mostafabiology@yahoo.com

addition to its anti-oxidative properties, treatment with vitamin E, incorporated in liposomes, has been shown to be beneficial in down-modulating airway inflammation induced by inhaled endotoxin and thereby providing significant protection against lung injury (Rocksén *et al.,* 2003).

Alpha-lipoic acid (ALA) is a naturally occurring dithiol compound synthesized from octanoic acid in the mitochondrion and acts as a coenzyme for the mitochondrial respiratory enzymes (Shay *et al.,* 2009). ALA in many tissues is rapidly converted to its redox couple, dihydrolipoic acid (DHLA). ALA and DHLA antioxidant properties are reflected by their direct ability to quench free radicals and indirect ability to recirculate cellular antioxidants (Aly *et al.,* 2009). ALA lipoic acid has a wide range of benefits; the most important one is its role as an antioxidant which equals to that of coenzyme Q 10, vitamin C and vitamin E (Bast and Haenen, 1998). In addition, ALA is both water and fat soluble, which makes it highly effective in reducing free radicals such as lipid peroxides in cellular membranes (Goraca *et al.,* 2009). Many studies have reported the effectiveness of ALA treatment in the prevention of pathologic conditions mediated by oxidative stress (Castro *et al.,* 2013). ALA also reduces apoptosis in liver cells by its anti- oxidative potential (Kaya-Dagistanli *et al.,* 2013). Moreover, ALA and DHLA derivatives exhibit anti-inflammatory effects (Kwiecień *et al.,* 2013).

The present study aimed to investigate and compare the ameliorative effects of both vitamin E and alpha lipoic acid on cytokines production and oxidative stress induced by lipopolysaccharides in experimental rats.

Materials and Methods

Animals

Fortymale albino rats (weighing 120-140 g) were obtained from the Experimental Animal Care Center, Faculty of Agriculture, Minia University, Minia, Egypt. All animals were weighed, housed in isolated polypropylene cages and fed on commercial rodent pellets and water under good hygienic laboratory conditions. Animals were maintained under standard conditions of temperature 24+1°C and 55+5% relative humidity with regular 12 hrs. light: 12 hrs. dark cycles. They were maintained for two weeks as an acclimation period before the beginning of the experiment.

Chemicals:

Lipopolysaccharides were extracted from E.coli serotype O127:B8, purchased from Sigma–Aldrich chemical St. Louis, MO, USA. Vitamin E is a lipid soluble vitamin that is known as α-tocopherol, purchased from oxford laboratory, Mumbai, India. Alpha lipoic acid was supplemented as thioctic acid, purchased from EVA Pharm Co., Egypt. TNF-α, IL-6 and IL-10 ELISA kits were purchased from Boster's rat kits, CA, USA. Liver MDA, GSH and catalase kits were purchased from Bio-diagnostic, Cairo, Egypt. ALT, AST, and ALP kits were purchased from Bio-Systems chemical company, Egypt. Other kits were purchased from Spectrum chemical company, Cairo, Egypt.

Experimental Design:

Animals were divided into four groups, each of ten rats. Control group, animals in this group were left for four weeks to start at the same date as treated groups, then, administered intraperitoneally (i.p) with saline (0.9%). Lipopolysaccharide (LPS) group, animals in this group were left for four weeks, then, were injected intraperitoneally with a single dose of LPS (1mg/kg body weight) (Wafaa *et al*., 2006). Alpha lipoic acid& LPS group (ALA& LPS), animals were pre-treated orally with (60 mg ALA /kg body weight) daily for four weeks, then, intoxicated with LPS (1mg/kg body weight) (Wafaa *et al*., 2006). Vitamin E & LPS group (Vit E + LPS), animals were treated orally with (40 mg Vit E /kg body weight) twice a week for four weeks (Karima, 2007), then, intoxicated with LPS (1mg/kg body weight).

All animals were sacrificed at two and six hours (five rats per each hour), after LPS injection in all treated groups. Blood samples from each rat were collected individually, from jugular vein, left to coagulate, then, centrifuged at 3000 rpm for 10 minutes to obtain serum for performing both immunological and biochemical investigations.

Markers of liver damage:

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to Gella *et al*. (1985) ;alkaline phosphatase (ALP) according to Glick *et al.* (1986).

Oxidative Stress Measurements:

Livers were removed aseptically from all the groups and were rinsed in isotonic saline solution and weighed. A 25% (w/v) tissue homogenate for each case was prepared in (0.05 M) phosphate buffer saline (pH 7.4) using a Potter Elvehjen homogenizer. An aliquot of the liver homogenate was used for the estimation of lipid peroxidation, reduced glutathione levels and catalase activity. Liver lipid peroxidation level (MDA) was measured using the method of Ohkawa *et al*. (1979), liver reduced glutathione level (GSH) was measured by using the method of Beutier *et al.,* (1963) and Liver catalase activity by using the method of Aebi (1984).

Immunological Studies:

Serum samples of all groups were used for the determination of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10) release by Enzyme-Linked Immune Sorbent Assay (ELISA) using Rat TNF- alpha PicoKine ™ ELISA Kit (EK0526), Rat IL-6 PicoKine ™ ELISA Kit (EK0412), and Rat IL-10 PicoKine ™ ELISA Kit(EK0418) from Boster Biological Technology Co., Ltd

Statistical analysis:

The collected data were coded, tabulated, and statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 20. Descriptive statistics were done for numerical data by mean and standard deviation. Analysis were done for parametric quantitative variables using one way ANOVA test and post Hoc Turkey's correction between groups. The level of significance was taken at (P value ≤ 0.05).

Results

Liver function tests:

LPS administration caused a significant marked rise (p<0. 001) in serum levels of ALT, AST and ALP chiefly at 6h when compared with saline control group. The activities of liver enzymes were decreased significantly (p<0. 001) on supplementation with ALA, Vit E before LPS injection (Table 1).

Parameter / Groups	ALT		AST		ALP	
	2h	6h	2h	6h	2h	6h
Saline	36.8 ± 1.92 ***	35.8 ± 1.78 ***	133.4 ± 2.41 *	134.4 ± 1.34 ***	187.6 ± 2.96 ***	188 ± 2.73 ***
LPS	45.6 ± 3.28	112 ± 7.58	141.2 ± 4.6	307.2 ± 5.06	204.8 ± 6.83	285.8 ± 6.05
ALA& LPS	41.6 ± 2.07	68.8 ± 3.03 ***	131.2 ± 2.38 **	220.6 ± 8.98 ***	193.8 ± 6.68 *	250 ± 1.87 ***
Vit E& LPS	43 ± 2.91	64.6 ± 5.89 ***	139.8 ± 5.06	219.8 ± 7.59 ***	198.3 ± 4.49	256.4 ± 3.51 ***

***P <0. 001 VS LPS & ** p <0. 01 VS LPS & * p <0. 05 VS LPS

(Table 1 Effect of LPS, Alpha lipoic acid & LPS and Vitamin E & LPS on serumalanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALP).

Effects on antioxidant activities:

LPS infusion caused a marked increase in lipid peroxidation when compared to control (P < 0.001).Treatment with ALA and Vit E resulted in a significant attenuation in MDA level when compared to LPS group. GSH levels and catalase activities were significantly decreased due to LPS injection when compared to control. GSH and CAT values were increased significantly in pretreated rats with ALA and Vit E when compared to LPS (Table 2).

Parameter / Groups	MDA		GSH		Cat	
	2h	6h	2h	6h	2h	6h
Saline	4.79 ± 0.36 **	4.75 ± 0.32 ***	11.45 ± 0.79 ***	11.52 ± 0.82 ***	1.807 ± 0.078 ***	1.829 ± 0.069 ***
LPS	5.81 ± 0.48	8.85 ± 0.66	8.28 ± 0.47	7.99 ± 0.05	1.118 ± 0.258	0.454 ± 0.087
ALA& LPS	5.33 ± 0.23	6.24 ± 0.43 ***	10.06 ± 0.26 ***	11.4 ± 0.41 ***	1.316 ± 0.107	1.465 ± 0.249 ***
Vit E& LPS	5.5 ± 0.38	6.94 ± 0.36 ***	9.1 ± 0.54	11.44 ± 0.56 ***	0.887 ± 0.091	1.428 ± 0.046 ***

***P <0. 001 VS LPS & ** p <0. 01 VS LPS & * p <0. 01 VS LPS

(Table 2 Effect of LPS, Alpha lipoic acid & LPS and Vitamin E & LPS on oxidative stress parameter in liver homogenates (Lipid peroxidation (MDA), reducedglutathione (GSH) and Catalase activity).

Serum pro and anti- inflammatory cytokines:

Rats challenged with LPS revealed a highly significant increase in serum TNF-αand IL-6 (pro-inflammatory cytokines) ($p<0.001$) when compared with saline control group. Pretreatment of rats with ALA and Vit E for 4weeks before LPS injection significantly reduced this increase ($p<0.001$) when compared with LPS group. IL-10 (anti-inflammatory cytokine) also increased in LPS group compared with the control group and it was also increased significantly in groups pretreated with ALA and Vit E (Table 3).

Parameter / Groups	TNF-α		IL-6		IL-10	
	2h	6h	2h	6h	2h	6h
Saline	63.6 ± 1.14 ***	64.8 ± 0.83	36.2 ± 1.92 ***	34.4 ± 1.81 ***	21.8 ± 1.3 ***	22.6 ± 1.14 ***
LPS	870 ± 27.38	62.4 ±2.51	438 ± 16.43	106 ± 6.36	297.4 ± 5.89	90.8 ± 4.38
ALA& LPS	380 ± 23.45 ***	63.4 ±1.94	256.4 ± 6.02 ***	95.4 ± 5.27 **	500.4 ± 14.74 ***	152.2 ± 3.96 ***
Vit E& LPS	525 ± 29.15 ***	64.6 ± 1.14	346 ± 23.02 ***	44 ± 1.41 ***	387.2 ± 9.01 ***	22 ± 0.71 ***

****P <0. 001 VS LPS & ** p <0. 01 VS LPS & * p <0. 01 VS LPS*

(Table 3 Effect of LPS, Alpha lipoic acid & LPS and Vitamin E & LPS onserum tumor necrosis factor-alpha (TNF-α), serum interleukin-6 (IL-6) and serum interleukin-10 (IL-10).

Discussion

Sepsis is a clinical syndrome that represents the systemic response to an infection and is characterized by systemic inflammation and widespread tissue injury (Matsuda *et al*., 1998). Several studies have demonstrated that LPS treatment causes steatosis, inflammatory reactions and hepatocyte damage in the liver of experimental animals (Depboylu *et al*., 2013). Circulating LPS binds to Toll-like receptor-4 (TLR-4) on hepatic phagocytes and macrophages, leading to their stimulation and subsequently tend to release reactive oxygen species (ROS), reactive nitric species (RNS), as well as, pro-inflammatory cytokines, such as TNF-α and IL-6 (Zhu andLei, 2011). Additionally, LPS induces the migration of activated polymorphonuclear leukocytes (PMNs) into the liver, which constitutes another source of free radicals (Zou *et al*., 2011).

In this study, it was observed that a single injection of LPS resulted in a hepatic injury as indicated by an elevation in the levels of serum ALT, AST, and ALP, all circulating markers of liver injury. These hepatic function marker enzymes are cytoplasmic in nature but are usually leaked into circulation when liver damage occurs due to an alteration in membrane permeability. This observation comes in accordance with Kaur et al. (2006) and Heibashy *et al*. (2013) who have shown that LPS induces hepatic damage and increases the level of serum aminotransferases.

On the other hand, pretreatment with ALA reduced the increase in ALT, AST and ALP levels due to reduction in oxidative stress induced due to LPS administration, by alleviating lipid peroxidation through free radical scavenging and these results come in accordance with Heibashy *et al*. (2013) and Tanaka *et al*. (2015). ALA also protects the integrity of cell membranes by interacting with other antioxidants, namely glutathione and vitamins E and C (Nordberg and Arner, 2001).

Also, Administration of vitamin E before LPS challenge resulted in a significant reduction in the serum aminotransferases levels and these results are in a harmony with the data of Sushma *et al.*(2010) and Hussein *et al.* (2012) Vitamin E also has been reported to confer protection against such changes in formaldehyde and monosodium glutamate induced-hepatotoxicity and oxidative stress in rats (Gulec *et al.,* 2006).

In this study, LPS induced lipid peroxidation which is an index of oxidative stress, and several studies have reported enhanced lipid peroxidation in many tissues (including liver, heart, brain, small intestine and stomach) of rats as a result of LPS administration (Sebai *et al.,* 2009). Under conditions of oxidative stress, reactive oxygen and nitrogen species (RONS) attack the polyunsaturated fatty acids (PUFAs) of cell membranes causing destabilization, disintegration and alteration in membrane fluidity and permeability, all events which increase the rate of protein degradation and eventually leads to cell lysis (Bharrhan *et al.,* 2010). Decomposition products of lipid hydroperoxides such as MDA can interact with protein and nucleic acids, leading to oxidative protein and DNA damage (Pari and Shagirtha, 2010).

Pre administration of ALA and vitamin E for 4 weeks in the LPS-challenged rats significantly decreased the formation of MDA in liver. Similarly, previous studies showed that treatment with ALA significantly

decreased the MDA level, which may be partly due to the ability of ALA to scavenge free radicals (Sena *et al*., 2007). This effect can be explained on the basis that, ALA or its reduced form dihydrolipoic acid(DHLA) can prevent lipid peroxidation and protein damage via interaction with vitamin C and glutathione. It has shown that ALA reduces the increased ROS generation and protein oxidation in the liver as a result of the potent antioxidant capacity of ALA (Tian *et al*., 2012).

In the present study, vitamin E supplementation showed an anti-peroxidative effect in the rat liver tissues by significantly decreasing the LPS-induced rise of liver MDA levels. This effect of vitamin E may be explained by its direct free radical scavenging property, which resulted in preserving cellular integrity (Sakamoto *et al.,* 1990), so it may protect against endotoxin-induced organ damage. The scavenging effect of vitamin E has been reported to attenuate endotoxin-induced oxidative stress in rat brain and sickness behavior in mice.

The impairment of the antioxidant defense system is a critical step in LPS-induced hepatic injury. Evidence has shown that LPS insult is characterized by change in tissue and circulating antioxidant enzymes levels, as well as non-enzymatic antioxidants, including reduced glutathione (GSH) (Kaur *et al*., 2006). GSH is essential for the regulation of a variety of cellular functions, playing an important role in intracellular protection against ROS and other free radicals (Naik *et al*., 2011). The increase in ROS generation in our study was reflected in a decrease in the concentration of catalase and GSH. This may result in hampered dismutation of superoxide anions and inefficient detoxification of H2O2 which results in formation of OH·ions that enhance the peroxidation of membrane lipids, thereby, lead to oxidative damage in many tissues.

The decrease in the content of GSH after LPS administration results in a parallel shift in the redox state of the thiol system to become more oxidized. However, vitamin E supplementation was observed to increase the levels of GSH and catalase activity in the LPS-challenged animals in the present investigation. Improvement of antioxidant status in vitamin E supplemented group is in agreement with Bansal *et al*. (2005). Vitamin E activity has been mainly related to a peroxidase-contained complex antioxidant system defense, such as catalase and glutathione peroxidase, therefore, an increase in these antioxidant enzymes after vitamin E administration was expected (Nordberg and Arner, 2001). Also, in this study we proved that ALA-treated rats demonstrated enhanced activity of catalase. Similarly, Akpinar *et al*. (2009) found that, ALA contributes to antioxidant defense by increasing catalase activity. Moreover, administration of ALA contributed to an increase in the level of GSH, thus improving the hepatic redox status (Akpinar *et al*. (2009). Both ALA and its derivate DHLA may act as extra-and intracellular redox couples and potent free radical scavengers. This may imply that ALA prevents the oxidation of free or protein-bound thiols (Mihai *et al*., 2010). The present results indicate that ALA improves a deficient thiol status by increasing the levels of hepatic GSH (Bertok, (2005).

Results from a previous study have shown that Kupffer cells activation during endotoxemia resulted in the secretion of a wide variety of cytokines, including TNF-α and IL-6. Up-regulation of cytokine production during LPS-induced endotoxemia is a well-known phenomenon, and evidence has shown that increased levels of pro-inflammatory cytokines from neutrophils in the liver were associated with liver cell damage (Hsieh *et al.,* 2008). Tumor necrosis factor-α, and IL-6 are two key cytokines involved in tissue damage during liver injury, although it has been suggested that TNF-α is the central mediator regulating other subsequent events (Jaeschke, 2000). TNF-α and IL-6 act as pleiotropic cytokines to exert either immunosuppressive or immune-stimulatory effects on a variety of cell types. In the present study, LPS significantly increased the level of hepatic TNF-α and IL-6 and these results are in agreement with that obtained with Chiao *et al.*(2005). Increased TNF-α has been repeatedly shown to play a pivotal role in LPS-induced liver injury. TNF-α is a multifunctional cytokine secreted by activated macrophages, monocytes, neutrophils and NK-cells. In addition to its direct cytotoxic effects, it is able to induce chemokines, macrophage chemotactic protein-1 and vascular cell adhesion molecule-1, which are the key to hyper inflammation and consequent liver damage. Moreover, TNF-α act as initiator in the cascade of endogenous mediators that direct the inflammatory and metabolic responses, eventually leads to severe shock and organ failure. Thus, the excessive production of pro-inflammatory cytokines is thought to contribute significantly to the lethality of liver injury (Inoue *et al.,* 2005).

In the present study, administration of vitamin E or ALA was able to reverse the increase in TNF-α and IL-6 and these results come in accordance with Bharrhan *et al*., (2010) and Goraca *et al*., (2009). IL-10 is an anti- inflammatory cytokine that has been reported to down-regulate TNF-α, as well as other cytokines production, by suppressing their gene expression in an autocrine-like feedback loop (Oberholzer *et al*., 2002). The anti- inflammatory actions of IL-10 appear to require induction of the enzyme heme-oxygenase-1 (HO-1) through a map kinase pathway involving the p38 kinases. HO-1 is induced by IL-10 and is also induced by oxidative stress (Lee and Chau, 2002). However, in this study, we observed that the level of IL-10 was significantly increased in response to ALA and vitamin E administration, indicating that the inhibition of TNF-α formation observed with ALA and vitamin E supplementation is not independent of the activation of the feedback loop of IL-10. The anti-inflammatory effect of vitamin E may be indirectly related to inhibition of chemotaxis of neutrophils through inhibition of protein kinase C, 5-lipoxygenase, tyrosine-kinase and cyclooxygenase, as has been suggested by Goraca *et al*., (2009). The present study showed that ALA prevented the increase of serum proinflammatory cytokines. Inhibition of an inflammatory mediator TNF-α as well as IL-6

points out an anti-inflammatory effect of ALA against LPS-induced liver sepsis. These results are supported by the ability of ALA to prevent the LPS-necrotic damage in rat liver. However, previous studies suggested that the hepatoprotective effect of ALA after LPS-induced oxidative stress may be related to inhibition of pro-inflammatory cytokines and induction of endogenous antioxidants (Singh *et al.,* 2005).

In conclusion, the results of the present study indicated that pretreatment with alpha lipoic acid and vitamin E were effective in reducing pro-inflammatory cytokines, liver marker enzymes and oxidative stress which may be efficient for the prophylactic management of LPS- induced liver injury.

References

Yongke, L., W. Xiaodong and I. Arthur, 2005. Lipopolysaccharide-induced liver injury in rats treated with the CYP2E1 inducer pyrazole. Am. Physiological Soc., 289: G308-G319

Kheir-Eldin, A.A., T. K.Motawi, M.Z. Gad and H.M. Abd-ElGawad, 2001. Protective effect of vitamin E, β-carotene and N-acetylcysteine from the brain oxidative stress induced in rats by lipopolysaccharide. Biochem. Cell Biol., 33: 475-482.

Pilkhwal, S., N. Tirkey, A. Kuhad and K. Chopra, 2010. Effect of bioflavonoid quercetin on endotoxin induced hepatotoxicity and oxidative stress in rat liver. Pharmacol & Therapeutics. 9: 47-53.

Wang, H., W. Wei, S. Zhang, Y. Shen, L. Yue, N. Wang and S. Xu, 2005. Melatonin-selenium nanoparticles inhibit oxidative stress and protect against hepatic injury induced by Bacillus Calmette-Guerin/lipopolysaccharide in mice. J Pineal Res., 39: 156-63.

Giriwono, P., H. Shirakawa, H. Hokazono, T. Goto and M. Komai, 2011. Fermented barley extract supplementation maintained anti-oxidative defence suppressing lipopolysaccharide-induced inflammatory liver injury in rats. Biosci, Biotech, and Biochem., 75: 1971-6.

Aoshiba, K., S. Onizawa, T. Tsuji and A. Nagai, 2009. Therapeutic effects of erythropoietin in murine models of endotoxin shock. Crit. Care Med., 37: 889-898.

James, P., M. Madhani, W. Roebuck, S. Jackson and H. Swartz, 2002. Endotoxin-induced liver hypoxia: defective oxygen delivery versus oxygen consumption. Nitric Oxide., 6: 18-28.

Roberts, R.A., P.E. Ganey, C. Ju, L.M. Kamendulis, I. Rusyn and J.E. Klaunig, 2007. Role of the Kuffer cell mediating hepatic toxicity and carcinogenesis. Toxicol. Sci., 96: 2-15.

Crimi, E., V. Sica, S. Williams-Ignarro, H. Zhang, A.S. Slutsky, L.J. Ignarro and C. Napoli, 2006. The role of oxidative stress in adult critical care. Free Radic. Biol. Med., 40: 398-406.

Ozsoy, N., A. Can, R. Yanardag and N. Akev, 2008. Antioxidant activity of Smilax excels a leaf extracts. Food Chem., 110: 571-583.

Kanter, M., O. Coskun, F. Armutcu, Y.H. Uz and G. Kizilay, 2005. Protective effects of vitamin C, alone or in combination with vitamin A on endotoxin-induced oxidative renal tissue damage in rats. J. Exp. Med., 206: 155-162.

Kaya, N.E., 2009. Alpha-tocopherol looking beyond an antioxidant. Molecular Vision., 15: 855-860.

Gurel, A., O. Coskun, F. Armutcu, M. Kanter and O. Ozen, 2005. Vitamin E against oxidative damage caused by formaldehyde in frontal cortex and hippocampus: biochemical and histological studies. Chem. Neuroanat., 29: 173-178.

Rocksén, D., B. Ekstrand-Hammarström, L. Johansson and A. Bucht, 2003. Vitamin E reduces trans endothelial migration of neutrophils and prevents lung injury in endotoxin-induced airway inflammation, Am J Respir Cell Mol Biol., 28: 199-207.

Shay, K.P., R.F. Moreau, E.J. Smith, A.R. Smith and T.M. Hagen, 2009. Alpha lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential. Biochim Biophys Acta., 1790: 1149-1160.

Aly, H.A., D.A. Lightfoot and H.A. El-Shemy, 2009. Modulatory role of lipoic acid on lipopolysaccharide-induced oxidative stress in adult rat Sertoli cells in vitro. Chem. Biol. Interac., 182: 112-118.

Bast, A. and G. Haenen, 1988. Interplay between lipoic acid and glutathione in the protection against microsomal lipid peroxidation. Biochim. Biophys. Acta., 963: 558-561.

Goraca, A., A. Piechota and H.Huk-Kolega, 2009. Effect of alpha-lipoic acid on LPS-induced oxidative stress in the heart. Physiol. Pharmacol., 60: 61-68.

Castro, M.C., M.L. Massa, G. Schinella and J. Gagliardino and F. Francini, 2013. Lipoic acid prevents liver metabolic changes induced by administration of a fructose-rich diet. J. Biochem. Biophys. Acta. 1830: 2226-2232.

Kaya-Dagistanli, F., G. Tanriverdi, A. Altinok, S. Ozyazgan and M. Ozturk, 2013. The effects of alpha lipoic acid on liver cells damages and apoptosis induced by polyunsaturated fatty acids. Food Chem. Toxicol., 53: 84-93.

Kwiecień, B., M. Dudek, A. Bilska-Wilkosz, J. Knutelska, M. Bednarski, I. Kwiecień, M. Zygmunt, M. Iciek, M. Sokołowska-Jeżewicz, J. Sapa and L. Włodek, 2013. In vivo anti-inflammatory activity of lipoic acid derivatives in mice. Postępy Hig. Med., 67: 331-338.

Wafaa, H., Z. Nashwah and H. Lobna, 2006. Prophylactic Effect of Antioxidants as Free Radical Scavengers in Endotoxemia. J. Drug Res. Egypt, 27: 1-2, 98.

Karima, M.M., 2007. Possible Prophylactic Effects of Vitamin E or Lycopene Treatment on Renal Toxicity Induced by CCL4Administration in Albino Rats. World Journal of Zoology, 2: 19-28.

Gella, F.J.,T. Olivella, M. Pastor, J. Arenas, R. Moreno, R. Durban and J.A. Gomez, 1985. A simple procedure for routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. J. Clin Chim Acta., 153: 241-247.

Glick, M.R., K.W. Ryder and S.A. Jackson, 1986. Graphical comparison of interferences in clinical chemistry instrumentation. J. Clin Chem, 32: 470-474.

Ohakawa, H., W. Ohhishi and K. Yagi, 1979. 95: 351.

Beultler, E., O. Duron and M. Kelly, 1963. Lab. Clin. Med. 61. 882.

Aebi, H., 1984. Methods Enzymol., 105: 121-126.

Matsuda, H., A. Ishikado and N. Nishida, 1998. Hepatoprotective, superoxide scavenging and antioxidative activities of aromatic constituents from the bark of *Betula platyphylla var. japonica.* Bio.org. Med. Chem. Lett., 8: 2939-2944.

Depboylu, B., M. Giriş, V. Olgaç, S. Dogru-Abbasoglu and M. Uysal, 2013. Response of liver to lipopolysaccharide treatment in male and female rats. Exp. Toxicol. Pathol., 65: 645-650.

Zhu, J.H. and X.G. Lei, 2011. Lipopolysaccharide-induced hepatic oxidative injury is not potentiated by knockout of GPX1 and SOD1 in mice. Biochem. Biophys. Res. Commun., 1: 559-563.

Zou, W., R.A. Roth, H.S. Younis, E. Malle and P.E. Ganey, 2011. Neutrophil-cytokine interactions in a rat model of sulindac-induced idiosyncratic liver injury. Toxicology, 290: 278-285.

Kaur, G., N. Tirkey and K. Chopra, 2006. Beneficial effect of hesperidin on lipopolysaccharide-induced hepatotoxicity. Toxicology, 226: 152-60.

Heibashy, M.I.A., G.M.A. Mazen and M.I. Shahin, 2013. The Curative Effects of some Antioxidants on Endotoxin Induced with Lipopolysaccharide in the Liver of Rats. J Am Sci., 9: 529-538.

Tanaka, Y., M. Kaibori, HMiki, R. Nakatake, K. Tokuhara, M. Nishizawa, T. Okumura and A. Kwon, 2015. Alpha-lipoic acid exerts a liver-protective effect in acute liver injury rats. J o f surgical research, 193: 675-683.

Nordberg, J. and E. Arner, 2001. Reactive oxygen species, antioxidants and the mammalian thioredoxin system. Free Rad. Biol., 31: 1287-1312.

Sushma, B., C. Kanwaljit and R. Praveen, 2010. Vitamin E Supplementation Modulates Endotoxin-induced Liver Damage in a Rat Model. Am. J. Biomed. Sci., 2: 51-62.

Hussein, S.A.,Y.A. El-Senosi, Omnia M. Abd El-Hamid and W.M.T. Bakr, 2012. Protective effect of vitamin E on biomarkers of oxidative stress and inflammatory response in a rat model of endotoxemia. B.V.M.J. 23: 121-136.

Gulec, M., A. Gurel and F. Armutcu, 2006. Vitamin E protects against oxidative damage caused by formaldehyde in the liver and plasma of rats. Mol. Cell Biochem, 290: 61-67.

Sebai, H., M. Ben-Attia, M. Sani, E. Aouani and N. Ghanem-Boughanmi, 2009. Protective effect of resveratrol in endotoxemia-induced acute phase response in rats. Arch. Toxicol., 83: 335-340.

Bharrhan, S., K. Chopra and P. Rishi, 2010. Vitamin E supplementation modulates endotoxin-induced liver damage in a rat model. Am J Biomed Sci., 2: 51-62.

Pari, L. and K. Shagirtha, 2010. Hesperetin protects against oxidative stress related hepatic dysfunction by cadmium in rats. Exp Toxicol Pathol, 64: 513-520.

Sena, C.M., E. Nunes, T. Louro, T. Proença and R.M. Seiça, 2007. Endothelial Dysfunction in Type 2 Diabetes: Effect of Antioxidants. Rev Port Cardiol., 26: 609-619.

Tian, Y.F., C.H. Hsieh, Y.J. Hsieh, Y.T. Chen, Y.J. Peng and P.S. Hsieh, 2012. α-Lipoic acid prevents mild portal endotoxemia-induced hepatic inflammation and β cell dysfunction. Eur. Clin. Invest., 42: 637-648.

Sakamoto, W., K. Fujie, H. Handa, T. Ogihara and M. Mino, 1990. In vivo inhibition of superoxide production and protein kinase C activity in macrophages from vitamin E-treated rats, Int J Vitam Nutr Res., 60: 338-342.

Kaur, G., N. Tirkey, S. Bharrhan, V. Chanana, P. Rishi and K. Chopra, 2006. Inhibition of oxidative stress and cytokine activity by curcumin in amelioration of endotoxin–induced experimental hepatotoxicity in rodents. Clin Exp Immunol., 145: 313-321.

Naik, S.R.,V.N. Thakare and S.R. Patil, 2011. Protective effect of curcumin on experimentally induced inflammation hepatotoxicity and cardiotoxicity in rats: Evidence of its antioxidant property. Exp Toxicol Pathol., 63: 419-431.

Bansal, A., M. Bansal, G. Soni and D. Bhatnagar, 2005. Protective role of vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. Chem-Biol Interact., 156: 101-111.

Nordberg, J. and E. Arner, 2001. Reactive oxygen species, antioxidants and the mammalian thioredoxin system. Free Rad. Biol., 31: 1287-1312.

Akpinar, D., P. Yargiçoğlu, N. Derin, Y. Alicigüzel and A. Ağar, 2009. The effect of lipoicacid on antioxidant status and lipid peroxidation in rats exposed to chronic restraint stress. Physiol Res.**,** 57: 893-901.

Mihai, B., C. Lăcătuşu, M. Graur, C. Cjevschi-Prelipceam and C. Mihai, 2010. Pharmacologic therapy in peripheral diabetic polyneuropathy. Rev. Med. Chir. Soc. Med. Nat. Iasi., 114: 332-341.

Bertok, L., 2005. Radio-detoxified endotoxin activates natural immunity. Pathophysiology, 12: 85-95.

Hsieh, C.H., M. Frink, Y.C. Hsieh, W. H.Kan, J.T. Hsu, M.G. Schwacha, M.A. Choudhry and I.H. Chaudry, 2008. The role of MIP-1α in the development of systemic inflammatory response and organ injury following trauma hemorrhage. Immunol., 181: 2806-2812.

Jaeschke, H., 2000. Reactive oxygen and mechanisms of inflammatory liver injury. J Gastroenterol. Hepatol., 15: 718-724.

Chiao, C., S. Lee, C. Wu and M. Su, 2005. Thaliporphine increases survival rate and attenuates multiple organ injury in LPS-induced endotoxaemia. Arch Pharmacol., 371: 34-43.

Inoue, K., H. Takano, A. Shimada, T. Morita, R. Yanagisawa, M. Sakurai, M. Sato, S. Yoshino and T. Yoshikawa, 2005. Cytoprotection by interleukin- against liver injury induced by lipopolysaccharide. Int. J. Mol. Med., 15: 221-224.

Goraca, A., H. Huk-Kolega, A. Kowalczyk and B. Skibska, 2015. Anti-oxidative and anti-inflammatory effects of lipoic acid in rat liver. Postepy Hig Med Dosw., 69: 270-276.

Oberholzer, A., C. Oberholzer and L. Moldawer, 2002. Interleukin-10: a complex role in the pathogenesis of sepsis syndromes and its potential as an anti-inflammatory drug. Crit Care Med., 30: S58-S63.

Lee, T.S. and L.Y. Chau, 2002. Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. Nat Med., 8: 240-246.

Goraca, A., A. Piechota and H. Huk-Kolega, 2009. Effect of alpha-lipoic acid on LPS-induced oxidative stress in the heart. J. Physiol Pharmacol., 60: 61-8.

Singh, U., S. Devaraj and I. Jialal, 2005. Vitamin E, oxidative stress, and inflammation, Annu Rev Nutr, 25: 151-174.