The Curative Effects of some Antioxidants on Endotoxin Induced with Lipopolysaccharide in the Liver of Rats

Heibashy, M.I.A.[1]; Mazen, G.M.A.[1] and Shahin, M.I.[2]

[1] Biological Applications Department, Nuclear Research Center, Atomic Energy Authority, Egypt
[2] Zoology Department, Women's Collage, Ain Shams University
Semsma.mona2000@yahoo.com

Abstract: This study was aimed to investigate the ameliorating effect of α-lipoic acid, L-carnitine or N-acetylcysteine and their mixture in salvaging endotoxin-induced hepatic dysfunction and oxidative stress in the liver of rats. Hepatotoxicity was induced by administering lipopolysaccharide (LPS) in a single dose of 5mg/kg intraperitoneally to the animals, which were being treated with α-lipoic acid, L-carnitine, N-acetylcysteine or their mixture daily for 30 days. The obtained data revealed that LPS induced a significant (p<0.05) increase in serum liver function tests (ALT, AST, ALP & γGT), cyclooxygenase-2 and resistin than those in control ones. Liver lipid peroxidation was significantly (p <0.05) elevated in LPS rats compared with those obtained in the control animals group. In addition, the levels of proinflammatory cytokines (IL-1β, IL-6 & TNF-α) were significantly (p <0.05) increased associated with a remarkable elevation in the level of serum plasminogen inhibitor-1 in LPS rats. On the other hand, LPS caused significant (p <0.05) decrease in liver antioxidant enzymes (glutathione, glutathione peroxidase, superoxide dismutase, xanthine dehydrogenase and xanthine oxidase) and serum total nitric oxide levels. After LPS rats group treated with α-lipoic acid, L-carnitine, N-acetylcysteine or their mixture for 30 days, a considerable amelioration effects in all previous studied parameters were pronounced dependent on certain mechanisms which were discussed according to available recent researches.
[Heibashy, M.I.A.; Mazen, G.M.A. and Shahin, M.I. **The Curative Effects of some Antioxidants on Endotoxin Induced with Lipopolysaccharide in the Liver of Rats.** *J Am Sci* 2013;9(12):529-538]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 70

Key words: Endotoxin, Lipopolysaccharide, α-lipoic acid, L-carnitine, N-acetylcysteine.

1- Introduction

Despite advances in antimicrobial therapy and medical support, septic shock remains a major cause of morbidity and mortality among hospitalized patients. Septicaemia causes by gram-negative pathogens; it is a dangerous infection which is associated with high incidence of liver dysfunction **(Kim & Han, 2000)**. The severe or acute hepatotoxicity is presumably due to massive release of endotoxin or lipopolysaccharide (LPS) into systemic circulation after bacterial killing **(Pilkhwal *et al.*, 2010)**. The direct toxic effects of endotoxin to variety of organs are probably due to increase production of reactive oxygen intermediates as O_2^-, peroxides and nitric oxide **(Kaur *et al.*, 2006)**. LPS directly causes liver injury by certain mechanisms involving inflammatory cells such as Kupffer cells and chemical mediators such as superoxide, nitric oxide, tumor necrosis factor-α and other cytokines **(Yongke *et al.*, 2005)**. The indirect cytotoxic effects are mediated through the action of endogenous mediators such as proinflammatory cytokines [tumor necrosis factor-α (TNF-α, interleukin-1 (IL-1) and interleukin-6 (IL-6)] produced and released by activated macrophages and neutrophils. Furthermore, LPS induced elevation in lipid peroxidation which is an index of oxidative stress and depending on both time and dose **(James *et al.*, 2002)**.

Alpha-lipoic acid (1,2-dithiolane-3-pentanoic acid; ALA), a disulphide derivative of octanoic acid and its reduced form dihydrolipoicacid (DHLA) are natural compounds widely distributed in plants and animals. They are synthesized through a reaction catalyzed by lipoic acid sysnthase within the mitochondria **(Wollin & Jones, 2003)**. The therapeutic actions of ALA are based on unique antioxidant ALA/DHLA system. Thus, DHLA is able to reduce not only reactive oxygen species (ROS) but also oxidize forms of other antioxidants. ALA regenerates other antioxidants and for this reason it is called an antioxidant of antioxidants **(Bilska *et al.*, 2008)**. Therefore, dietary lipoic acid is effective in attenuating oxidative stress induced by drugs **(Amudha *et al.*, 2006)** and aging **(Arivazhagan *et al.*, 2002)**.

ALA is also used as modulator in several liver disorders such as alcohol induced liver damage, mushroom poisoning, metal intoxication and chloroform poisoning **(Basamante *et al.*, 1998)**. ALA elevates the hepatic GSH content due to the presence of thiol groups. Free thiols represent essential precursors or intermediates in GSH synthesis and degrading pathways as well as in the metabolism of

several agents used in medical treatments **(Lilling & Holmgren, 2007)**.

L-carnitine(β-hydroxy-(γ-N-trimethylamino) butyrate, carnitine), is an essential factor for the transfer of long-chain fatty acids from the cytosol to mitochondria for subsequent β-oxidation **(Bahcecioglu *et al.*, 1999)**. **Hatamkhani *et al.* (2013)** recorded that supplementation of carnitine to septic patients could increase the rate of oxidation of fatty acids and normalize lipid metabolism. Furthermore, carnitine administration led to the symptoms of carniine-deficient humans **(Worthley *et al.*, 1983)** and elevated the survival of endotoxic rats **(Takeyama *et al.*, 1989)**. Also, carnitine administration to LPS-injected rats increased survival and food consumption but decreased plasma triglycerides and hepatic lipogenesis. Mechanisms of carnitine effects in sepsis are still unknown, as *in vitro* rates of oxidation in isolated mitochondria from livers of LPS-injected rats were not increased by *in vivo* administration of carnitine **(Gallo *et al.*, 1993)**.

N-acetylcysteine (NAC) is derived from the sulfur-containing amino acid cysteine. It is a precursor of intracellular glutathione (GSH) which behaves as an antioxidant as well, functioning to remove toxic peroxides. As a potent antioxidant, NAC directly scavenges hydrogen peroxide, hydroxyl free radicals and hypochloric acid **(Wang *et al.*, 2007)**. NAC is readily absorbed, quickly converted to L-cysteine and then intracellular glutathione, thus, replenishing and maintaining healthy contents of glutathione **(Heibashy, 2005)**. Also, NAC inhibits LPS-induced inducible nitric oxide synthase, TNF-α expression and NF-κB activity **(Verhasselt *et al.*, 1999)**.

In the light of these studies, the current work was designed to scrutinize the hepatoprotective and antioxidant potentials of alpha-lipoic acid, L-carnitine or N-acetylcysteine and their mixture against oxidative stress and cytokine disturbance of endotoxin-induced experimental hepatotoxicity with lipopolysaccharide in rats.

2- Material and Methods

Forty two male albino rats obtained from the Animal House of Sera and Antigens Center, Cairo, were used in this study. The animals were housed in the vivarum of Biological Applications Department, Nuclear Research Center, Atomic Energy Authority and kept under hygienic managerial and environmental conditions. They were fed to appetite on a standard laboratory animal diet according to **NRC (1977)**. Food and tap water were served *ad libitum* with fresh supplies presented daily. Animals were then divided randomly into six groups, a control and five experimental groups, each of seven male albino rats on the base of the body weight, 120 g in average (120±10g).

Experimental design:-

A comparison was occurred between normal control rats group (C) and five LPS-administrated animals groups which divided as follow:-

1- Normal control rats group: These animals injected with saline solution (0.9%NaCl).

2- Lipopolysaccharide (LPS) rats group: These animals injected intraperitoneally (i.p) with a single dose of LPS (5mg/kg body weight) according to **Kaur *et al.* (2006)**. However, LPS (serotype *Escherichia coli* 0111:B4 containing not less than 500,000 EU/mg; Sigma Chemical Co.; USA) was prepared in pyrogen-free water for injection. The drug solutions were made freshly at the beginning of each experiment.

3- Alpha-lipoic acid rats group (LPS+ALA): These animals treated i.p with 100mg ALA /kg body weight after 1 hr of single dose of LPS which induced endotoxin for 30 days as described by **Goraca *et al.* (2009)**. ALA was purchased from Sigma Chemical Co.; USA.

4- L-carnitine rats group (LPS+Car): These rats treated i.p with 200mg L-carnitine/kg body weight after induction of endotoxin as previous described for 30 days as described by **Winter *et al.* (1995)**. L-carnitine was purchased from MEPACO, Egypt.

5- N-acetylcysteine rats group (LPS+NAC): These animals treated i.p with 25mg NAC/kg body weight after endotoxin induction as mentioned before for 30 days as described by **Alipour *et al.* (2007)**. NAC was purchased from Sigma Chemical Co.; USA.

6- Mixture rats group (LPS+Mix): These animals treated with a mixture from previous antioxidant drugs after LPS induction as above described.

At the end of experimental period, the rats were overnight fasted and killed by head decapitation. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (γ-GT) activities were assessed kinetically using commercial kit from Randox Ltd., Co. (UK). The levels of cytokine profile [(Rat interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α) were determined using enzyme immunoassay (EIA) techniques (IBL Gesellschaft, Hamburg, Germany). Also, rat plasminogen activator inhibitor-1 level was measured using an ELISA kit purchased from American Diagnostica, Inc.; (USA). The activity of rat cyclooxygenase (COX) was determined using enzyme immunoassay (EIA) techniques (IBL Gesellschaft, Hamburg, Germany). Serum rat total nitric oxide (TNO) as a free radical was estimated using a commercial ELISA kit (Assay Designs, Inc.; Germany). However, serum resistin level was determined by radioimmunoassay (RIA) specific kits

for rats using solid phase component system (ICN Pharmaceuticals Inc, USA).

A midline abdominal incision was performed, their livers were harvested, perfused with cold isotonic saline, dried carefully on filter papers, weighed and were deep-frozen until further liver analysis. The content of glutathione (GSH) and the activities of glutathione peroxidase (GPx), superoxide dismutase (SOD), xanthine dehydrogenase (XDH) and xanthine oxidase (XO) were determined using enzyme immunoassay (EIA) techniques (IBL Gesellschaft, Hamburg, Germany). In addition, the lipid peroxidation (LPO) was estimated using commercial ELISA kit (Oxis International, Inc, USA).

Statistical differences between the means were assessed by analysis of variance (ANOVA) followed by Duncan's multiple range test according to **Duncan (1955)** and **Snedecor & Cochran (1982)** using a computer program (Costate). Values of $P < 0.05$ were considered statistically significant.

3- Results and Discussion

Sepsis is a clinical syndrome that represents the systemic response to an infection and is characterized by systemic inflammation and widespread tissue injury. Bacterial LPS (endotoxin) induces extensive damage to a variety of organs, including liver, due to the increase production of reactive oxygen intermediates and a resultant rise in lipid peroxidation. Endotoxin accumulates in tissues rich in cells of the reticuloendothelial system such as liver and spleen **(Matsuda *et al.*, 1998)**. In the current study, rats injected with a single dose of LPS showed a significant ($p<0.05$) elevation in serum activities of AST, ALT, ALP and γGT which are the circulating markers of hepatocyte injury. This elevation of hepatic enzymes was coupled with a marked decrease in the content of glutathione and the activities of antioxidant enzymes (glutathione peroxidase, superoxide dismatase, xanthine dehydrogenase and xanthine oxidase) in the liver associated with a considerable increase in hepatic oxidative stress as evident by a marked elevation in lipid peroxidation level (Tables 1,2 & 3). These results are in harmony with that obtained by **Kaur *et al.* (2006)** and **Pilkhwal *et al.* (2010)**.

There are many mechanisms by which LPS can induce oxidative stress. LPS stimulates Kupffer cells, the resident macrophage in the liver. Physiologically, macrophages serve as the first-line defense against invading pathogens by (1) overproducing of superoxide anion (NO_2^-) *via* activation of NADPH-oxidase localized in its plasma membrane; (2) inducing the expression of inducible nitric oxide synthase (iNOS) activity and (3) generating highly toxic peroxynitrite (ONOO-) to kill the invading pathogen **(Srisook *et al.*, 2005)**.

Table (1): The curative effects of some antioxidants on endotoxin induced with lipopolysaccharide on serum liver enzymes activities

Parameters \ Groups		Normal Control	Toxic LPS	LPS + ALA	LPS + CAR	LPS + NAC	LPS + Mixture
AST (U/L)	Mean ±SE	125.734 ± 1.302 ^{A}	191.531 ± 2.694 ^{B}	152.657 ± 1.578 ^{C}	154.562 ± 1.591 ^{C}	153.789 ± 1.602 ^{C}	128.019 ± 1.347 ^{A}
ALT (U/L)	Mean ±SE	24.819 ± 0.372 ^{A}	51.218 ± 0.754 ^{B}	35.047 ± 0.521 ^{C}	34.982 ± 0.548 ^{C}	35.178 ± 0.516 ^{C}	26.319 ± 0.381 ^{D}
ALP (U/L)	Mean ±SE	26.789 ± 0.392 ^{A}	68.098 ± 0.923 ^{B}	40.245 ± 0.609 ^{C}	40.203 ± 0.623 ^{C}	44.457 ± 0.706 ^{D}	28.107 ± 0.417 ^{E}
GGT (U/L)	Mean ±SE	15.616 ± 0.192 ^{A}	52.137 ± 0.764 ^{B}	32.119 ± 0.5481 ^{C}	31.993 ± 0.568 ^{C}	38.107 ± 0.627 ^{D}	20.229 ± 0.278 ^{E}

- Values are expressed as mean ± SE
- $^{A, B, C, D, E, F}$ Means with a common superscript within a row are significantly different ($P<0.05$).

The involvement of ROS in tissue damage has been reported in humans with septic shock and in animal models of endotoxic shock **(Planas & Garcia, 1997 and Olszanecki *et al.*, 2006)**. **Cowley *et al.* (1998)** reported depression of total antioxidant potential in patients with sepsis and organ dysfunction associated with poor survival. Also, **Nielsen *et al.* (1997)** indicated that free radicals induce lipid peroxidation in cells and malondialdehyde formed during oxidative degeneration is regarded as an indicator of lipid peroxidation. Moreover, peroxidation of membrane lipids accompanying sepsis has been shown as an early contributing organ injury **(Goraca & Jozefowicz-Okonkwo, 2007)**.

GSH is one of the body's most important antioxidant responsible for free radical scavenging in all cell types **(Kumaran *et al.*, 2004)**. GSH is essential for the protection of thiol and other nucleophilic groups in proteins from the toxic

metabolites and lower GSH contents under conditions of intracellular stress lead to oxidation and damage of lipids, proteins and DNA by ROS **(Nordberg & Arner, 2001)**. This study showed that LPS treatment decreased GSH content and glutathione peroxidse activity in liver (Table 2). The observed decrease in glutathione content may result from (1) decrease availability of substrates needed for glutathione synthesis, particularly cysteine, which is considered to be the most limiting amino acid; (2) increase activity of GPx and glutathione transferase (GST) in liver tissue; and (3) high concentrations of H_2O_2 and other oxidants produced in septic cells. The decrease in GSH content and GP_X activity in the liver of endotoxic rats constitute evidence of strong oxidative stress and a breakdown of the redox balance in cells of endotoxic rats **(Tsiotou *et al.*, 2005)**. Similarly, **Reddy *et al.* (2006)** reported that the decrease in the GSH content and the increase in GSSG concentration as well as a depletion in the GSH/GSSG ratio were observed during the initial phase of septic shock (during the first hours after infection).

Table (2): The curative effects of some antioxidants on endotoxin induced with lipopolysaccharide on liver antioxidants enzymes activities and lipid peroxidation levels.

Groups / Parameters		Normal Control	Toxic LPS	LPS + ALA	LPS + CAR	LPS + NAC	LPS +Mixture
Glutathione (nmol/g tissue)	Mean ±SE	20.652 ± 0.316 ^{A}	7.549 ± 0.154 ^{B}	14.209 ± 0.254 ^{C}	14.231 ± 0.263 ^{C}	11.776 ± 0.202 ^{D}	17.937 ± 0.284 ^{E}
Glutathione peroxidase (nmol/mg protein/min)	Mean ±SE	5.387 ± 0.141 ^{A}	2.318 ± 0.087 ^{B}	3.925 ± 0.102 ^{C}	3.968 ± 0.109 ^{C}	3.543 ± 0.098 ^{D}	4.862 ± 0.118 ^{E}
Superoxide dismatase (NU/mg protein/30 min)	Mean ±SE	7.613 ± 0.178 ^{A}	4.477 ± 0.103 ^{B}	5.996 ± 0.142 ^{C}	6.004 ± 0.136 ^{C}	5.988 ± 0.139 ^{C}	7.256 ± 0.0163 ^{D}
Xanthine dehydrogenase (μg/mg protein/60min)	Mean ±SE	3.263 ± 0.078 ^{A}	1.263 ± 0.043 ^{B}	2.554 ± 0.053 ^{C}	2.602 ± 0.043 ^{C}	2.558 ± 0.043 ^{C}	3.259 ± 0.043 ^{A}
Xanthine oxidase (μg/mg protein/60 min)	Mean ±SE	5.442 ± 0.091 ^{A}	3.312 ± 0.058 ^{B}	4.661 ± 0.079 ^{C}	4.658 ± 0.089 ^{C}	4.649 ± 0.089 ^{C}	5.389 ± 0.089 ^{A}
Lipid peroxidation (nmol/100 mg tissue)	Mean ±SE	126.572 ± 1.421 ^{A}	273.128 ± 3.174 ^{B}	192.011 ± 2.109 ^{C}	189.982 ± 2.048 ^{C}	191.307 ± 2.078 ^{C}	123.973 ± 1.668 ^{A}

- Values are expressed as mean ± SE
- $^{A, B, C, D, E, F}$ Means with a common superscript within a row are significantly different (P<0.05).

Superoxide dismutase (SOD) is an enzyme extensively used as biochemical indicator of pathological states associated with oxidative stress. It protects the organism against the deleterious effects triggered by the superoxide radical thereby preventing free radical chain reaction **(Ritter *et al.*, 2003)**. Table (2) showed a significant (p<0.05) decrease in the activity of SOD after LPS treatment in hepatic tissue of rats. The reduction in SOD activity may be due to the direct damaging effect of free radicals on the enzyme **(Omar *et al.*, 2012)**. Furthermore, the decrease of SOD activity may result from a suppression of SOD synthesis due to a genetic defect or leak of SOD out of cell due to increase production of oxygen radical causing cell membrane damage and inactivation of SOD by increment intracellular H_2O_2 level **(Suzuki *et al.*, 1991)**. This finding is in accordance with those previously described for SOD activity of heart in LPS induced septic rats **(Goraca *et al.*, 2009)**.

Lipid peroxidation is known to cause cellular injury by inactivation of membrane enzymes and receptors, depolymerization of polysaccharides as well as protein cross-linking and fragmentation **(Zhao *et al.*, 2008)**. In the current study, LPS rats showed a significant (p<0.05) elevation in the level of lipid peroxidation when compared to their corresponding normal control rats group (Table 2). The elevation of lipid peroxidation levels may be resulted from oxidative damage to the liver tissue. These data are in harmony with those obtained by **Goraca *et al.* (2013)**. Furthermore, LPS possesses prooxidative activity due to its ability to induce excessive ROS and the release of proinflammatory cytokines such as TNF-α, IL-1β and IL-6 **(Melo *et al.*, 2010)**. ROS and cytokines lead to cellular injury by impairment of vital macromolecules such as protein and lipid leading to the multiple organ failure syndrome. A study by **Gonzalo *et al.* (2010)** showed an increase in the formation of products of oxidative damage to protein (carbonyls) and lipids (malondialdehyde and 4-hydroxyalkenals) in the intestine and blood after LPS administration in rabbits.

Lysosomes and lysosomal enzymes are the main factors playing a vital role in tissue injury and repair, inflammation, phagocytosis, intracellular

degeneration, cancer and rheumatoid arthritis. It was further postulated that the lysosomal enzymes are released in inflammation diseases to stimulate the synthesis of prostaglandins. Extracellular release of such enzymes may be crucial to pathogenesis of tissue injury and inflammation **(Rasool *et al.*, 2006 and Sabina *et al.*, 2010)**, it is likely that a reduction in the release of such enzymes would prove beneficial. As shown by the results, the data indicated that administration of LPS led to cause significant increment in the levels of IL-1β, IL-6, TNF-α and COX-2 along with a significant decline in NO level as compared with the corresponding control ones (Tables 3 & 4). These results are in agreement with that obtained with **Chiao *et al.* (2005)**. The authors reported that activated Kupffer cells were shown to increase proinflammatory cytokines such as TNF-α and IL-6. The role of IL-6 in inflammatory injury remains controversial. **Inoue *et al.* (2005)** showed that IL-6-deficient mice were more prone to LPS damage whereas in other studies, IL-6 increase in liver was found to be detrimental **(Guler *et al.*, 2004)**. Proinflammatory TNF-α and IL-1β act as initiators in the cascade of endogenous mediators that direct the inflammatory and metabolic responses, eventually leading to severe shock and organ failure. Thus, the excessive production of proinflammatory cytokines is thought to contribute significantly to the lethality of sepsis **(Campanile *et al.*, 1996)**.

In addition to inflammation, elevated PAI-1 levels are associated with a predisposition to thrombosis in a number of clinical conditions, including sepsis. It has been shown that PAI-1 levels correlate well with the severity of the infection during sepsis **(Mavrommatis *et al.*, 2001)**. Although, the mechanism(s) by which PAI-1 is elevated by Gram-negative bacterial products are not fully understood, previous work in rodents indicated that proinflammatory cytokines such as TNF-α, IL-1β and IL-6 might be critically involved **(Seki & Gelehrter, 1996)**. Judging from the data in table (4), serum PAI-1 level was elevated after LPS treatment when compared to their corresponding control rat. These results are in agreement with that obtained data by **Fearns & Loskutoff (1997)** who demonstrated that the induction of PAI-1 caused by bacterial endotoxin is mediated by TNF-α. IL-1 and TNF-α can stimulate the release of PAI-1 by endothelial cells. Thus, these results suggested that TNF-α increment of PAI-1 production promotes fibrin deposition and injury in this animal model.

Table (3): The curative effects of some antioxidants on endotoxin induced with lipopolysaccharide on serum cytokine profile.

Parameters \ Groups		Normal Control	Toxic LPS	LPS + ALA	LPS + CAR	LPS + NAC	LPS + Mixture
IL-1ß (pg/ml)	Mean ±SE	3.684 ± 0.039 ^{A}	10.112 ± 0.188 ^{B}	5.239 ± 0.089 ^{C}	5.252 ± 0.097 ^{C}	5.244 ± 0.092 ^{C}	3.976 ± 0.053 ^{D}
IL-6 (pg/ml)	Mean ±SE	10.502 ± 0.079 ^{A}	31.056 ± 0.268 ^{B}	17.498 ± 0.268 ^{C}	15.329 ± 0.268 ^{D}	19.107 ± 0.268 ^{E}	10.756 ± 0.268 ^{A}
TNF-α (pg/ml)	Mean ±SE	5.169 ± 0.049 ^{A}	22.506 ± 0.123 ^{B}	14.009 ± 0.085 ^{C}	12.506 ± 0.074 ^{D}	15.783 ± 0.092 ^{E}	8.179 ± 0.063 ^{F}

- Values are expressed as mean ± SE

- $^{A, B, C, D, E, F}$ Means with a common superscript within a row are significantly different (P<0.05).

One mediator molecule involved in the inflammatory responses is nitric oxide **(Boveris *et al.*, 2002)**. In the current study, serum total nitric oxide was decreased in rats treated by LPS when compared to control rats group (Table 4). It is convenient to point out that total nitric oxide, in addition to its role as inflammatory mediator, can also act as a free radical either directly or through generation of peroxynitrites through its interaction with superoxide anion (O_2^-) **(Szabo, 2003)**. Furthermore, an excess of nitric oxide and peroxynitrites can negatively affect mitochondrial function and phosphatidylcholine (PC) synthesis **(Sastre *et al.*, 2000)**.

Resistin is up-regulated by proinflammatory cytokines including TNF-α, IL-6 and IL-1β. It promotes the synthesis of these cytokines by NF-kB activation **(Shoelson *et al.*, 2007)**. The major findings of this study indicated that LPS rats group showed a remarkable elevation in the resistin level (Table 4). It was shown that resistin enhances inflammatory cell recruitment and liver damage caused by LPS. Moreover, **Yagmur *et al.* (2006)** reported the correlation between serum TNF-α level (which was one of the parameters indicating chronic inflammatory condition) and serum resistin in patients with chronic liver diseases (Chronic hepatitis B or C virus infection, alcoholism, autoimmune hepatitis, primary biliary cirrhosis and primary sclerosing cholangitis). Exposure of the liver to low levels of LPS is common and occurs through multiple means, including increased LPS translocation from

the intestinal lumen into the portal venous blood **(Ganey & Roth, 2001)**. LPS administration is also an experimental model of infection and inflammation, which also induces insulin resistance in rodents **(Rajala *et al.*, 2002 and Sugita *et al.*, 2002)**.

Alpha-lipoic acid, known as thioctic acid, is a naturally occurring compound that is synthesized by plants and animals, including humans **(Yi & Maeda, 2005)**. It contains two sulfur molecules that can be oxidized or reduced. This feature allows ALA to function as a cofactor for several important enzymes as well as a potent antioxidant **(Ozkan *et al.*, 2005)**. In the current work, LPS rats treated by alpha-lipoic acid recorded a significant (p<0.05) decrease in the levels of liver functions (ALT, AST, ALP & GGT), lipid peroxidation, COX-2, serum cytokine profile (IL-1β, IL-6 & TNF-α), plasminogen activator inhibitor-1 and resistin associated with a significant amelioration were occurred in the activity of liver antioxidant enzymes and level of serum total nitric oxide (Tables 1-4). These effects may be attributed to reduction in oxidative stress induced by LPS by alleviating lipid peroxidation through free radical scavenging or by enhancing the synthesis of antioxidants containing -SH groups and GSH which then detoxify free radicals **(Goraca *et al.*, 2009)**.

Table (4): The curative effects of some antioxidants on endotoxin induced with lipopolysaccharide on serum plasminogen activator inhibitor-1, cyclooxygenase, total nitric oxide and resistin levels.

Parameters \ Groups		Normal Control	Toxic LPS	LPS + ALA	LPS + CAR	LPS + NAC	LPS + Mixture
PAI-1 (ng/ml)	Mean ±SE	16.347 ± 0.096 ^{A}	271.229 ± 3.586 ^{B}	105.618 ± 1.117 ^{C}	141.506 ± 1.843 ^{D}	189.098 ± 2.152 ^{E}	62.172 ± 0.825 ^{F}
COX-2 (ng/mg)	Mean ±SE	59.784 ± 0.604 ^{A}	100.548 ± 1.427 ^{B}	81.219 ± 0.864 ^{C}	76.626 ± 0.795 ^{D}	84.158 ± 0.902 ^{E}	60.033 ± 0.742 ^{A}
TNO (µmol/L)	Mean ±SE	21.379 ±0.348 ^{A}	10.604 ±0.187 ^{B}	15.288 ±0.259 ^{C}	17.329 ±0.296 ^{D}	15.302 ±0.261 ^{C}	21.565 ±0.354 ^{A}
Resistin (ng/ml)	Mean ±SE	3.329 ±0.038 ^{A}	6.341 ±0.087 ^{B}	5.103 ±0.064 ^{C}	4.497 ±0.059 ^{D}	5.116 ±0.071 ^{E}	3.809 ±0.047 ^{F}

- Values are expressed as mean ± SE
- $^{A, B, C, D, E, F}$ Means with a common superscript within a row are significantly different (P<0.05).

Alpha-lipoic acid exerts its effect mainly through its reduced form, dihydrolipoic acid (DHLA), a conversion to which is catalyzed by lipoamide dehydrogenase. This endogenous thiol antioxidant quenches reactive oxygen species (singlet oxygen, H_2O_2, hydroxyl radicals), regenerates reduced glutathione (GSH) and chelates metals such as iron, copper, mercury and cadmium, which are known to mediate free-radical production in biological systems. Both ALA and DHLA also protect the integrity of cell membranes by interacting with other antioxidants, namely glutathione and vitamins E and C **(Nordberg & Arner, 2001)**. It has been also shown that lipid peroxyl radicals can be directly scavenged by lipoic acid during course of lipid peroxidation **(Packer *et al.*, 1995)**.

Treatment with ALA after LPS challenge restored the reduce GSH level significantly suggesting that this antioxidant might modulate liver anti-oxidative defense by elevation of tissue glutathione redox status. Also, the increase of GSH in liver tissue after ALA administration may be due to the direct action of DHLA, which is a potent reducing agent and therefore converts GSSG to GSH **(Lu, 2008)**. Other authors indicated that LA is able to correct deficient thiol status of the cell by increasing *de novo* synthesis of cellular GSH by improving cystine utilization **(Han *et al.*, 1997)**. Moreover, GSH can react as a non-enzymatic antioxidant by direct interaction of -SH group with ROS, or can be involved in an enzymatic detoxification reaction for ROS, as a cofactor or coenzyme. All these effects of ALA in LPS-induced sepsis are in accordance with the findings of ALA's antioxidant properties. Moreover, this antioxidant can also inhibit acute inflammatory response *in vitro* and *in vivo* by activating the PI3/Akt pathway **(Zhang *et al.*, 2007)**.

Furthermore, alpha-lipoic acid prevented the LPS-induced NO and prevented the increase of serum cytokine. Inhibition of an inflammatory mediator TNF-α as well as IL-1β 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 livers and prevention of the increase in the activities of the liver enzyme markers ALT, AST and γ-GT. **Lee *et al.* (2004)** showed that the increase in γ-GT is a strong evidence and marker of liver oxidative damage.

The pathophysiology of bacterial endotoxin mediated tissue damage may involve the interplay of reduce host carnitine levels and pathogenic requirement of carnitine for growth and survival in the host. The endogenous carnitine pool could be a major determinant of mounting an effective immune and

inflammatory response towards invading pathogens **(Famularo *et al.*, 2004)**. By reviewing tables (1-4), the supplementation of L-carnitine to the LPS rats led to a considerable correction in all studied parameters. These results were confirmed by **Penn *et al.* (1999)** and **Trumbeckaite *et al.* (2001)**. They suggested the possible beneficial effect of carnitine may be related to an improved pyruvate oxidation, Krebs cycle and flux through glutamate dehydrogenase.

L-carnitine therapy to the LPS rats group led to a remarkable reduction in the levels of cytokines (TNF-α, IL-lβ and IL-6) (Table 3). These results suggested that carnitine plays a role in controlling the level of circulating cytokines, which in turn have an effect on lipid metabolism. In fact, **Delogu *et al.* (1993)** noted that carnitine reduces circulating cytokines in surgical patients and also reduces TNF-α secretion by stimulated human polymorphonuclear cells **(Fattorossi *et al.*, 1993).**

Also, enhanced TNF-α production can increase the production of reactive oxygen species by macrophages **(Goossens *et al.*, 1995)**. This can stimulate lipid peroxidation, which is thought to have an important pathogenic role in alcohol-induced liver damage **(Hoek & Pastorino, 2002)**. Moreover, **Bahcecioglu *et al.* (1999)** reported that the ethanol-induced an increase in lipid peroxidation which is significantly blunted by carnitine. In experimental model of oxidative stress, carnitine has been demonstrated to protect against lipid peroxidation **(Loster & Bohm, 2001)**.

L-carnitine has been shown to have effective hydrogen peroxide and superoxide anion scavenging abilities **(Ferrari *et al.*, 2004)**. The authors attributed these results to the antioxidant powerful of L-carnitine which acts to minimize the interaction between nitric oxide and superoxide, ultimately leading to decrease formation of peroxynitrite and maintenance of nitric oxide, hence, carnitine may lead to both the production of nitric oxide as well as the maintenance of existing nitric oxide.

In the current study, the supplementation of N-acetyl cysteine (NAC) to LPS rats caused a marked amelioration effects in all studied parameters (Tables 1-4). These results were confirmed by **Gul *et al.* (2011)**. They suggested the protective effect of N-acetyl cysteine is based on diverse mechanisms, of which the most important are: (1) to facilitate the synthesis of depleted glutathione; (2) to serve as a specific substrate for hepatic microsomal glutathione transferase, thereby improving oxygenation; (3) increasing the blood flow by increasing the soluble nitric oxide activity in the glutamylcyclase system; (4) antioxidant and immunomodulatory actions. However, NAC has been utilized in an experimental form, encapsulated in liposomes, in laboratory animals, where it confers hepatoprotection against the toxic effects of lipopolysaccharides **(Sotelo *et al.*, 2009 and Heibashy *et al.*, 2012)**.

N-acetylcysteine acts as a highly efficient free scavenger of the toxic hydroxyl and peroxyl radicals, increases protein anabolism and decreases its catabolism. Also, NAC has been used to regenerate oxidative phosphorylation complexes in mitochondria from age-related decline in function by sulfhydryl group action rather than antioxidant effect. In study carried by **Ortolani et al. (2002)**, they used GSH and NAC for prevention of free radical injury in the early phase of septic shock. High-dose NAC treatment added to GSH was found to significantly decrease the perioxidative stress in early septic shock patients.

Moreover, NAC treatment has an effect on suppressed the release of inflammation markers in organ suffered from endotoxin shock. **Victor *et al.* (2003)** found that treatment by 150mg/kg intraperitoneal NAC for 30 minutes after the lipopolysaccharide (LPS) procedure was led to reduce reactive oxygen species, TNF-α, MDA levels and the oxidized reduced GSH rate. NAC was shown to prolong the survival of rats.

On the basis of previous investigations and present data in this work, the maximum corrections in all studied parameters were recorded in LPS plus mixture rats group due to the potential powerful of antioxidants (ALA, Carnitine & NAC) properties (pharmakinetic and pharmadynamic). So, it is possible to conclude that α-lipoic acid, L-carnitine, N-acetyl cysteine or their mixture can prevent the LPS induced oxidative liver damage as well as inflammatory stress in liver by alleviating lipid peroxidation through free radical scavenging or by enhancing the synthesis of antioxidants and improves glutathione redox system which then detoxify free radicals. Also, this study can practically help to encourage the clinical use of this compound as a treatment for endotoxemia.

References

1. Alipour, M.; Omri, A.; Smith, M. and Suntres, S.: Prophylactic effect of liposomal N-acetylcysteine against LPS-induced liver injuries. J. Endotoxin Res., 13(5): 297-304 (2007).
2. Amudha, G.; Josephine, A. and Varalakshmi, P.: Role of lipoic acid in reducing the oxidative stress induced by cyclosporineA. Clin. chimica. Acta., 372: 134-139(2006).
3. Arivazhagan, P.; Thilakaathy, T.; Ramanthan, K.; Kumaran, S. and Panneerslvam, C.: Effect of L-α-lipoic on the status of lipid peroxidation and protein oxidation in various brain regions of aged rats. J. Nutr. Biochem., 13: 619-624 (2002).
4. Bahcecioglu, I.; Demir, A.; Ustundag, B.; Ilhan, M.; Baydas, G.; Canatan, H. and Ozercan I.:

Protective effect of L-carnitine on alcoholic fatty liver in rats. Med. Sci. Res., 27: 475-478 (1999).
5. Basamante, J.; Lodge, J.; Marcocci, L.; Tritschler, H.; Packer, I. and Rihn, B.: Alpha lipoic acid in liver metabolism and diseases. Free Rad. Biol. Med., 24: 60-65 (1998).
6. Bilska, A.; Dudek, M.; Iciek, M.; Kwiecien, I.; Sokolowska-Jezewicz, M.; Filipek, B. and Wlodek, L.: Biological actions of lipoic acid associated with sulfane sulfur metabolism. Pharmcol. Rep., 60: 225-232 (2008).
7. Boveris, A.; Alvarez, S. and Navarro, A.: The role of mitochondrial nitric oxide synthase in inflammation and septic shock. Free Radic. Biol. Med., 33: 1186-1193 (2002).
8. Campanile, F.; Giampietri, A.; Grohmann, U.; Belladonna, M.; Fioretti, M. and Puccetti, P.: Evidence for tumor necrosis factor alpha as a mediator of the toxicity of a cyclooxygenase inhibitor in gram-negative sepsis. Eur. J. Pharmacol.; 307: 191-199 (1996).
9. Chiao, C.; Lee, S.; Wu, C. and Su, M.: Thaliporphine increases survival rate and attenuates multiple organ injury in LPS-induced endotoxaemia. Naunyn Schmiedebergs Arch Pharmacol., 371: 34-43 (2005).
10. Cowley, H.; Bacon, P.; Goode, H.; Webster, N.; Jones, J. and Menon, D.: Plasma antioxidant potential in sever sepsis: a comparison of survivors and non-survivors. Crit. Care Med., 26: 705-709 (1998).
11. Delogu, G.; De Simone, C.; Famularo, G.; Fegiz, A.; Paoletti, F. and Jirillo, E.: Anaesthetics modulate tumour necrosis factor alpha: Effects of L-carnitine supplementation in surgical patients. Preliminary results. Mediators of Inflammation 2: S33–S36 (1993).
12. Duncan, D.B.: Multiple range and multiple s-testes .Biometr., 11: 1-42 (1955).
13. Famularo, G.; De Simone, C.; Trinchieri, V. and Mosca, L.: Carnitine and its congeners: a metabolic pathway to the regulation of immune response and inflammation. Ann. NY Acad. Sci., 1033:132-138 (2004).
14. Fattorossi, A.; Biselli, R.; Casciaro, A.; Tzantzoglou, S. and De Simone, C.: Regulation of normal human polymorphonuclear leucocytes by carnitine. Mediators of Inflammation 2: S37–S41(1993).
15. Fearns, C. and Loskutoff, D.: Induction of plasminogen activator inhibitor-1 gene expression in murine liver by lipopolysaccharide. Cellular localization and role of endogenous tumor necrosis factor-alpha. Am. J. Pathol., 150**:**579-590 (1997).
16. Ferrari, R.; Merli, E.; Cicchitelli, G.; Mele, D., Fucili, A. and Ceconi, C.: Therapeutic effects of L-carnitine and propionyl-L-carnitine on cardiovascular diseases: a review. Ann. NY Acad. Sci., 1033, 79-91 (2004).
17. Gallo, L.; Tlan, Y.; Orfalian, Z. and Fiskum, G.: Amelioration of lipopolysaccharide-induced sepsis in rats by free and esterified carnitine. Med. Inflamm., 2: S51-S56 (1993).
18. Ganey, P. and Roth, R.: Concurrent inflammation as a determinant of susceptibility to toxicity from xenobiotic agents. Toxicology 169: 195–208 (2001).
19. Gonzalo, S.; Grasa, L.; Arruebo, M.; Plaza, M. and Murillo, M.: Inhibition of p38MAPK improves intestinal disturbances and oxidative stress induced in a rabbit endotoxemia model. Neurogastroenterol Motil., 22: 564-572 (2010).
20. Goossens, V.; Grooten, J.; De Vos, K. and Fiers, W.: Direct evidence for tumour necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. Proceedings of National Acad. Sciences USA, 92: 8115–8119 (1995).
21. Goraca, A. and Jozefowicz-Okonkwo, G.: Protective effects of early treatment with lipoic acid in LPS-induced lung injury in rats. J. Physiol. Pharmacol., 58 (3): 541-549 (2007).
22. Goraca, A.; Huk-Kolega, H.; Kleniewska, P.; Piechota-Polañczyk, A. and Skibska, B.: Effects of lipoic acid on spleen oxidative stress after LPS administration. Pharmacological Rep., 65: 179-186 (2013).
23. Goraca, A.; Piechota, A. and Huk-Kolega, H.: Effect of alpha-lipoic acid on LPS-induced oxidative stress in the heart. J. Physiol. Pharmacol., 60(1): 61-68 (2009).
24. Gul, M.; Ayan, M.; Seydanoglu, A.; Cander, B.; Girisgin, S.; Erayman, I. and Erdem, S.: The effect of N-acetyl cysteine on serum glutathione, TNF-α and tissue malondialdehyde levels in the treatment of sepsis. Turkish J. Trauma Emerg. Surg., 17(4): 293-297 (2011).
25. Guler, R.; Olleros, M.; Vesin, D.; Parapanov, R.; Vesin, C.; Kantengwa, S.; Rubbia-Brandt, L.; Mensi, N.; Angelillo-Scherrer, A.; Martinez-Soria, E.; Tacchini-Cottier, F. and Garcia, I.: Inhibition of inducible nitric oxide synthase protects against liver injury induced by mycobacterial infection and endotoxins. J. Hepatol., 41(5): 773-81(2004).
26. Han, D.; Handelman, G. and Marcocci, L.: Lipoic acid increases de novo synthesis of cellular glutathione by improving cysteine utilization. Biofactors, 6: 321-338 (1997).
27. Hatamkhani, S.; Karimzadeh, I.; Elyasi, S.; Farsaei, S. and Khalili, H.: Carnitine and Sepsis: A Review of an Old Clinical Dilemma. J. Pharm. Pharm Sci., 16(3): 414-423 (2013).
28. Heibashy, M.; Mazen, G. and Ibrahim, A.: The levels of cytokines and S100β which associated with the pathogenesis of hepatic encephalopathy in rats: role of lactulose and N-acetylcysteine in its treatment. Isotope Rad. Res., 44 (4): 771-788 (2012).

29. Heibashy, M.I.A.: Role of N-acetyl Cystein or Vitamin E and Their Mixture in Prevention of Hepatotoxicity Induced by Paracetamol in Male Albino Rats. Egyptian Soc. Nucl. Sci. Appl., 38 (2): 269–278 (2005).
30. Hoek, J. and Pastorino, J.: Ethanol, oxidative stress, and cytokine-induced liver cell injury. Alcohol, **27**: 63-68 (2002).
31. Inoue, K.; Takano, H.; Shimada, A.; Morita, T.; Yanagisawa, R.; Sakurai, M.; Sato, M.; Yoshino, S. and Yoshikawa, T.: Cytoprotection by interleukin-6 against liver injury induced by lipopolysaccharide. Int. J. Mol. Med., 15(2): 221-224 (2005).
32. James, P.; Madhani, M.; Roebuck, W.; Jackson, S. and Swartz, H.: Endotoxin-induced liver hypoxia: defective oxygen delivery versus oxygen consumption. Nitric Oxide. 6:18–28 (2002).
33. Kaur, G.; Tirkey, N. and Chopra, K.: Beneficial effect of hesperidin on lipopolysaccharide-induced hepatotoxicity. Toxicology, 226: 152-60 (2006).
34. Kim, Y. and Han, S.: Superoxide reactivates nitric oxide-inhibited catalase. Biol. Chem. 381: 1269-1271(2000).
35. Kumaran, S.; Savitha, S.; Anusya Devi, M. and Panneerselvam, C.: L-carnitine and DL-*a*-lipoic acid reverse the agerelated deficit in glutathione redox state in skeletal muscle and heart tissues. Mech. Ageing Dev., 125: 507-512 (2004).
36. Lee, D.; Blomhoff, R. and Jacobs, D.: Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radic Res., 38(6): 535-539 (2004).
37. Lilling, C. and Holmgren, A.: Thioredoxin and related molecules from biology to health and disease. Antioxid. Redox. Sign., 9: 25-47(2007).
38. Loster, H. and Bohm, U.: L-carnitine reduces malondialdehyde concentrations in isolated rat hearts in dependence on perfusion conditions. Mol. Cell Biochemi., 217: 83-90(2001).
39. Lu, S.: Regulation of glutathione synthesis. Mol. Aspects Med., (5): 5- 12 (2008).
40. Matsuda, H.; Ishikado, A. and Nishida, N.: Hepatoprotective, superoxide scavenging and antioxidative activities of aromatic constituents from the bark of Betula platyphylla var. japonica. Bioorg. Med. Chem. Lett., 8: 2939-2944 (1998).
41. Mavrommatis, A.; Theodoridis, T.; Economou, M.; Kotanidou, A.; Ali, M.; Christopoulou-Kokkinou, V. and Zakynthinos, S.: Activation of the fibrinolytic system and utilization of the coagulation inhibitors in sepsis: comparison with severe sepsis and septic shock. Intensive Care Med**.,** 27:1853–1859 (2001).
42. Melo, E.; Barbeiro, H.; Ariga, S.; Goloubkova, T.; Curi, R.; Valasco, I.; Vasconcelos, D. and Soriano, F.: Immune cells and oxidative stress in the endotoxin tolerance mouse model. Braz. J. Med. Biol. Res., 43: 57-67(2010).
43. Nielsen, F.; Mikkelsen, B.; Nielson, J.; Andersen, H. and Grandjean, P.: Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. Clin. Chem., 47: 1209-1214 (1997).
44. Nordberg, J. and Arner, E.: Reactive oxygen species, antioxidants and the mammalian thioredoxin system. Free Rad. Biol., 31: 1287-1312 (2001).
45. NRC National Research Council: Nutrient Requirements of Domestic Animals, National Academy of Science. Washington, DC. U.S.A. (1977).
46. Olszanecki, R.; Gebska, A.; Jawien, J.; Jakubowski, A. and Korbut, R.: Inhibation of NOS-2 induction in LPS-stimulated J 774.2 cells by 1,5-isoquinolinediol an inhibitor of PARP. J. Physiol. Pharmacol., 57: 109-117 (2006).
47. Omar, H.; Saad El-dien, H.; Saeed, M.; Al-Salahy, M. and Abel-Tawab, H.: Alpha-lipoic acid and N-acetyl cysteine ameliorates oxidative stress and hepatic injury in alloxan-induced diabetic rabbits. Intern. J. Diab. Res., 1(1): 7-17 (2012).
48. Ortolani, O.; Conti, A.; De Gaudio, A.; Moraldi, E. and Novelli, G.: Glutathione and N-acetylcysteine in the prevention of free radical damage in the initial phase of septic shock. Recenti. Prog. Med., 93:125-9 (2002).
49. Ozkan, Y.; Yilmaz, O.; Ozturk, A. and Ersan, Y.: Effects of triple antioxidant combination (vitamin E, vitamin C and alpha-lipoic acid) with insulin on lipid and cholesterol levels and fatty acid composition of brain tissue in experimental diabetic and non-diabetic rats. Cell Biol. Int., 29(9):754-760 (2005).
50. Packer, L.; Witt, E. and Tritschler, H.: Alpha-lipoic acid as a biological antioxidant. Free Radic. Biol. Med., 19: 227-250 (1995).
51. Penn, D.; Zhang, L.; Bobrowski, P.; Quinn, M. and McDonough, K.: Carnitine deprivation adversely affects cardiac performance in the lipopolysaccharide and hypoxia/reoxygenation-stressed piglet heart. Shock, 11:120-126(1999).
52. Pilkhwal, S.; Tirkey, N.; Kuhad, A. and Chopra, K.: Effect of bioflavonoid quercetin on endotoxin-induced hepatotoxicity and oxidative stress in rat liver. Iranian J. Pharmacol. Therapeutics, 9: 47-53 (2010).
53. Planas, M. and Garcia, A.: Lipid peroxidation in clinical patients. Nutr. Hosp., 12:233-236 (1997).
54. Rajala, M.; Lin, Y.; Ranalletta, M.; Yang, X.; Qian, H.; Gingerich, R.; Barzilai, N. and Scherer, P.: Mol. Endocrinol., 16: 1920-1930 (2002).
55. Rasool, M.; Sabina, E. and Lavanya, B.: Anti-inflammatory effect of Spirulinafusiformison adjuvant-induced arthritis in mice. Biol. Pharm. Bull., 29(1): 2483-2487(2006).
56. Reddy, M.; Mahipal, S.; Subhashini, J.; Reddy, M.; Roy, K.; Reddy, G.; Reddy, P. and Reddanna, P: Bacterial lipopolysaccharide-induced oxidative stress in the impairment of steroidogenesis and

spermatogenesis in rats. Reprod. Toxicol., 22: 493-500 (2006).
57. Ritter, C.; Andrades, M. and Frota, M.: Oxidative parameters and mortility in sepsis induced by cecal ligation and perforation. Intensive Care Med., 29: 1782-1789 (2003).
58. Sabina, E.; Rasool, M.; Mathew, l.; EzilRani, P. and Indu, H.: 6-Shagaol inhibits monosodium urate crystal-induced inflammation- An *in vitro* and *in vitro* study. Food Chem. Toxicol., 48: 229-235 (2010).
59. Sastre, J.; Pallardo, F. and Vina, J.: Mitochondrial oxidative stress plays a key role in aging and apoptosis. IUBMB Life, 45: 427-435(2000).
60. Seki, T. and Gelehrter, T.: Interleukin-1 induction of type-1 plasminogen activator inhibitor (PAI-1) gene expression in the mouse hepatocyte line, AML 12. J. Cell Physiol., 168**:** 648–656 (1996).
61. Shoelson, S.; Herrero, L. and Naaz, A.: Gastroenterology, 132(6): 2169-1275 (2007).
62. Snedecor, G.W. and Cochran, W.G.: "Statistical Methods" 7th ed., Twa State University Press, Ames, lowa, U.S.A. (1982).
63. Sotelo, N.; Durazo, M.; Gonzalez, A. and Dhanakotti, N.: Early treatment with N-acetylcysteine in children with acute liver failure secondary to hepatitis A. Ann. Hepaol., 8(4):353-358 (2009).
64. Srisook, K.; Kim, C. and Cha, Y.: Role of NO in Enhancing the Expression of HO-1 in LPS-Stimulated Macrophages. Methods Enzymol., 396: 368-77 (2005).
65. Sugita, H.; Kaneki, M.; Tokunaga, E.; Sugita, M.; Koike, C.; Yasuhara, S.; Tompkins, R. and Martyn, J.: Am. J. Physiol. Endocrinol. Metab., 282: E386–E394 (2002).
66. Suzuki, Y.; Tsuchiya, M. and Packer, L.: Thioctic acid and dihydroliloic acid are noval antioxidants which interact with reactive oxygen species. Free Radic. Res. Commun., 15: 255-263(1991).
67. Szabo, C.: Multiple pathways of peroxynitrite cytotoxicity. Toxicol. Lett., 140: 105-112 (2003).
68. Takeyama, J.; Takagi, D.; Matsuo, N.; Kitazawa, Y. and Tanaka, T.: Altered hepatic fatty acid metabolism in endotoxicosis: effect of L-carnitine on survival. Am. J. Phvsiol., 256: E31-E38 (1989).
69. Trumbeckaite, S.; Opalka, J.; Neuhof, C.; Zierz, S. and Gellerich, F.: Different sensitivity of rabbit heart and skeletal muscle to endotoxin-induced impairment of mitochondrial function. Eur. J. Biochem., 268:1422-1429 (2001).
70. Tsiotou, A.; Sakorafas, G.; Anagnostopoulos, G. and Bramis, J.: Septic shock: current pathogenetic concepts from a clinical perspective. Med. Sci. Monit., 11: RA76–RA85 (2005).
71. Verhasselt, V.; Vanden-Berghe, W.; Vanderheyde, N.; Willems, F.; Haegeman, G. and Goldman, M.: N-acetyl-L-cysteine inhibits primary human T cell responses at the dendritic cell level: association with NF-kappaB inhibition. J. Immunol., 162: 2569–74 (1999).
72. Victor, V. and De Fuente, M.: Several functions of immune cells in mice changed by oxidative stress caused by endotoxin. Physiol Res., 52(6):789-96 (2003).
73. Wang, H.; Xu, D.; Lu, J.; Zhao, L.; Zhang, C. and Wei, W.: N-Acetylcysteine attenuates lipopolysaccharide-induced apoptotic liver damage in D-galactosamine-sensitized mice. Acta. Pharmacol. Sin., 28(11): 1803-1809 (2007).
74. Winter, B.; Fiskum, G. and Gallo, L.: Effects of L-carnitine on serum triglyceride and cytokine levels in rat models of cachexia and septic shock. British Journal of Cancer 72: 1173–1179 (1995).
75. Wollin, S. and Jones, P.: Alpha-lipoic acid and cardiovascular disease. J. Nutr., 133: 3327-3330 (2003).
76. Worthley, L.; Fishlock, R. and Snoswell, A.: Carnitine deficiency with hyperbilirubinemia, genralized skeletal muscle weakness and reactive hypoglycemia in a patient on longterm total parenteral nutrition: Treatment with intravenous Lcarnitine. J. Parenter. Enter. Nutr., 7: 176-180 (1983).
77. Yagmur, E.; Trautwein, Ch.; Gressner, A. and Tacke, F.: Resistin serum levels are associated with insulin resistance, disease severity, clinical complications, and prognosis in patients with chronic liver diseases. Am. J. Gastroenterol., 101:1244-1252 (2006).
78. Yi, X. and Maeda, N.: Endogenous production of lipoic Acid is essential for mouse development. Mol. Cell Biol., 25(18):8387-92 (2005).
79. Yongke, L.; Xiaodong, W. and Arthur, I.: Lipopolysaccharide-induced liver injury in rats treated with the CYP2E1 inducer pyrazole. J. Am. Physiological Soc., 289 (2): G308-G319 (2005).
80. Zhang, W.; Wei, H.; Hagen, T. and Fre, B.: a-Lipoic acid attenuates LPS-induced inflammatory responses by activating the phosphoinositide 3-kinase/Akt signaling pathway. Proc. Natl. Acad. Sci. USA.; 104: 4077-4082 (2007).
81. Zhao, L.; Chen, Y. and Wang, H.: Reactive oxygen species contribute to lipopolysaccharide-induced tetragenesis in mice. Toxicol. Sci., (103): 149-157 (2008).

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