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

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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.


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 O₂⁻; 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 synthase 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 carnitine-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 vivarium 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 intraperitonealey (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+C): 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 \( \gamma \)GT 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 dismutase, 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 \( (\text{NO}_2^-) \) 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 (ONO-\text{O}) 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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Normal Control</th>
<th>Toxic LPS</th>
<th>LPS + ALA</th>
<th>LPS + CAR</th>
<th>LPS + NAC</th>
<th>LPS + Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AST (U/L) Mean +SE</td>
<td>125.734 ± 1.302</td>
<td>191.531 ± 2.694</td>
<td>152.657 ± 1.578</td>
<td>154.562 ± 1.591</td>
<td>153.789 ± 1.602</td>
<td>128.019 ± 1.347</td>
</tr>
<tr>
<td></td>
<td>ALT (U/L) Mean +SE</td>
<td>24.819 ± 0.372</td>
<td>51.218 ± 0.754</td>
<td>35.047 ± 0.521</td>
<td>34.982 ± 0.548</td>
<td>35.178 ± 0.516</td>
<td>26.319 ± 0.381</td>
</tr>
<tr>
<td></td>
<td>ALP (U/L) Mean +SE</td>
<td>26.789 ± 0.392</td>
<td>68.098 ± 0.923</td>
<td>40.245 ± 0.609</td>
<td>40.203 ± 0.623</td>
<td>44.457 ± 0.706</td>
<td>28.107 ± 0.417</td>
</tr>
<tr>
<td></td>
<td>GGT (U/L) Mean +SE</td>
<td>15.616 ± 0.192</td>
<td>52.137 ± 0.764</td>
<td>32.119 ± 0.548</td>
<td>31.993 ± 0.568</td>
<td>38.107 ± 0.627</td>
<td>20.229 ± 0.278</td>
</tr>
</tbody>
</table>

* Values are expressed as mean ± SE
* A, B, C, D, E, F Means with a common superscript within a row are significantly different \( (P<0.05) \).
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 peroxidase 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 H₂O₂ and other oxidants produced in septic cells. The decrease in GSH content and GPx 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.

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

- Values are expressed as mean ± SE
- 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 H₂O₂ 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 Gonzalez 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Normal Control</th>
<th>Toxic LPS</th>
<th>LPS + ALA</th>
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<th>LPS + NAC</th>
<th>LPS + Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β (pg/ml)</td>
<td>Mean ±SE</td>
<td>3.684 ± 0.039 A</td>
<td>10.112 ± 0.188 B</td>
<td>5.239 ± 0.089 C</td>
<td>5.252 ± 0.097 C</td>
<td>5.244 ± 0.092 C</td>
<td>3.976 ± 0.053 D</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>Mean ±SE</td>
<td>5.169 ± 0.049 A</td>
<td>22.506 ± 0.123 B</td>
<td>14.099 ± 0.085 C</td>
<td>12.506 ± 0.074 D</td>
<td>15.783 ± 0.092 E</td>
<td>8.179 ± 0.063 F</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>Mean ±SE</td>
<td>10.502 ± 0.079 A</td>
<td>31.056 ± 0.268 B</td>
<td>17.498 ± 0.268 C</td>
<td>15.329 ± 0.268 D</td>
<td>19.107 ± 0.268 E</td>
<td>10.756 ± 0.268 D</td>
</tr>
</tbody>
</table>

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- 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 (O₂⁻) (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 thiocotic 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 antioxidants 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Normal Control</th>
<th>Toxic LPS</th>
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<th>LPS + Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ±SE</td>
<td>16.347 ± 0.096 A</td>
<td>271.229 ± 3.586 B</td>
<td>105.618 ± 1.117 C</td>
<td>141.506 ± 1.843 D</td>
<td>189.098 ± 2.152 E</td>
<td>62.172 ± 0.825 F</td>
</tr>
<tr>
<td>COX-2 (ng/ml)</td>
<td>Mean ±SE</td>
<td>59.784 ± 0.604 A</td>
<td>100.548 ± 1.427 B</td>
<td>81.219 ± 0.864 C</td>
<td>76.626 ± 0.798 D</td>
<td>84.158 ± 0.902 E</td>
<td>60.033 ± 0.742 A</td>
</tr>
<tr>
<td>TNO (µmol/L)</td>
<td>Mean ±SE</td>
<td>21.379 ± 0.348 A</td>
<td>10.604 ± 0.187 B</td>
<td>15.288 ± 0.259 C</td>
<td>17.329 ± 0.296 D</td>
<td>15.302 ± 0.261 C</td>
<td>21.565 ± 0.354 A</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>Mean ±SE</td>
<td>3.329 ± 0.038 A</td>
<td>6.341 ± 0.087 B</td>
<td>5.103 ± 0.064 C</td>
<td>4.497 ± 0.059 D</td>
<td>5.116 ± 0.071 E</td>
<td>3.809 ± 0.047 F</td>
</tr>
</tbody>
</table>

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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, H2O2, 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 peroxy 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-1β 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 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 peroxy 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 peroxidative 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


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