Gastro Protective, Antiapoptotic and Anti-inflammatory Effect of Alpha-lipoic Acid on Ethanol Induced Gastric Mucosal Lesions in Rats

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ABSTRACT

Excessive alcohol consumption can lead to gastric ulcer and the present work was aimed to evaluate the potential beneficial effect of alpha lipoic acid (ALA) administration on ethanol induced gastric mucosa erosion in rats. This study was carried out on 60 male rats. The rats were divided into four equal groups of 15 rats each. Group I: (Control group): Received no drugs. Group II: (Ulcereated non-treated group): Administered with a single oral dose of 1 mL rat⁻¹ of absolute ethanol for gastric ulcer induction. Group III: (Ulcereated+ALA protected group): Received alpha lipoic acid (100 mg kg⁻¹ b.wt. day⁻¹) orally for 7 days before ethanol administration for the gastric erosion induction. Group IV: (Ulcereated+ALA treated group): Received alpha lipoic acid as in group III and the treatment was continued for 10 days later. Blood samples for serum separation were collected at the 8th and 18th days from the onset of treatment with ALA for the determination of serum Nitric Oxide (NO), Sialic Acid (SA), tumor necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6) and L-Malondialdehyde (L-MDA). Also, gastric tissue specimens were collected for determination of (L-MDA), vitamin C, Glutathione peroxidase (GPx), Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GR), reduced glutathione (GSH), DNA-fragmentation and myeloperoxidase (MPO) activity. The results showed that ethanol induced gastric damage caused significant decreased in serum (NO) and (SA) concentrations and in gastric tissue vitamin C level, GPx, SOD and CAT activities. On the other hand, a marked increase in TNF-α, IL-6, MPO, L-MDA, GR and DNA-fragmentation were observed in ethanol induced gastric damage. Pretreatment of ALA was able to mitigate gastric mucosa damage induced by ethanol through increasing of SA, vitamin C, SOD, CAT, GSH in addition to decreasing DNA-fragmentation and MPO in gastric tissue. The results of the present study suggest that, ALA may be effective in enhances the healing of gastric ulcers by its radical scavenging and antiapoptotic activity, adjusting the pro-inflammatory cytokine, inhibited neutrophil accumulation and regenerating endogenous antioxidant mechanisms.

Key words: α-lipoic acid, ethanol, apoptosis, pro-inflammatory cytokines, antioxidant enzymes

INTRODUCTION

Gastritis is an inflammation, irritation, or erosion that occurs when the endogenous defensive mechanisms of mucosal barrier cannot properly protect the organ. Usually, exposure to excess acid and pepsin causes insult on the gastrointestinal wall (Khazaei and Salehi, 2006), for more than a century, peptic ulcer disease has been a major cause of morbidity and mortality (Umamaheswari et al., 2007).
Gastric ulcer is a common disease with various etiologies, such as gastric hydrochloric acid, free oxygen radicals, ethanol and among them alcohol ingestion is an important contributor to gastric ulceration (Ham and Kaunitz, 2007). Excessive ethanol ingestion, serving as the main incentive of gastric ulcer in humans, causes acute gastric mucosal lesions through the neutrophil infiltration, release of pro-inflammatory cytokines, as well as the expression of nuclear factor-κB (NF-κB), etc., (Allavena et al., 2008; Liu et al., 2012).

The activated neutrophils will increase the production of pro-oxidative and pro-inflammatory enzymes and free radicals which lead to oxidative burst (Chatterjee et al., 2007). On the other hand, cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) play important roles in the pathogenesis of acute gastric lesions induced by ethanol (Choi et al., 2010).

Administration of absolute ethanol into the gastric lumen induced gross lesions in the glandular part of the stomach (Natale et al., 2001). Intragastrically administered EtOH rapidly penetrates the gastrointestinal mucosa, causing membrane damage, exfoliation of cells and erosion. The increase in mucosal permeability together with the release of vasoactive products from mast cells, macrophages and blood cells may lead to vascular injury, necrosis and ulcer formation. Thus, generation of free radicals by the metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells has been suggested as one of the mechanisms responsible for gastro duodenal injury (Pihan et al., 1987). The effects of alcohol on the gastric mucosa are dose-dependent and the damage appears as early as 30 min after ingestion and reaches a peak at about 60 min (Knoll et al., 1998).

Oxidative stress and depletion of anti-oxidants have been considered a crucial step in alcohol-induced mucosal damage and so they have been widely investigated in a number of studies (Arafa and Sayed-Ahmed, 2003). Overproduction of Reactive Oxygen Species (ROS) has been concerned as one of the major pathogenic factors that directly results in oxidative damage, including lipid peroxidation, protein oxidation and DNA damage, which can lead to cell death (Ali and Harty, 2009).

Alpha-lipoic acid (ALA) and its reduced form dihydro-lipoic acid are present in all prokaryotic and eukaryotic cells. Lipoic acid was once considered a vitamin but now it is commonly accepted that it can be synthesized de novo in human cells. It has long been known as a coenzyme of multienzymatic complexes catalyzing the decarboxylation of alpha keto acids. In addition, ALA is involved in the regulation of carbohydrate and lipid metabolism (Malinska and Winiarska, 2005). Moreover, Jones et al. (2002) demonstrated that, alpha lipoic acid able to neutralize free radicals and recycle or regenerate several other important antioxidants, including vitamin C and glutathione. Also, alpha lipoic acid pretreatment effectively counteracts the deleterious effect of ethanol-induced acute gastric mucosal injury (Sehirli et al., 2008). Accordingly, the purpose of the present study was to investigate the effect of alpha-lipoic acid against ethanol-induced gastric mucosal lesions in rats. Also, to determine whether alpha-lipoic acid when administered to ulcerated rats would attenuate the oxidative stress in gastric tissue, beneficial for the prevention and treatment of gastric ulcer complications and provide therapeutic alternatives for repairing gastric mucosal lesions.

MATERIALS AND METHODS
Experimental animals: A total number of 60 male albino rats, 12-16 weeks old and average body weight 200-250 g were used in the experimental investigation of this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout
the period of experiment. The animals were fed on constant ration and water was supplied *ad libitum*. The animals were left 14 days for acclimatization before the beginning of the experiment.

**Ethanol-induced gastric mucosal lesions**: Rats were fasted for 18 h and allowed free access of water prior to the administration of ethanol for gastric ulcer induction. All the rats except those of the control group orally administrated with absolute ethanol at a dose of 1 mL rat⁻¹ (Sehirli et al., 2008).

**Experimental design**: Rats were randomly divided into four main equal groups, 15 rats each, placed in individual cages and classified as follow.

**Group I (normal control group)**: Received no drugs. This group was divided into 2 subgroups:

- **Subgroup (a)**: Included 7 rats sacrificed at the 8th day of the experiment, served as the normal control rats for early ulcer group
- **Subgroup (b)**: Included 8 rats sacrificed at the 18th day of the experiment, served as the normal control rats for non-treated late ulcer group

**Group II (ulcerated non-treated group)**: Included 15 rats, administrated once orally with 1 mL rat⁻¹ absolute ethanol for induction of gastric ulcer. This group was divided into 2 subgroups:

- **Subgroup (a)**: Consisted of 7 rats served as early ulcerated non-treated group, for comparison with alpha lipoic acid protected group. This group received absolute ethanol at a dose of (1 mL rat⁻¹) on empty stomach and the rats were sacrificed after 1 h later of ethanol administration
- **Subgroup (b)**: Consisted of 8 rats served as, late ulcerated non-treated group, for comparison with alpha lipoic acid treated group. This group received absolute ethanol at a dose of (1 mL rat⁻¹) on empty stomach and the rats were left free and sacrificed ten days later of ethanol administration

**Group III (alpha lipoic acid protected rats group)**: Comprised 15 male rats received alpha lipoic acid (100 mg kg⁻¹ b.wt. day⁻¹) orally for seven days prior absolute ethanol administration. One hour after the administration of ethanol the animals were sacrificed.

**Group IV (alpha lipoic acid treated rats group)**: Included 15 male rats received alpha lipoic acid orally (100 mg kg⁻¹ b.wt. day⁻¹) for seven days before ethanol administration and the treatment were continued with ALA for ten days later.

**Sampling**: Blood samples and tissue specimens (gastric tissues) were collected 1 h after administration of ethanol in normal control group (subgroup a), ulcerated non-treated group (subgroup a) and alpha lipoic acid protected ulcer group at 8 days from the onset of treatment with α-lipoic acid. Also, blood samples and tissue specimens (gastric tissues) were collected after 17 days from the onset of treatment with α-lipoic acid in normal control group (subgroup b), ulcerated non-treated group subgroup (b) and alpha lipoic acid treated ulcer group.
**Blood samples:** Blood samples for serum separation were collected by ocular vein puncture at the end of each experimental period in dry, clean and screw capped tubes and serum were separated by centrifugation at 2500 rpm for 15 min. The clean, clear serum was separated by automatic pipette and received in dry sterile samples tube and kept in a deep freeze at -20°C until used for subsequent biochemical analysis. All serum samples were analyzed for NO, SA, TNF-α, IL-6 and L-MDA.

**Tissue samples (gastric tissue):** After seven and seventeen days of treatment with alpha-lipoic acid the rats were sacrificed by cervical decapitation. The stomach was quickly removed and opened along the greater curvature using a scraper, cleaned by rinsing with cold saline and stored at -20°C for subsequent biochemical analyses.

**Stomach tissue preparation:** Briefly, gastric tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 rpm for 15 min at 4°C then the resultant supernatant were used for the determination of the following parameters: Superoxide dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx), Glutathione reductase (GR), L-malondialdehyde (L-MDA), reduced glutathione (GSH), myeloperoxidase (MPO), vitamin C and DNA fragmentation.

**Biochemical analysis:** Serum NO, SA, TNF-α, IL-6 and L-MDA, gastric tissue vitamin C, GPx, SOD, CAT, GR, GSH, DNA fragmentation and MPO were determined according to the methods described by Vodovotz (1996); Human Sialic acid (SA) Elisa kit (Cat. No. CSB- E09605h) (Beyaert and Fiers, 1998; Chan and Perlstein, 1987; Mesbah et al., 2004); Rat Vitamin C, VC ELISA kit (Cat. No.E0913r) (Gross et al., 1967; Kakkar et al., 1984; Luck, 1974; David and Richard, 1983; Moron et al., 1979; Shi et al., 1996) and Rat Myeloperoxidase ELISA kit (Kamiya Biomedical Company, Cat. No. KT-60345), respectively.

**Statistical analysis:** The obtained data were statistically analyzed by one-way Analysis of Variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science (SPSS, 2009). Values of p<0.05 were considered to be significant.

**RESULTS**

**Effect of alpha-lipoic acid treatment on serum parameters of ethanol-induced gastric mucosal erosions in rats:** The obtained results demonstrated in Table 1 revealed that, a significant decrease in serum NO and SA concentrations were observed in ethanol-induced ulcer group in the protective period and this decrease become non-significant in the treatment period. However, a significant increase in serum TNF-α, IL-6 and L-MDA concentrations were observed in ethanol-induced gastric ulcer group in both protective and treatment period when compared with normal control rats group. Pretreatment with ALA in ethanol–induced ulcerated rats resulted in a non-significant increase in serum NO level and significant increase in SA concentration in protective period. However, treatment with ALA to ethanol-induced ulcerated rats caused a non-significant decrease in serum NO level with significant increase in serum SA level in treatment period. Also, administration of ALA resulted in significant increase in serum TNF-α in protective
Table 1: Effect of alphalipoic acid administration on serum biochemical parameters of ethanol-induced gastric mucosal erosions in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental periods</th>
<th>Animals groups</th>
<th>Protective period</th>
<th>Treatment period</th>
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<td>Normal</td>
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<td></td>
<td>control group</td>
<td>ulcerated group</td>
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<tr>
<td>Nitric oxide (NO) (mmol L⁻¹)</td>
<td>21.82±0.69⁴</td>
<td>15.61±0.68⁴</td>
<td>19.74±1.99⁴</td>
<td>32.76±3.58⁴</td>
</tr>
<tr>
<td>Sialic acid (mg mL⁻¹)</td>
<td>39.29±1.80⁴</td>
<td>25.06±0.77⁴</td>
<td>34.89±0.96⁴</td>
<td>31.97±2.52⁴</td>
</tr>
<tr>
<td>TNF-α (pg mL⁻¹)</td>
<td>42.45±4.63³</td>
<td>67.30±3.27³</td>
<td>82.76±2.38³</td>
<td>63.90±2.27³</td>
</tr>
<tr>
<td>Interleukin-6 (IL-6) (pg mL⁻¹)</td>
<td>36.56±3.40³</td>
<td>70.10±4.35³</td>
<td>64.01±5.81³</td>
<td>52.94±4.56³</td>
</tr>
<tr>
<td>L-MDA (mmol L⁻¹)</td>
<td>64.57±3.48³</td>
<td>94.13±2.38³</td>
<td>99.22±3.96³</td>
<td>86.29±5.34³</td>
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</table>

Data are presented as (Mean±S.E). SE: Standard error. Mean values with different superscript letters in the same row are significantly different at p<0.05.

Table 2: Effect of alpha-lipoic acid administration on gastric tissue parameters of ethanol-induced gastric mucosal erosions in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental periods</th>
<th>Animals groups</th>
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<tr>
<td></td>
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<td>Protective period</td>
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<tr>
<td></td>
<td></td>
<td>Normal control group</td>
</tr>
<tr>
<td>L-MDA (mmol g⁻¹ tissue)</td>
<td>56.68±4.59⁴</td>
<td>90.75±5.25⁰</td>
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<tr>
<td>Vitamin C (mg g⁻¹ tissue)</td>
<td>17.47±0.74⁴</td>
<td>9.24±0.60⁶</td>
</tr>
<tr>
<td>GPx (ng g⁻¹ tissue)</td>
<td>28.26±1.06⁴</td>
<td>22.10±0.99⁰</td>
</tr>
<tr>
<td>SOD (u g⁻¹ tissue)</td>
<td>64.30±1.92⁴</td>
<td>37.54±3.10³</td>
</tr>
<tr>
<td>CAT (mmol g⁻¹ tissue)</td>
<td>52.09±1.64³</td>
<td>39.63±2.99⁰</td>
</tr>
<tr>
<td>GR (ng g⁻¹ tissue)</td>
<td>2.36±0.69⁴</td>
<td>2.85±0.69⁰</td>
</tr>
<tr>
<td>GSH (mg g⁻¹ tissue)</td>
<td>2.70±0.79⁴</td>
<td>3.90±0.29⁰</td>
</tr>
<tr>
<td>DNA-fragmentation (cells well⁻¹ tissue)</td>
<td>38.34±6.49⁴</td>
<td>439.39±34.61¹</td>
</tr>
</tbody>
</table>

Data are presented as (Mean±S.E). SE: Standard error. Mean values with different superscript letters in the same row are significantly different at p<0.05.

period and this increase become non-significant in treatment period. Moreover, a non-significant decrease in serum IL-6 and non-significant increase in serum L-MDA after ALA administration in both protective and treatment period compared with control ulcerated non-treated groups.

**Effect of alpha-lipoic acid treatment on gastric tissue parameters of ethanol-induced gastric mucosal erosions in rats:** The obtained data presented in Table 2 revealed that, a significant increase in gastric tissue L-MDA, GR, DNA-fragmentation and MPO activity and significant decrease in GPx, SOD and CAT activities were observed in ethanol-induced ulcerated group in protective and treatment period accompanied with a significant decrease in vitamin C concentration in protective period followed by a non-significant in treatment period. However, a non-significant increase in gastric tissue GSH level was observed in ethanol-induced ulcerated
group in protective period and this increase become significant in treatment period when compared with normal control groups. Administration of alpha-lipoic acid to ethanol-induced ulcerated rats resulted in significant increase in gastric tissue vitamin C level. Meanwhile, a significant decrease in DNA-fragmentation, a non-significant increase CAT activity and a non-significant decrease L-MDA concentration were observed in protective and treatment period. A non-significant decrease in gastric tissue GPx activity was observed in protective period and this decrease become significant in treatment period. Also, a significant increase in gastric tissue SOD activity was observed in protective period and this increase become non-significant in treatment period. A significant decrease in gastric tissues GR and MPO activities were observed in protective period followed by a non-significant increase in GR activity and a non-significant decrease in MPO activity in treatment period. In addition to, a non-significant increase in gastric tissue GSH level was observed in protective period and this increase become significant in treatment period when compared with control non-treated ulcerated groups.

**DISCUSSION**

The ethanol model is widely used to assess the protective and healing activity of many drugs in ulcer studies (Choi et al., 2010). Due to its ability to reduce endogenous NO level and blood flow in gastric mucosa, which leads to a serious hemorrhagic necrosis and consequently depletes gastric mucus constituents (Kryger et al., 2000), resulting in an increased flow of Na⁺ and K⁺, elevated pepsin secretion, loss of H⁺ ions and histamine into the lumen (Szabo, 1987).

The obtained data in Table 1 revealed a significant decrease in serum NO and SA concentrations in protective period and this decrease was non significant in treatment period. Lower nitrites level in alcoholics than in control group might result from endothelium dysfunction, or decreased NOS reaction on stimuli (Enomoto et al., 2000), or NO consumption in free radicals reactions with peroxynitrites (ONOO⁻) overproduction (Banan et al., 2000). Potentially formed during NO reaction with free radicals overproduced during ethanol metabolism (Zima et al., 2001). The levels of sialic acid were found to be reduced in ethanol treated ulcers. The decrease in the glycoprotein moieties in the gastric mucosa may be attributed to the decreased activity of defense mechanisms as a result of damage to the gastric mucosa (Amudhan and Begum, 2008).

Pretreatment with ALA exhibited a non-significant increase in serum NO concentration. These results are nearly similar to those recorded by Shay et al. (2008) who reported that, because decreasing the synthesis and release of NO is the main factor to promote endothelial dysfunction, studies has been shown that LA improves endothelial NO synthesis and thus improving endothelial function. Nitric oxide has an important role in maintaining gastric mucosal integrity. Inhibition of gastric NO formation decreases gastric blood flow, deprives the tissue of oxygen and increases mucosal vulnerability to intragastric administration of irritants that mildly damage the gastric mucosa. Moreover, in the stomach NO regulation of the mucosal haemodynamics, including blood flow and hemoglobin oxygen saturation, was shown to be responsible for its important contribution to the maintenance of mucosal integrity. These aspects of NO deprivation may certainly contribute to the severity of the gastric injury induced by iodoacetamide. Inhibition of NO formation was shown to aggravate gastric mucosal injury induced by ethanol. Decrease in the availability of NO induces a decrease in the resting mucosal blood flow, resulting in tissue hypoxia (Masuda et al., 1995). The cumulative data thus show that endogenous NO is an essential protective factor in the pathogenesis of gastric injury induced by agents such as ethanol and iodoacetamide (Karmeli et al., 1996). On another hand, continuously production of NO and
superoxide anion is likely during inflammation and pathological conditions. They react together to form peroxynitrite. The scavenging effect on superoxide anion by NO may be a mechanism by which tissues of host are protected from the deleterious effects of superoxide and superoxide derived reactive oxygen species (Beckman et al., 1990). Peroxynitrite is a potent and versatile oxidant that can attack relatively slowly, a wide range of biological targets. Furthermore, peroxynitrite is toxic by more direct oxidative mechanisms. It modifies, tyrosine in proteins to create nitrotyrosine leaving a footprint detectable in vivo. Nitration of structural proteins including neurofilament and actin can disrupt thiol oxidation with major pathological consequences (Beckman and Koppenol, 1996). Data of present study indicate ALA non-significantly decrease NO overproduction in treatment period may inhibit peroxynitrite anion formation which has potent oxidative and cytotoxic activities, maintenance of intracellular antioxidant status and protected against ethanol-induced gastric mucosa damage. Also, DeMarco et al. (2004) reported that, α-LA is able to decrease the synthesis of NO by preventing the upregulation of iNOS. Another explanation for the reduction of NO level might be due to the direct scavenging effect of NO by the sulphydryl group of α-LA (Biewenga et al., 1997).

Sialic acid is the generic term given to a family of acetylated derivatives of neuraminic acid which occur mainly at terminal positions of glycoprotein and glycolipid oligosaccharide side-chains. Several biological functions have been suggested for SA, such as stabilizing the conformation of glycoproteins and cellular membranes, assisting in cell-cell recognition and interaction, contributing to membrane transport, providing binding sites for ligands for the membrane receptor functions and affecting the function, stability and survival of glycoproteins in blood circulation (Sumangala et al., 1998). In present study ALA may be help in protective of gastric mucosa from ethanol-induced gastric mucosa erosion due to its positive effect on increasing serum sialic acid concentration in protective and treatment period.

Mucus secretion is a crucial factor in the protection of gastric mucosa from the gastric lesions and has been regarded as an important defensive factor in the gastric mucus barrier. A decrease in the synthesis of sulphated mucus glycoprotein has been implicated in the etiology of gastric ulcer (Younan et al., 1982). Mucus serves as first line of defense against ulcers. Mucus is secreted by the mucus neck cells and covers the gastric mucosa. The increase in total carbohydrate: Protein (TC: P) ratio is the direct reflection of mucin activity, which is indicated by the enhanced level of individual mucopolysaccharides like hexose, hexosamine, fucose and sialic acid (Goel et al., 1994). Protection may be due to SA are known to exist in animals and occupy the terminal position of many glycoproteins. An earlier study suggested that OH reacted with a wide range of sugars including mannitol, fructose, galactose and sialic acid (Anbar and Neta, 1967). Therefore, it seems that high content of sugars in mucus secretions should give them a substantial capacity to scavenge hydroxyl radicals (Cross et al., 1984). However, most SA is abundant as the terminal sugar of sialeglycoprotein and sialoglycolipids in vivo. Further, a recent report indicated that the glycosidic linkage of sialic acid is a potential target for superoxide and other related ROS (Eguchi et al., 2005). Mucin acts as a sacrificial scavenger for OH and its protective function is exerted by the direct reaction with its sialic acids (Ogasawara et al., 2007).

Chronic ethanol treatment elevates endotoxin level and endotoxin activates kupfer's cells to produce free radicals via NADPH oxidase. The free radicals activate nuclear factor-kappa B (NF-kB), leading to an increase in production of tumor necrosis factor alpha (TNF-α), followed eventually by tissue damage (Dey and Cederbaum, 2006). The present study suggests that increased in TNF-α and non-significant decreased in IL-6 in protected and treated period may be
needed more dose or long time of treatment with LA for inhibited these proinflammatory cytokine. ALA dose-dependently inhibited TNF-α-induced I kappa B kinase activation, subsequent degradation of I kappa B (Al Rasheed et al., 2012).

The obtained data in Table 2 revealed a significant increase in gastric tissue L-MDA, GR, DNA-fragmentation and MPO activity in ethanol-induced ulcerated group. In addition to, a significant decreased in GPX, SOD and CAT activities were observed in protective and treatment period accompanied with a significant decrease in vitamin C in protective period followed by a non-significant increases in treatment period. In addition to, a non-significant increase in gastric tissue GSH level in protective period followed by a significant increase in treatment period. Malondialdehyde is the final product of lipid peroxidation and is used to determine lipid peroxidation levels (Johansen et al., 2005). There is consensus that the deleterious effects of ethanol on gastric mucosa are consequence of enhanced lipid peroxidation. The presence of oxygen free radicals that cause lipid peroxidation have been reported in the pathogenesis of gastric mucosal lesions induced by ulcer inducing agents such indomethacin, alcohol and aspirin in rats (Takeuchi et al., 1989). Free oxygen radicals initiate lipid peroxidation by removing one hydrogen atom from polyunsaturated fatty acids with the subsequent formation of hydro peroxides. As a result of these reactions, the membrane fluidity and membrane integrity of cells are impaired, leading to disintegration of cells and cell death. These subcellular structures that are released into the extracellular environment trigger several inflammatory events and further worsen the ongoing damage (Esrefoglu et al., 2006). In the present study ALA non-significantly decreased L-MDA level in gastric tissue. Similarly, Arivazhagan et al. (2002) suggested that, DL-α-lipoic acid is found in human and animal tissue, where it is a latent antioxidant that mainly suppresses lipid peroxidation following oxidative stress and improves the levels of other antioxidants. The present observation also showed that, protection and treated with LA associated with a non-significant increase in serum MDA may due to decrease of antioxidant enzymes in blood due to increasing of free radical and may also depended on the LA dose, where Lipoic acid dose-dependently decreased plasma creatinine level and lipid peroxidation (Somani et al., 2000).

Vitamin C is a water-soluble antioxidant present in the circulation and tissues (Kucharz, 1992). It scavenges and destroys the free radicals in combination with glutathione. The observed decreased in these antioxidants in ulcerated rats may be due to increased utilization in scavenging the free radicals (Prakash et al., 2008). The release of oxygen-derived free radicals (ROS) has drawn attention as a possible pathogenic factor of gastric mucosal injury associated with ethanol consumption (Smith et al., 1996). To scavenge ROS, gastric cell have several enzymatic and non-enzymatic antioxidants including CAT, SOD, GPx, myeloperoxidase (MPO) and endogenous GSH but excessive generation of ROS enhance lipid peroxidation and depletes these antioxidants enzymes. Superoxide produced by peroxidase in the stomach tissues might damage cell membranes and cause ulcer by increasing MDA level (Cadirei et al., 2007). SOD is considered as the first line of defense against the deleterious effects of oxygen radicals in the cells and it scavenges ROS by catalyzing the dismutation of superoxide to H2O2 (Okado-Matsumoto and Fridovich, 2001). There is evidence to indicate that ethanol significantly depresses SOD activities (Rukkumani et al., 2004).

Ethanol inhibited SOD and thus superoxide radicals could not convert to H2O2. The inhibition of SOD activity may result in an increased flux of superoxide in cellular compartments which may be the reason for the increased lipid peroxidative indices. In this context, present result is near similarly with that reported by Megala and Geetha (2010). Moreover, ethanol decreased the gene expression and the activity of SOD in the gastric mucosa, suggesting that the suppression of key
mucosal antioxidant enzyme, along with the elevation of lipid peroxidation, play an important role in the pathogenesis of these lesions (Brzozowski et al., 1998). In the present study, SOD activity decreased significantly in the ETOH treated group of animals, which might be due to an excessive formation of superoxide anions. These excessive superoxide anions might inactivate SOD and decrease its activity. In the absence of adequate SOD activity, superoxide anions are not dismutase into H$_2$O$_2$, which is the substrate for the H$_2$O$_2$ scavenging enzymes CAT and GPx. These result in inactivation of the H$_2$O$_2$ scavenging enzymes CAT and GPx, leading to a decrease in their activities (Panda et al., 2012). The increase in glutathione appears to result in efficient glutathione recycling. Although the increase in the activity of GR can promote the recycling of glutathione for the active detoxification of xenobiotics, the decrease in GPx activity may attenuate the radical scavenging function (Oh et al., 1998), GR accelerating the conversion of GSSG to GSH and enhancing the detoxification of reactive metabolites by conjugation with GSH (Panda et al., 2012).

Pretreatment with ALA in ethanol-induced ulcerated rats resulted in significantly increase vitamin C and SOD activity accompanied with non-significant increase of CAT and GSH concentration. Meanwhile, significantly decreased GR, DNA-fragmentation and MPO activity accompanied with non-significantly decrease GPX activity. Antioxidant effects of LA is based on their interactions with peroxyl radicals, which are essential for the initiation of lipid peroxidation; and ascorbyl radicals of vitamin C. DHLA, can recycle ascorbyl radicals and reduce dehydroascorbate generated in the course of ascorbate oxidation by radicals. Therefore, DHLA may act as a strong chain-breaking antioxidant and may enhance the antioxidant potency of other antioxidants like vitamin C in both the aqueous and in hydrophobic membrane phase (Padayatty et al., 2002). Vitamin C is an excellent hydrophilic antioxidant in plasma, because it disappears faster than other antioxidant when plasma is exposed to reactive oxygen species (Frei et al., 1989). The elevated levels of vitamin C and SOD activities in ALA-supplemented group play a protective role against oxidative stress. ALA replenishes vitamin C, glutathione and vitamin E through the reduction of their radicals via the redox cycle (Packer et al., 2001). GR activity decrease in protected period may due to the over production of free radical and hydrogen peroxide. GSH is required to maintain the normal reduced state and to counteract the deleterious effects of oxidative stress. During the reduction of hydrogen peroxide, GSH is oxidized to GSSG. When GSSG levels are enhanced, the GSH-reductase activity was activated to convert GSSG in GSH (Cui et al., 2011). The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues (Kojio, 2004). Therefore, the reduction in the GR activity may result in a number of deleterious effects due to the accumulation of superoxide radicals and H$_2$O$_2$ in protective period. Meanwhile, after treatment with LA the concentration of GSH increased and superoxide radicals decreased. Therefore, GR activity increased in treatment period. Glutathione peroxidase plays a primary role in minimizing oxidative damage. (GPx), an enzyme with selenium and Glutathione-s-transferase (GST) works together with glutathione in the decomposition of H$_2$O$_2$ or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione (Freeman and Crapo, 1982). Reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme (Hodgson and Fridovich, 1975). Also, decline in the GPX activity may be due to over production of free radical induced cells damage. It is now known that, when there is an imbalance between free radical production and antioxidant defenses, ‘oxidative stress’ occurs resulting in deregulation of cellular functions (Bandyopadhyay et al., 1999).

In present study, pretreatment with ALA associated with increased SOD activity in gastric tissue. The enzymatic antioxidant defence systems are the natural protectors against lipid
peroxidation. They include superoxide dismutase and glutathione peroxidase (Koppenol, 1981). Superoxide dismutase is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O$_2$ and to the less reactive species H$_2$O$_2$. Peroxide can be destroyed by CAT or GPX reactions (Teixeira et al., 1998). First, SOD converts the superoxide anion to hydrogen peroxide in a cellular antioxidant reaction. Thereafter, GSH-Px detoxify hydrogen peroxide produced (Jaeschke, 1995). It has been suggested that LA can reduce oxidized GSH and increase the GSH status, which in turn exhibits increased free radical scavenging property, so LA indirectly influences the activity of SOD thereby preventing the deleterious effect of superoxide radical formed. That causes activation of SOD (Selvakumar et al., 2005). Glutathione and glutathione-related enzymes play a key role in protecting the cells against the damaging effects of reactive oxygen species. Intracellular GSH can act as a reductant, reducing hydrogen peroxide and lipid hydroperoxides directly to H$_2$O, a reaction catalyzed by GSH-Px. Depletion of intracellular GSH, under conditions of continuous intracellular oxidative stress, leads to oxidation and damage of lipids, proteins and DNA by the reactive oxygen species (Nordberg and Arner, 2001). Alpha-lipoic acid was found to prevent GSH depletion by scavenging reactive oxygen species (Lu and Liu, 2002). Therefore, it inhibits the oxidative damage of cellular macromolecules. Also, α-LA can increase GSH levels by increasing cysteine uptake, which is a rate limiting step for GSH biosynthesis (Han et al., 1997).

The present study indicates that EtOH exposure increases the apoptotic DNA fragmentation ratio of the gastric mucosa, which seems to be responsible of severe injury. Furthermore, it was investigated that ROS may cause DNA-fragmentation (Bagchi et al., 1999). Under normal physiological conditions, the balance between gastric epithelial cell proliferation and death is of great importance in maintaining gastric mucosal integrity. Since, the balance between cell apoptosis and cell proliferation has important role to keep the gastric mucosa healthy (Kalia et al., 2000). Since, the gastric epithelial cells proliferate in the lower part of the glandular neck and migrate up the crypt towards the surface and then are shed into the lumen by apoptosis (Ohkura et al., 2003). Disturbance of this balance could result in either cell loss, leading to mucosal damage and ulcer formation, or cell accumulation, leading to cancer development (Kohda et al., 1999). The gastric mucosal hemorrhage evoked by extra amounts of alcohol is initiated by the microcirculatory damage of the gastrointestinal mucosa, namely a disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting. It has been shown that EtOH dramatically increases the low level of spontaneous apoptosis in gastric tissues (Chattopadhyay et al., 2004), which normally occurs to protect against the survival and expansion of genetically damaged cells. LA treatment, in the present study, suppressed the high percentage of DNA fragmentation in the gastric mucosa while gastric epithelial integrity was maintained. Similarly, Vincent et al. (2005) demonstrated that, application of the antioxidant LA in animal and cell culture models decreases oxidative stress and supports the endogenous antioxidant systems potently- and apoptosis-related cell death in tissues exposed to oxidant injury. In accordance with the previous studies, current findings revealed that oxidative stress-induced apoptotic DNA fragmentation in the gastric mucosa, which seems to be responsible for acetic acid-induced chronic gastric damage, was prevented by LA treatment. Recent findings provide evidence that LA enhances the healing of chronic gastric ulcers by its radical scavenging and antiapoptotic activity, as well as by regenerating endogenous antioxidant mechanisms (Karakoyun et al., 2009).

Myeloperoxidase is an essential enzyme for normal neutrophil function, released into extracellular fluid as a response to various stimulatory substances. MPO activity is considered as
an index for the evaluation of neutrophil infiltration. In the present study, a significant increase in gastric tissue MPO activity was observed in ethanol-induced ulcerated group. The elevated activity of MPO in the gastric mucosa indicates oxidative injury induced by ethanol involves the contribution of neutrophil accumulation (Amudhan and Begum, 2008). The increase in enzyme activity level may be associated with increase in the levels of neutrophil infiltration and \( \text{H}_2\text{O}_2 \) in the gastric damaged tissues administered with ethanol (Megala and Geetha, 2010). Also, one mechanism in the pathogenesis of mucosal lesions provoked by ethanol may be circulating neutrophils (Kvietys et al., 1990). The leukocytes might create gastric ulcerations through various mechanisms, such as the production of reactive oxygen metabolites or the release of proteases and lipid mediators (Zimmerman and Granger, 1994). Moreover, activated neutrophils produce many enzymes and free radicals that damage the gastric mucosa, neutrophil is considered as an aggressive factor in ulcer formation (Fukumura et al., 1995).

The gastric injury induced by EtOH involves toxic oxygen metabolites. Since one of the sources of oxygen radicals in gastric mucosal injury induced by EtOH in rats seems to be the neutrophils (Vazquez-Ramirez et al., 2006). In the present study, the role of neutrophils was assessed by tissue-associated MPO activity, demonstrating a significant elevation in both periods of ulcer. On the other hand, ALA treatment inhibited the increase in MPO activity and restored it to control levels, suggesting that the neutrophil infiltration could be delimited by the antioxidant agent. These findings are in parallel with the CL data, defining the role of neutrophils in the release of ROS (Karakoyun et al., 2009). In accordance with present data, it was shown that ALA and DHLA are powerful scavengers of HOCl, an oxidant produced by neutrophils (Biewenga et al., 1994). Furthermore, ALA pretreatment was previously shown to offer gastric mucosal protection against ethanol-induced acute lesions by a neutrophil-dependent mechanism (Sehirli et al., 2008).

CONCLUSION

In conclusion, the present study demonstrated that, alpha-lipoic acid possesses significantly gastroprotection and treatment effects against gastric ulcer and oxidative damage in gastric tissue induced by ethanol in rats. Since, \( \alpha \)-lipoic acid was able to ameliorate serum biochemical parameters, enzymatic and non-enzymatic antioxidant defense system, mucus secretion and prevent DNA fragmentation in gastric tissue. Based on the data of the current study, the effect of alpha-lipoic acid against ethanol-induced gastric lesions can be attributed to the inhibitory effects on neutrophil infiltration and its reduction of pro-inflammatory cytokines as well as its antiapoptotic effect.

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