



## PROTECTIVE ROLE OF ALPHA-LIPOIC ACID ON SOME BIOCHEMICAL MEMBERS OF ETHANOL INDUCED GASTRIC MUCOSAL LESIONS IN RATS

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### ABSTRACT

In the present study, the protective effect of alpha lipoic acid (ALA) administration on nitric oxide (NO), sialic acid (SA), vitamin C, antioxidant enzymes, reduced glutathione (GSH), and DNA-fragmentation in ethanol-induced gastric mucosa erosion rats have been evaluated. This study was carried out on 45 male rats. The rats were divided into three equal groups of 15 rats each. Group I :( Control group): received no drugs. Group II :( ulcerated non-treated group): Administration of a single oral dose of 1 ml/rat of absolute ethanol for ulcerated induction and Group III :( ulcerated + ALA protected group): received ALA orally in a daily dose of 100 mg/kg body weight for seven days prior ethanol administration. Ethanol-induced a significant decreased in serum (NO) and (SA) and vitamin C, GPX, SOD and CAT activities in gastric mucosa tissue. Also, marked increase of GR activity and DNA-fragmentation and, non-significantly increases on GSH concentration. Pretreatment with ALA to ethanol-induced ulcerated rats significantly increase SA, vitamin C and SOD activity. Also, non-significantly increase of NO, CAT and GSH concentration. Meanwhile, significantly reduced GR and DNA-fragmentation was observed as well as non-significantly decrease GPX activity. These results suggest that, ALA may be effective in enhances the healing of gastric ulcers by its radical scavenging and antiapoptotic activity, as well as by regenerating endogenous antioxidant mechanisms.

**KEY WORDS:**  $\alpha$ -lipoic acid; Ethanol; Gastric mucosal injury; Apoptosis; Antioxidant enzymes.

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### 1- 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 exceed acid and pepsin causes insult on the gastrointestinal wall [1], for more than a century, peptic ulcer disease has been a major cause of morbidity and mortality [2].

Administration of absolute ethanol into the gastric lumen induced gross lesions in the glandular part of the stomach [3]. 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 [4]. The effects of alcohol on the gastric mucosa are dose-dependent, and the damage appears as early as 30 minutes after ingestion and reaches a peak at about 60 minutes [5].

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 [6]. 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 [7].

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 multi-enzymatic complexes catalyzing the decarboxylation of alpha keto acids. In addition, ALA is involved in the regulation of carbohydrate and lipid metabolism [8]. It is said that alpha lipoic acid able to neutralize free radicals, and recycle or regenerate several other important antioxidants, including vitamin C and glutathione [9]. Also, alpha lipoic acid pretreatment effectively counteracts the deleterious effect of ethanol-induced acute gastric mucosal injury [10]. Therefore, in light of abovementioned findings, the aim of the present study was to investigate whether and to what extent LA, would provide protection against gastric mucosal injury.

## 2. MATERIAL AND METHODS:

### 2.1. *Experimental animals:*

A total number of 45 Male albino rats, 12-16 weeks old and average body weight 200-250 gm were used in the experimental investigation of this study, and obtained from the Laboratory Animals Research Center, Faculty of Veterinary Medicine, Benha University, housed in separate wire mesh cages, exposed to good ventilation, humidity and to a 12-hr light - dark cycle, and provided with a constant

supply of standard pellet diet and fresh, clean drinking water *ad libitum*.

### 2.2. *Ulcerated Induction:*

After 7 days from the onset of treatment with  $\alpha$ -lipoic acid. Rats were fasted for 18 hours and allowed free access of water. Fasting for 18 hours before gastric ulcer induction to ensure empty stomach. The ulceration was induced by the administration of absolute ethanol orally at a dose of 1 ml of absolute ethanol to each rat according to [10].

### 2.3. *Experimental design:*

Rats were randomly divided into three main equal groups, 15 rats each, placed in individual cages and classified as follow:

Group I (control normal group): received no drugs, served as control non-treated for all experimental groups.

Group II (ulcerated non-treated group): received no drugs and served as ethanol-induced gastric mucosa erosion groups.

Group III (ulcerated alpha lipoic acid protected group): received alpha lipoic acid orally in a daily dose of 100 mg/kg body weight for seven days before ethanol induced gastric erosion.

### 2.4. *Sampling:*

Random blood samples and tissue specimens (gastric mucosa tissue) were collected one hour after administration of ethanol from all animals groups (control and experimental groups) after 7 days from the onset of treatment with  $\alpha$ -lipoic acid.

### 2.5. *Blood samples:*

Blood samples for serum separation were collected by ocular vein puncture at the end of experimental period in dry, clean, and screw capped tubes and serum were separated by centrifugation at 2500 r.p.m for 15 minutes. 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 the

Nitric oxide (NO) and Sialic acid (SA) analysis.

#### 2.6. Tissue samples (gastric mucosal tissue):

At the end of experimental period the rats were sacrificed. The abdomen was opened and the stomach was rapidly excised gently, rinsed with ice-cold isotonic saline, cleared off blood, photographed and immediately transferred into ice-cold isotonic saline again, then blotted between 2 filter papers. These tissue samples were quickly frozen in a deep freezer at (-20 °C). Stomach tissues were divided into appropriate portions, and 0.5 gm from each were homogenized in 5 ml - 10 % (w/v)-cold phosphate buffer saline (PBS) per gram tissue, using tissue homogenizer, and then centrifuged at 10,000 r.p.m for 20 minutes at 4°C, the resulting supernatant was assayed for the determination of vitamin C, antioxidant enzymes (glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR)), reduced glutathione (GSH) and DNA fragmentation.

#### 2.7. Biochemical analysis:

Serum (NO), (SA) and stomach vitamin C, (GPX), (SOD), (CAT) and (GR)), (GSH) and DNA fragmentation were analyzed according to the methods described by [11]; Human Sialic acid (SA) Elisa kit (Cat. no. CSB- E09605h); Rat Vitamin C, VC ELISA kit (Cat .No.E0913r); [12]; [13]; [14]; [15]; [16] and [17].

#### 2.8. 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, 13.0 software, 2009). Values of  $P < 0.05$  were considered to be significant.

### 3. RESULTS:

The obtained data in table (1) revealed a significant decrease in serum NO and SA in ethanol- induced ulcerated group. Pretreatment with ALA in ethanol – induced ulcerated in rats resulted in non-significant increase in serum NO accompanied with significant increase in SA. The obtained data in table (2) revealed a significant decreased in gastric mucosa tissue vitamin C, GPX, SOD and CAT accompanied with significant increase of GR activity and DNA-fragmentation and, non-significantly increases on GSH in ethanol- induced ulcerated group. Pretreatment with ALA in ethanol – induced ulcerated in rats resulted in significantly increase vitamin C and SOD activity accompanied with non-significant increase of CAT and GSH concentration. Meanwhile, significantly reduced in GR and DNA-fragmentation accompanied with non-significantly decrease GPX activity.

### 4. DISCUSSION:

The ethanol model is widely used to assess the protective and healing activity of many drugs in ulcer studies [18]. 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 [19].

The obtained data in table (1) revealed a significant decrease in serum NO and SA. Lower nitrites level in alcoholics than in control group might result from endothelium dysfunction, or decreased NOS reaction on stimuli [20], or NO consumption in free radicals reactions with peroxynitrites (ONOO-) overproduction [21], potentially formed during NO reaction with free radicals overproduced during ethanol metabolism [22]. The levels of sialic acid were found to be significantly 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

[23]. Pretreatment with ALA exhibited a non-significant increase in serum NO concentration these results are nearly similar to those reported by Shay et al. [24], 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 [25]. 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 [26].

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[27]. In present study ALA may be help in protective of gastric mucosa from ethanol-induced gastric erosion due to its positive effect on increasing serum sialic acid concentration in protected rats groups. 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 [28].

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 [29]. Protection against experimental ulcer 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 [30]. Therefore, it seems that high contents of sugars in mucus secretions should give them a substantial capacity to scavenge hydroxyl radicals [31]. However, most SA is abundant as the terminal sugar of sialoglycoprotein 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 [32]. Mucin acts as a sacrificial scavenger for -OH and its protective function is exerted by the direct reaction with its sialic acids [33].

The obtained data in (Table 2) revealed that, a significant decreased in gastric mucosa tissue of vitamin C level, GPX, SOD and CAT activities with significant increase in GR activity and DNA-fragmentation as well as, non-significant increase in GSH contents were observed.

Vitamin C is a water-soluble antioxidant present in the circulation and tissues [34]. It scavenges and destroys the free radicals in combination with glutathione. The observed decrease in these antioxidants in ulcerated rats may be due to increased utilization in scavenging the free radicals [35]. The release of oxygen-derived free radicals (ROS) has drawn attention as a possible pathogenic factor of gastric mucosal injury associated with ethanol consumption [36]. 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 H<sub>2</sub>O<sub>2</sub> [37]. It has been reported that ethanol inhibited SOD and thus superoxide radicals could not convert to H<sub>2</sub>O<sub>2</sub>. 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, the obtained results are near similarly with that reported by Megala and Geetha [38]. 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 [39]. In the present study, SOD activity decreased significantly in the ETOH treated group of rats, 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 dismutated into H<sub>2</sub>O<sub>2</sub>, which is the substrate for the H<sub>2</sub>O<sub>2</sub> scavenging enzymes CAT and GPx. This results in inactivation of the H<sub>2</sub>O<sub>2</sub> scavenging enzymes CAT and GPx, leading to a decrease in their activities [40]. 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 [41], GR accelerating the conversion of GSSG to GSH and enhancing the detoxification of reactive metabolites by conjugation with GSH [40]. The observed results also indicate 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 [42]. 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 [43]. 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 [44]. Disturbance of this balance could result in either cell loss, leading to mucosal damage and ulcer formation, or cell accumulation, leading to cancer development [45].

Pretreatment with ALA in ethanol-induced ulcerated rats resulted in significant increase in vitamin C concentration and SOD activity accompanied with a non-significant increase in CAT activity and GSH concentration. Meanwhile, a significant decrease in GR and DNA-fragmentation accompanied with a non-significant decrease in GPX activity were observed in ALA pretreatment rats groups. Antioxidant effects of LA is based on their interactions with peroxy radicals, which were essential for the initiation of lipid peroxidation and ascorbyl radicals of vitamin C. Dihydrolipoic acid (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 [46]. The elevated levels of vitamin C and SOD activities in ALA-treated 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 [47].

Glutathione reductase activity was decreased in protected rats group. Such decreased may be 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 [48].

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<sub>2</sub>O<sub>2</sub> or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione [49]. Reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme [50]. 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 [51].

In the present study, pretreatment with ALA associated with SOD increased in gastric mucosal tissue. The enzymatic antioxidant defence systems are the natural protectors against lipid peroxidation. They include superoxide dismutase and glutathione peroxidase [52]. First, SOD converts the superoxide anion to hydrogen peroxide in a cellular antioxidant reaction. Thereafter, GSH-Px detoxify hydrogen peroxide produced [53]. 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 [54]. 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<sub>2</sub>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 [55]. Alpha-lipoic acid was found to prevent GSH depletion by scavenging reactive oxygen species [56]. Therefore, it inhibits the oxidative damage of cellular macromolecules. Also,  $\alpha$ -LA can increase GSH levels by increasing cysteine uptake, which is a rate limiting step for GSH biosynthesis [57].

Lipoic acid 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. [58] who 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

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mechanisms [59]. In conclusion, the present study demonstrated that alpha-lipoic acid pretreatment provided an effective protection against ulcer and oxidative damage in gastric mucosa 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 mucosa tissue. We recommended that, administration of diet rich in the antioxidant alpha lipoic acid is very important for protection of different body tissue, especially gastric mucosa tissue, against oxidative stress or even inflammation or erosion.

Table 1: Effect of Alpha-lipoic acid pretreatment on serum Nitric oxide and Sialic acid concentrations in ethanol-induced gastric mucosal erosion in rats:

	Nitric oxide mmol/l	Sialic acid mg/ ml
Control normal group	21.62± 0.99a	39.29±1.89a
Control ulcerated group	15.61± 0.68b	25.96±0.77c
Ulcerated+ $\alpha$ -lipoic acid protected group	19.74± 1.99ab	34.89±0.96b

Table 2: Effect of Alpha-lipoic acid pretreatment on gastric mucosa tissue parameters of ethanol-induced gastric mucosal erosion in male rats:

	Vit C ng/g tissue	GPx ng/g tissue	SOD u/g tissue	CAT mmol/min /g tissue	GR ng/min /g tissue	GSH ng/g tissue	DNA- fragmentation cells/ well. tissue
Control Normal Group	17.47±0.74 a	28.26±1.06 a	64.30±1.92 a	52.09±1.64 a	2.36±0.06 b	2.70± 0.75b	38.34±6.46c
Control ulcerated group	9.28± 0.69c	22.10±0.99 b	37.54±3.31 c	39.63±2.29 b	2.85±0.06 a	3.90±0.29a b	439.39±34.61 a
Ulcerated + $\alpha$ -lipoic acid protected group	14.43±0.28 b	21.41±1.04 b	51.60±1.21 b	40.44±2.04 b	2.29±0.15 b	5.19± 0.21a	143.99±30.85 b

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## الدور الوقائي لحمض ألفا ليبويك على بعض الدلالات الكيميائية الحيوية لتقرح جدار البطانة المخاطية للمعدة المحدث تجريبيا بالكحول الايثيلي في الفئران

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### الملخص العربي

في هذه الدراسة تم تقييم التأثير الوقائي لحمض ألفا ليبويك على التغيرات في مستوى أكسيد النيتريك وحمض السياليك، فيتامين ج، الإنزيمات المضادة للأكسدة، الجلوتاثيون المختزل وتفتيت الـ دي ان ايه في دم وأنسجة الجرذان المستحدث فيها تآكل جدار البطانة المخاطية للمعدة بالكحول الايثيلي. هذا وقد أستخدم لأجراء هذه الدراسة عدد 45 من ذكور الجرذان البيضاء أعمارهم تتراوح من 12-16 أسبوع وأوزانها من 200-250 جرام وقد قسمت إلي ثلاث مجموعات متساوية اشتملت كل مجموعة على عدد خمسة عشر فأر وتم توزيعها كالاتي: المجموعة الأولى: (المجموعة الضابطة): اشتملت على 15 فأر لم تعطى أي أدوية واستخدمت كمجموعة ضابطة للمجموعات الأخرى. المجموعة الثانية: (المجموعة المحدث بها مرض قرحة المعدة): تكونت من 15 فأر تم اعطاؤها الكحول الايثيلي عن طريق الفم بتركيز 100% بمعدل (1 مل / فأر). المجموعة الثالثة: (مجموعة حمض ألفا ليبويك والمحدث بها مرض قرحة المعدة): اشتملت على 15 فأر تم تجريبها حمض ألفا ليبويك يوميا عن طريق الفم بجرعة مقدارها (100 مللي جرام/ كيلوجرام) لمدة 7 ايام وفي اليوم الثامن تم تجريبها الكحول الايثيلي عن طريق الفم بتركيز 100% بمعدل (1 مل / فأر) لإحداث تآكل في جدار البطانة المخاطية للمعدة. وقد تم تجميع عينات الدم والأنسجة في اليوم الثامن من بداية التجربة بعد ساعة من تجريبها بالكحول الايثيلي. وقد أسفرت نتائج التحليل البيوكيميائي عن وجود انخفاض معنوي في كلا من أكسيد النيتريك وحمض السياليك بالمصل بالإضافة إلى مستوى فيتامين ج وأيضاً نقص معنوي في نشاط الجلوتاثيون بيروكسيداز و سوبر أكسيد ديسميوتيز و الكاتاليز في نسيج المعدة مع حدوث زيادة في نشاط انزيم الجلوتاثيون ريدكتاز و دي ان ايه فرجمنتاشن في المجموعه المحدث بها قرحة معدية. كما أوضحت النتائج أن مجموعة الجرذان المحدث بها مرض القرحة بالمعدة والتي تم وقايتها بـ حمض ألفا ليبويك أظهرت زيادة في كلا من أكسيد النيتريك وحمض السياليك بالدم بالإضافة الى فيتامين ج و سوبر أكسيد ديسميوتيز و الكاتاليز بالمعدة في حين انخفض مستوى نشاط انزيم الجلوتاثيون ريدكتاز و دي ان ايه فرجمنتاشن. وأوضحت الدراسة أن استخدام حمض الفالبيويك كماده واقية مضادة للأكسدة كان لها دور فعال في حماية الغشاء المبطن للمعدة من التآكل والتقرح المحدث تجريبيا في الجرذان باستخدام الكحول الايثيلي المطلق وأدى استخدامه كذلك الى الحفاظ على نسب القياسات البيوكيميائية في الدم والأنسجة لما يقارب النسب الطبيعية، ويرجع ذلك إلى نشاط حمض الفالبيويك المضاد للأكسدة ولتكسير الـ دي ان ايه وللموت الخلوي للخلايا. لذلك توصي الدراسة بضرورة استغلال تلك المزايا الهائلة لحمض الفالبيويك كماده وقائية مضادة للأكسدة وإدخاله كماده فعالة في صناعة العقاقير الطبية المستخدمة في وقاية وعلاج المعدة من الالتهابات والقروح.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(2):165-176, ديسمبر 2013)