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Effect of *Helicobacter Pylori* Eradication Therapy and some Antioxidants on Ulcer Healing Rates in Patients with *Helicobacter pylori*-associated Duodenal Ulcer.

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Research Article

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ABSTRACT

The aim of this study is to evaluate the status of endogenous antioxidant defense system in *H. pylori*-infected endoscopically diagnosed chronic duodenal ulcer patients through the assessment of certain oxidative stress biomarkers. These biomarkers namely plasma malodialdehyde (MDA) and blood activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) are to be evaluated before and after the administration of the histamine H₂ antagonist nizatidine alone or with either vitamin E (α -tocopherol) or thioctic acid (α -lipoic acid) or a combination of both. The same biomarkers were also assessed before and after administration of *H. pylori* eradication therapy. The effect of antioxidant and *H.pylori* eradication therapies on ulcer healing rates was also investigated. Moreover, the efficacy of *H.pylori* eradication by combined use of nizatidine with the macrolide antibiotic "clarithromycin" and amoxicillin was also evaluated in close association to ulcer healing. *H.pylori*-infected duodenal ulcer patients are subjected to massive oxidative stress as reflected by a significant decrease of erythrocytic GPx and SOD activities and elevation of plasma MDA levels. Treatment of *H.pylori*-infected duodenal ulcer patients with nizatidine alone failed to produce any significant change in any of the measured oxidative stress biomarkers. Treatment with nizatidine in combination with either vitamin E, thioctic acid or a combination of both resulted in a significant increase in erythrocytic GPx and SOD activities and a significant decrease in plasma MDA level. Some post-treatment values of certain oxidative stress biomarkers (e.g. MDA, GPx and SOD) were not significantly different from normal control values following oral treatment with *H.pylori* eradication therapy or nizatidine combinations with either vitamin E, thioctic acid or a combination of both. The use of triple *H.pylori* eradication therapy consisting of nizatidine (300mg once daily for 6-weeks), clarithromycin (500mg twice daily for 10 days) and amoxicillin (1000mg twice daily for 10 days) resulted in 100% eradication rate of the organism in *H.pylori*-infected duodenal ulcer patients. Attenuation of oxidative stress in *H.pylori*-infected peptic ulcer patients either by combined nizatidine/antioxidant treatment or by *H.pylori* eradication therapy was associated with higher ulcer healing rates (on a percentage basis) as compared with patients treated with nizatidine alone. In patients receiving *H.pylori* eradication therapy, the ulcer healing rate was 100% compared to 60% in those treated with nizatidine alone and 86.67%, 73.34% and 93.34% in patients treated with nizatidine in combination with either vitamin E, thioctic acid or a combination of both respectively.

INTRODUCTION

Peptic ulcer is a common serious disease that affects 10% of adult population worldwide. The symptoms range from mild irritation and pain to haemorrhage and possibly perforation. The pathogenesis of gastroduodenal mucosal injury is multifactorial and involves an imbalance between several aggressive and protective factors.

Among the aggressive factors excess acid, pepsin, *H.pylori* infection, tobacco smoking, non-steroidal anti-inflammatory drugs (NSAIDs), bile reflux and oxidative stress are of outstanding importance. The most important protective factors are the mucus-bicarbonate barrier, regional blood flow and cytoprotective prostaglandins [1,2,3].

A peptic ulcer is defined as a disruption of the mucosal integrity of the lower oesophagus, stomach and duodenum [4,5]. Ulcers in the stomach or duodenum may be acute or chronic. Both types can penetrate the muscularis mucosa, but the acute one shows no evidence of fibrosis [4].

Helicobacter pylori (HP) is a gram-negative spiral-shaped organism closely associated with gastritis and peptic ulceration. The bacterium lives underneath the adherent mucus gel layer of the stomach especially in the antrum but always outside the surface epithelium [6]. It has the property of ammonia production from urea through the activity of its urease enzyme. The organism protects itself from the bactericidal effect of gastric acidity partly by the mucus gel layer and partly by ammonia production [7].

Mucosal epithelial and lamina propria endothelial cells are the prime targets for the destructive actions of HP colonization. The bacterium secretes a chemotactic factor for neutrophils and monocytes [8,9], resulting in their activation and the generation of reactive oxygen species (ROS) including superoxide anion and hydroxyl radicals, hydrogen peroxide and hypochlorous acid [10,11].

Helicobacter pylori can be diagnosed either by invasive or non-invasive methods. The invasive methods are biopsy-based and include culture on special media [11], staining of tissue sections [12] and biopsy urease test. In the latter test, biopsy tissue is put into a small amount of urea solution with an indicator; if HP is present, the pH changes from acid to alkaline (within few minutes to 2 hours) due to the generation of ammonia from urea under the influence of HP urease [13]. The non-invasive methods are the most preferable methods and include urea breath tests in which C¹⁴ or C¹³-labelled urea is fed to the patient and the emission of the isotope as CO₂ is measured in the breath. Patients infected with HP give high readings of the isotope because of the breakdown of urea to CO₂ (and ammonia) through the action of HP urease [14]. Serology is another non-invasive technique where immunological assays can be used to detect antibodies (IgG, IgM or IgE) to HP or its urease in body fluids [15]. Also, rapid bed-side stool antigen tests for *H.pylori* detection are now available for commercial use [16].

ROS are implicated in the pathogenesis of acute and chronic peptic ulceration being detrimental to the integrity of the gastroduodenal mucosa and more crucial than acid hypersecretion in this respect [17]. When these radicals were removed by radical scavengers, it was possible to treat effectively acute and chronic peptic ulceration [18] and stimulate the healing of refractory ulcers [19]. ROS are also involved in the progression and relapse of *H.pylori*-associated peptic ulceration and exert an adverse effect on ulcer healing [20].

Durak et al., [21], demonstrated that superoxide dismutase (SOD) activity was elevated in the gastric mucosa of patients with acute peptic ulcer reflecting a possible role for ROS in the stimulation of endogenous antioxidant enzymes in the acute phase of the disease. In another study by Kedziora et al., [22], raised plasma level of malondialdehyde (MDA), a marker of lipid peroxidation was detected in association with reduced blood SOD activity in peptic ulcer patients. The same author concluded that, in spite of similar range of superoxide anion generation in peptic ulcer patients and healthy controls, the injury to patients by the superoxide anion radical is more destructive to gastroduodenal mucosa due to the decreased enzymatic antioxidant defence together with increased lipid peroxidation [22]. Reduced activities of other enzymatic antioxidants namely, catalase and glutathione peroxidase (GPx) were also reported in peptic ulcer patients suggestive of enhanced free radical activity in acid-peptic disorders [23].

The major lipid-soluble antioxidant in biological membranes is d- α -tocopherol, often called vitamin E. There are at least five different mechanisms by which vitamin E exerts its antioxidant functions. First, reduction of localized oxygen concentrations so that oxidation by molecular oxygen is less likely to occur. Second, prevention of chain reaction initiation by scavenging initiating ROS. Third, binding of transition metal ion catalysts to prevent generation of initiating ROS. Fourth, decomposition of peroxides so that they can not be converted further to active radical species that act as initiators. Fifth, chain-breaking to prevent continued hydrogen abstraction by active radical species [24].

Vitamin E supplementation is recommended in chronic diseases, such as ischaemic heart disease [25], atherosclerosis [26], diabetes [27], Parkinson's disease [28], Alzheimer's disease [29]. Moreover, α -tocopherol exhibits a pronounced protective effects against arthritis [30], cancer [31], aging [32], cataract [33], haemolysis [34] and uraemia [35]. Significant immune-enhancing effects of vitamin E have been reported, including increased interleukin-2 (IL-2) production, and increased T cell proliferation [24, 36]. α -Tocopherol also protects protein thiol groups and thereby acts as a sparing agent during circumstances of increased oxidative stress *in vivo* [37].

Thioctic (α -Lipoic) acid is both water- and fat-soluble antioxidant that is directly (*by removing reactive species*) and indirectly (*by chelating transition metal ions*) involved in the protection of biological components from the damage of oxidative stress. Thioctic acid interacts with its antioxidant partners, vitamins E and C, and helps conserve their antioxidant power by regenerating (recycling) them from their oxidized forms. Lipoic acid also increases the body's GSH levels by facilitating the production of this major intracellular antioxidant. In addition, both lipoic acid and GSH act synergistically to protect biological membranes against lipid peroxidation [38]. Thioctic acid has been shown to be effective in a wide range of clinical conditions including diabetes and its complications, heart diseases, cancer, heavy metal intoxication, HIV infection, cataract, liver diseases, neurodegenerative diseases and age-related pathologies [39].

It is well established that the inhibition of gastric acid secretion will promote healing of most duodenal ulcers within 4 to 8 weeks [40,41]. The histamine H₂ receptor antagonists (e.g., ranitidine, famotidine, nizatidine and roxatidine) continue to be used for the treatment of peptic ulcer disease and are effective in preventing its relapse. They are generally well tolerated with rare minor adverse effects. H₂ antagonists may occasionally interfere with the absorption, metabolism and excretion of some drugs [42]. Studies have shown that nizatidine is a potent inhibitor of basal, nocturnal and stimulated acid secretion [43]. By suppressing the acid output of the parietal cells efficiently, H₂ antagonists have shown to increase the gastric concentrations of certain antibiotics in a manner similar to proton pump inhibitors [44]. A number of H₂ receptor antagonists have been used for the eradication of *HP* either in combination with bismuth salts and antibiotics achieving eradication rates of 85-94% [45] or with antibiotics only achieving eradication rates of 78-95% [46,47].

Various antibiotics used either singly or in combination with bismuth compounds, H₂ receptor antagonists and/or proton pump inhibitors (PPIs) have been tried for eradication of *HP* [48]. Studies from various centers have revealed a wide diversity of eradication rates ranging from 15% at best when a single antibiotic is used to over 95% when antibiotic combinations are used [49].

Clarithromycin is an effective macrolide antibiotic with desirable attributes for *HP* eradication. It is acid-stable, soluble at lower pH, penetrates in high concentrations into gastric tissue and mucus and acts both at the extracellular and intracellular levels exerting excellent antimicrobial activity. Clarithromycin achieves higher concentrations in macrophages in a way that augments the body's immune response against *HP*. Clarithromycin was also found to reduce significantly anti-*HP* IgG levels within two months from the documented eradication of *HP* [50]. Resistance to erythromycin is still relatively low [43].

Amoxicillin was used extensively in combination with bismuth salts as well as in dual, triple and quadruple *HP*-eradication regimens with proton pump inhibitors or H₂ receptor antagonists. Resistance of *HP* to amoxicillin was found to be very low (0.3%) in strains tested in Japan [51].

MATERIALS AND METHODS

Study Subjects

Seventy five non-smoking, *H.pylori*-positive, endoscopically diagnosed, male cases with duodenal ulcer and 15 healthy *H.pylori*-negative, male controls with a matched age (25-50 years) and a body mass index (22.48 ± 0.67 Kg/m²) were recruited in the present study. All cases were admitted to the endoscopy unit at El-Matariya Teaching Hospital for routine investigation of dyspepsia. Patients were subjected to full history taking and clinical examination stressing the onset and duration of symptoms.

Exclusion Criteria

Female patients, smokers, gastric and mixed ulcer patients, patients with GERD or chronic diseases, subjects with chronic NSAIDs intake or regular intake of acid-inhibitory drugs, antibiotics or antioxidants in the preceding one month of the study.

Drugs, Assay Kits and Diagnostics

Nizatidine was obtained from Eli Lilly Company. Clarithromycin was obtained from Abbot Company, Italy. Amoxicillin was obtained from GSK Company, Egypt. Vitamin E was obtained from Mepaco Company, Egypt. Thioctic acid was obtained from EVA Company, Egypt. Oxltek glutathione peroxidase kits (ZeptoMatrix Corp., USA). Rapid urease test for the detection of *H.pylori* in gastric mucosal biopsies, (hpfast[®], CheckMed Inc., USA). Serological test for anti-*H.pylori* IgG HelicoTest[®] (Hexal Pharma Group, Oberhaching, Austria).

Study Design

The study subjects were classified into the following 6 groups:

Group I comprises 15 healthy *H.pylori*-negative subjects serving as the control group.

Group II comprises 15 *H.pylori*-positive duodenal ulcer patients treated with nizatidine alone (300 mg once daily at bedtime for 6 weeks).

Group III comprises 15 *H.pylori*-positive duodenal ulcer patients treated with nizatidine (300 mg once daily at bedtime for 6 weeks) in combination with vitamin E (400 mg once daily for 6 weeks).

Group IV comprises 15 *H.pylori*-positive duodenal ulcer patients treated with nizatidine (300 mg once daily at bedtime for 6 weeks) in combination with thiocetic acid (300 mg once daily for 6 weeks).

Group V comprises 15 *H.pylori*-positive duodenal ulcer patients treated with nizatidine (300 mg once daily at bedtime for 6 weeks) in combination with vitamin E (400 mg once daily for 6 weeks) and thiocetic acid (300 mg once daily for 6 weeks).

Group VI comprises 15 *H.pylori*-positive duodenal ulcer patients treated with *H.pylori* eradication therapy consisting of nizatidine (300 mg at bed time for 6 weeks) in combination with clarithromycin (500 mg twice daily for the first 10 days of therapy) and amoxicillin (1000 mg twice daily for the first 10 days of therapy).

Ulcer healing rates were assessed by a repeat endoscopy after 6 weeks of regular drug therapy. Complete ulcer healing was indicated by white scar formation.

Specimen Collection

Immediately prior to endoscopy and after drug treatment, venous blood samples were collected after 10-12 overnight fast and kept frozen after separation into plasma and erythrocyte fractions awaiting analysis. Because of the patchy distribution of *H. pylori* infection, two biopsy specimens were collected from each patient, one from the immediate prepyloric area and the other from the proximal antrum on the lesser curvature.

Methods

- Blood glutathione peroxidase (GPx) activity was determined according to the method of Paglia and Valentine^[52], using the commercial Oxltek glutathione peroxidase kits (Cat. No. 0805002, ZeptoMatrix Corp., USA).
- Blood superoxide dismutase (SOD) activity was assayed according to the method of Marklund and Marklund^[53].
- Plasma malodialdehyde concentration was determined according to the method of Yagi^[54].
- *H.pylori* was detected in gastric mucosal biopsies according to the method of Genta and Graham^[12] using the commercial *hpf* rapid urease test (CheckMed Inc., USA).
- Specific IgG to *H.pylori* was detected serologically according to the method of Viara and Holton^[55], by using the commercial qualitative HelicoTest® (Hexal Pharma Group, Oberhaching, Austria).

Statistical Analysis

Data were analyzed using SPSS for Windows (version 10). All data were expressed as mean \pm standard error. A paired Student t-test was used to determine the difference between means of the same group before and after drug therapy. Means of pre-treatment and post-treatment groups were compared with that of the control group using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test. The means of different groups were compared using the Student (t) test. The level of significance was set at $p < 0.05$.

Results

Seventy five non-smoking, *H.pylori*-positive, endoscopically diagnosed, male cases with duodenal ulcer and 15 healthy *H.pylori*-negative, male controls were recruited in this open-label clinical pharmacology study.

H.pylori eradication

In the current study a course of triple drug regimen consisting of nizatidine (300mg once daily for 6 weeks), clarithromycin (500 mg twice daily for 10 days) and amoxicillin (1000mg twice daily for 10 days) was used to eradicate *H.pylori*. This regimen achieved 100% eradication rate as indicated by negative rapid urease tests.

Oxidative stress biomarkers in untreated *H.pylori*-infected duodenal ulcer patients as compared to normal controls:

As shown in Table (1) and Figure (1,2 and 3), the mean values of plasma MDA levels and blood GPx and SOD activities in untreated *H.pylori*-infected duodenal ulcer patients are significantly different from that of the control group.

Table 1: Oxidative stress biomarkers in normal controls and untreated *H.pylori*-infected duodenal ulcer patients.

Group	(n)	MDA ($\mu\text{mol/L}$)	GPx (U/gHb)	SOD (U/ml)
Control	15	1.35 \pm 0.10	51.10 \pm 2.20	63.19 \pm 1.76
Duodenal Ulcer Patients	75	3.98 \pm 0.20*	27.17 \pm 1.61*	37.23 \pm 1.75*

Values are expressed as means \pm SEM. (*)Significantly different from normal controls at $p \leq 0.05$ (unpaired Student t- test). MDA : plasma malondialdehyde, GPx : blood glutathione peroxidase, SOD : blood superoxide dismutase.

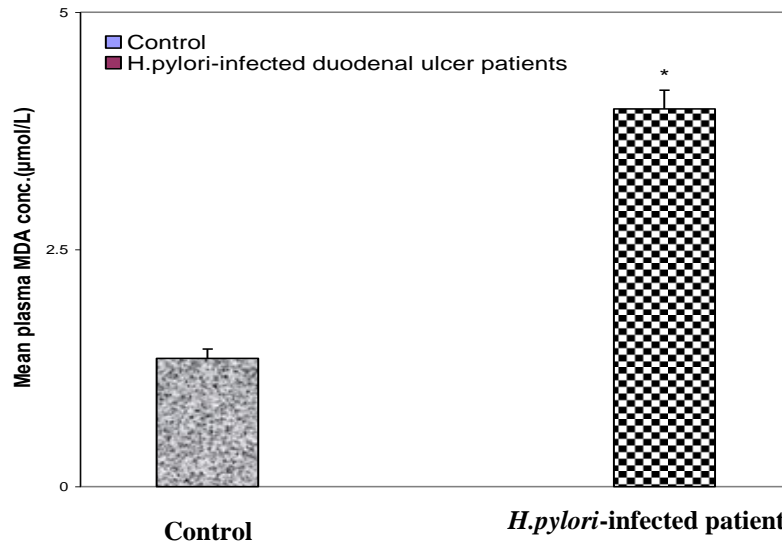


Figure (1): Plasma MDA Concentration ($\mu\text{mol/L}$) in normal controls and untreated duodenal ulcer patients. Values are expressed as means \pm SEM. (*)Significantly different from normal controls at $p \leq 0.05$ (unpaired Student t- test). MDA, malondialdehyde.

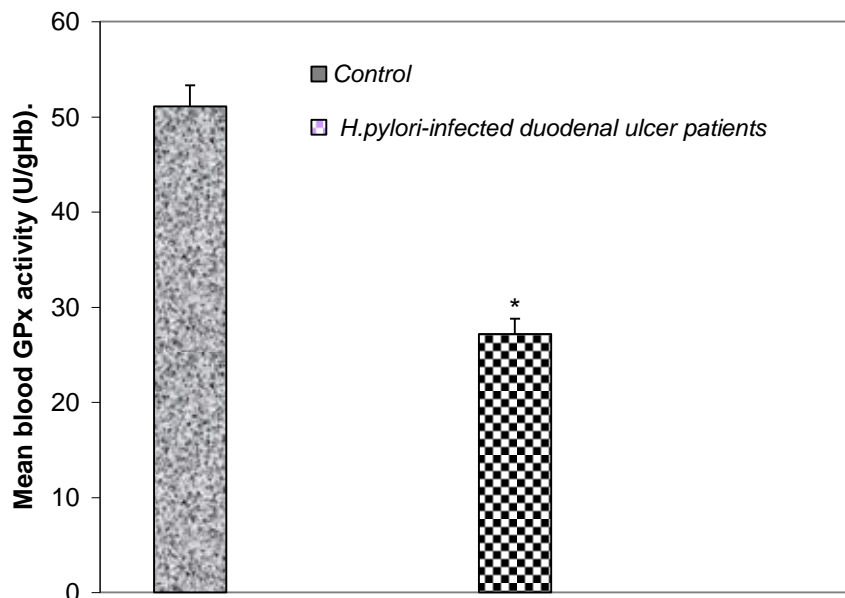


Figure (2): Blood GPx activity (U/gHb) in normal controls and untreated duodenal ulcer patients. Values are expressed as means \pm SEM. (*)Significantly different from normal controls at $p \leq 0.05$ (unpaired Student t- test). GPx, glutathione peroxidase.

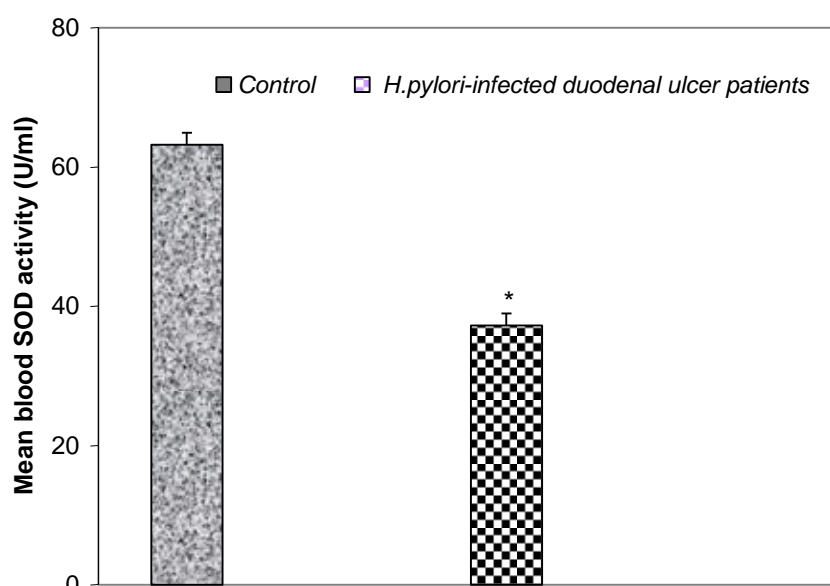


Figure (3): Blood SOD activity (U/ml) in normal controls and untreated duodenal ulcer patients. Values are expressed as means ± SEM. (*) Significantly different from normal controls at $p \leq 0.05$ (unpaired Student t- test). SOD, superoxide dismutase.

Effect of antioxidant therapy on oxidative stress biomarkers in *H.pylori*-infected duodenal ulcer patients as compared to pretreatment and normal control levels:

Table (2) and Figures (4,5,6) depict the effect of 6-weeks oral treatment with *H.pylori* eradication therapy, nizatidine alone or in combination with either vitamin E or thioctic acid or a combination of both on plasma MDA concentration and blood GPx and SOD activities in *H.pylori*-infected duodenal ulcer patients as compared with pretreatment and normal control values. Administration of *H.pylori* eradication therapy or nizatidine with both vitamin E and thioctic acid to the *H.pylori*-infected duodenal ulcer patients normalized plasma MDA levels and blood GPx and SOD activities as compared to normal control group. However, administration of nizatidine alone failed to alter significantly any of the oxidative stress biomarkers. On the other hand, co-administration of nizatidine with either vitamin E or thioctic acid resulted in a significant reduction in plasma MDA level and blood GPx and SOD activities as compared with pretreatment level, yet this effect was still significantly higher than the normal baseline values.

Table 2: Effect of 6-weeks oral treatment with *H.pylori* eradication therapy⁽¹⁾, nizatidine alone⁽²⁾ and in combination with either vitamin E⁽³⁾ or thioctic acid⁽⁴⁾ or a combination of both⁽⁵⁾ on plasma MDA conc. (µmol/L) and blood GPx and SOD activities in *H.pylori*-infected duodenal ulcer patients as compared with pre-treatment and normal control values.

Parameter	MDA (µmol/L).		GPx (U/gHb)		SOD (U/ml)	
	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T
Control	1.35 ± 10		51.10 ± 2.20		63.19 ± 1.76	
Drug Treatment	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T
<i>H.pylori</i> ET	4.09 ± 0.18*	1.56 ± 0.16 [®]	27.70 ± 1.63*	46.00 ± 2.22 [®]	38.58 ± 2.20*	59.30 ± 2.50 [®]
Nizatidine	4.18 ± 0.18*	3.79 ± 0.17*	28.20 ± 1.66*	28.50 ± 1.15*	36.61 ± 1.78*	37.47 ± 1.75*
Nizatidine + VE	3.74 ± 0.23*	2.25 ± 0.12 [®]	26.00 ± 1.53*	36.50 ± 2.05 [®]	37.43 ± 1.64*	45.16 ± 1.93 [®]
Nizatidine + TA	3.89 ± 0.24*	2.29 ± 0.14 [®]	26.70 ± 1.55*	42.40 ± 2.16 [®]	36.50 ± 1.54*	44.62 ± 1.90 [®]
Nizatidine + VE + TA	4.06 ± 0.19*	1.45 ± 0.07 [®]	27.50 ± 1.36*	46.20 ± 2.18 [®]	37.77 ± 1.53*	58.70 ± 2.00 [®]

Values are means ± SEM of 15 observations. Significantly different from normal controls (*), and pre-treatment (®) values, $p \leq 0.05$ (A paired Student t-test was used to determine the difference between means of the same group before and after drug therapy, means of pre-treatment groups were compared with one another and with that of the control group using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test).

Key:

ET, eradication therapy, VE, vitamin E, TA, thiocotic acid, T, treatment.

- (1) Nizatidine (300mg tablets once daily for 6-weeks) plus clarithromycin (500mg tablets twice daily for 10 days) and amoxicillin (1000mg capsules twice daily for 10 days).
- (2) Nizatidine 300 mg tablets at bedtime.
- (3) Nizatidine 300 mg at bedtime plus vitamin E 400 mg capsules once daily for 6 weeks.
- (4) Nizatidine 300 mg at bedtime plus thiocotic acid 300 mg tablets once daily for 6 weeks.
- (5) Nizatidine 300 mg at bedtime plus vitamin E 400 mg once daily and thiocotic acid 300 mg once daily for 6 weeks.

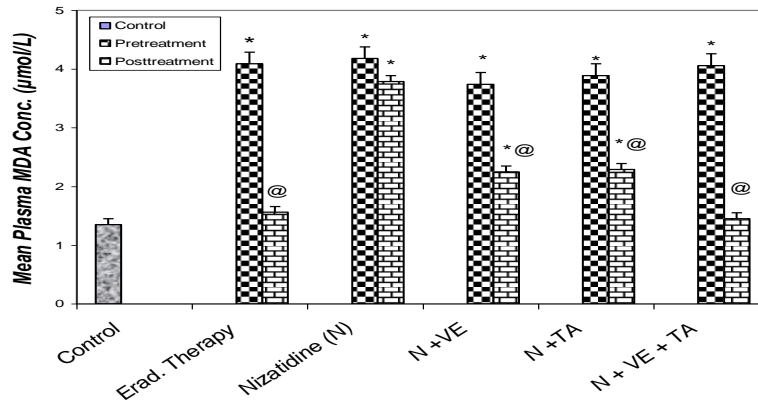


Figure (4) : Effect of 6-weeks oral treatment with *H.pylori* eradication therapy⁽¹⁾, nizatidine alone and in combination with either vitamin E or thiocotic acid or a combination of both on plasma MDA conc. (µmol/L) in *H.pylori*-infected duodenal ulcer patients as compared with pre-treatment and normal control values. Values are means ± SEM of 15 observations. Significantly different from normal controls (*), and pre-treatment (@) values, $p \leq 0.05$ (A paired Student t-test was used to determine the difference between means of the same group before and after drug therapy, means of pre-treatment groups were compared with one another and with that of the control group using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test). ET, eradication therapy, VE, vitamin E, TA, thiocotic acid.
(1) Nizatidine (300mg tablets once daily for 6-weeks) plus clarithromycin (500mg tablets twice daily for 10 days) and amoxicillin (1000mg capsules twice daily for 10 days).

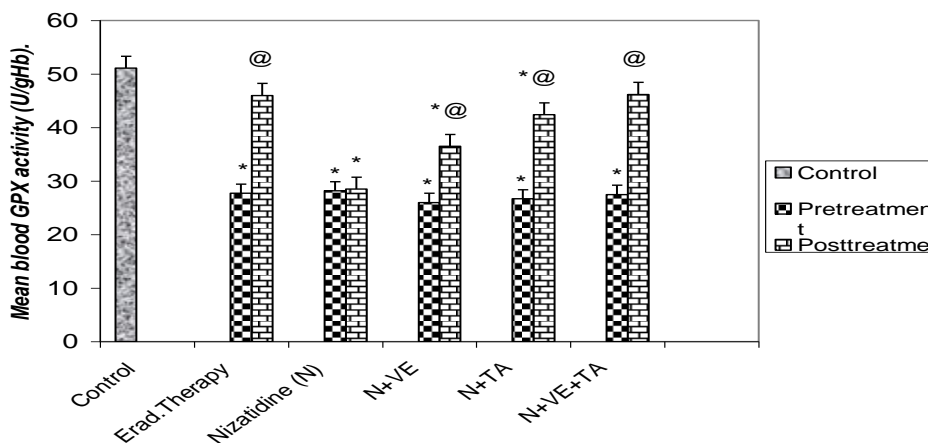


Figure (5) : Effect of 6-weeks oral treatment with *H.pylori* eradication therapy⁽¹⁾, nizatidine alone and in combination with either vitamin E or thiocotic acid or a combination of both on blood GPx activity in *H.pylori*-infected duodenal ulcer patients as compared with pre-treatment and normal control values. Values are means ± SEM of 15 observations. Significantly different from normal controls (*), and pre-treatment (@) values, $p \leq 0.05$ (A paired Student t-test was used to determine the difference between means of the same group before and after drug therapy, means of pre-treatment groups were compared with one another and with that of the control group using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test). ET, eradication therapy, VE, vitamin E, TA, thiocotic acid,
(1) Nizatidine (300mg tablets once daily for 6-weeks) plus clarithromycin (500mg tablets twice daily for 10 days) and amoxicillin (1000mg capsules twice daily for 10 days).

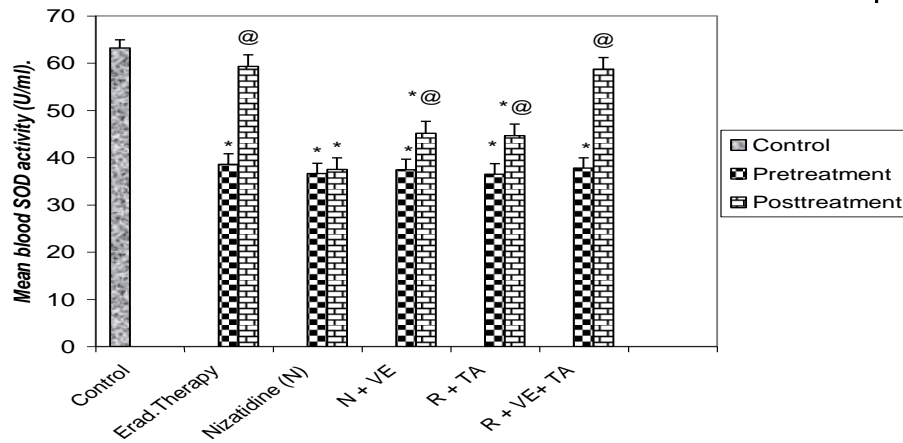


Figure (6) : Effect of 6-weeks oral treatment with *H.pylori* eradication therapy⁽¹⁾, nizatidine alone and in combination with either vitamin E or thioctic acid or a combination of both on blood SOD activity in *H.pylori*-infected duodenal ulcer patients as compared with pre-treatment and normal control values. Values are means \pm SEM of 15 observations. Significantly different from normal controls (*), and pre-treatment (@) values, $p \leq 0.05$ (A paired Student t-test was used to determine the difference between means of the same group before and after drug therapy, means of pre-treatment groups were compared with one another and with that of the control group using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test). ET, eradication therapy, VE, vitamin E, TA, thioctic acid.

⁽¹⁾ Nizatidine (300mg tablets once daily for 6-weeks) plus clarithromycin (500mg tablets twice daily for 10 days) and amoxicillin (1000mg capsules twice daily for 10 days).

Effect of combined nizatidine-antioxidant therapy and *H.pylori* eradication therapy on ulcer healing rates in *H.pylori*-infected duodenal ulcer patients:

Table (3) and Figure (7) show the ulcer healing rates in *H.pylori*-infected duodenal ulcer patients following 6-weeks oral treatment with *H.pylori* eradication therapy, nizatidine alone or in combination with either vitamin E or thioctic acid or a combination of both (as revealed by 8-weeks followup endoscopy). The use of *H.pylori* eradication therapy or combined use of nizatidine with either vitamin E, thioctic acid or a combination of both resulted in remarkably higher ulcer healing rates in duodenal ulcer patients (100, 86.67, 73.34, 93.34%) compared to the regular use of nizatidine alone (60%).

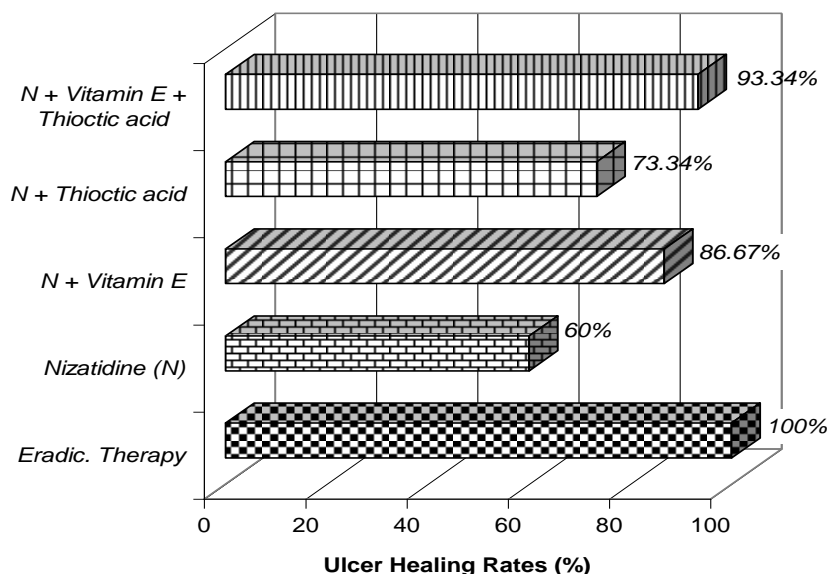


Figure (7) : Ulcer healing rates (%)* in *H.pylori*-infected duodenal ulcer patients following 6-weeks oral treatment with *H.pylori* eradication therapy, nizatidine (N) alone and in combination with either vitamin E or thioctic acid or a combination of both.

*Complete ulcer healing is evidenced endoscopically by white scar formation (Complete re-epithelization).

Table 3: Ulcer healing rates in *H.pylori*-infected duodenal ulcer patients following 6-weeks oral treatment with *H.pylori* eradication therapy, nizatidine alone and in combination with either vitamin E or thioctic acid or a combination of both.

Drug regimen	N	N°	Healing rate (%)
<i>H.pylori</i> eradication therapy	15	15	100
Nizatidine	15	9	60
Nizatidine + VE	15	13	86.67
Nizatidine + TA	15	11	73.34
Nizatidine + VE + TA	15	14	93.34

N : total number of patients undergoing 8-week follow-up upper GIT endoscopy.

N°: number of patients showing complete ulcer-healing as evidenced endoscopically by white scar formation. VE, vitamin E, TA, thioctic acid.

DISCUSSION

In the current study, a significant increase in plasma MDA (a marker of lipid peroxidation) level was observed in *H.pylori*-infected duodenal ulcer patients compared to normal controls. This observation is consistent with previous studies [67,68,69]. The increase in plasma MDA in those patients may be attributed to *H.pylori*-induced chemotactic factors that attract and activate neutrophils and monocytes [8,9] resulting in the generation of ROS in the circulation and gastroduodenal mucosa [10,11]. The ROS produced include the superoxide anion ($O_2^{\cdot-}$) radicals [22], the hydroxyl ($\cdot OH$) radicals, hydrogen peroxide [70] and the highly cytotoxic peroxynitrite ($ONOO^-$) anion [71]. Furthermore, *H.pylori* may exacerbate FR-mediated mucosal damage by degrading the surface mucus layer, which is known to have FR-scavenging activity [72]. Lipid peroxidation in the gastroduodenal mucosa of *H.pylori*-infected peptic ulcer patients is also aggravated by the depletion of many mucosal micronutrient antioxidants including α -tocopherol, β -carotene [73] and vitamin C [74]. In addition, Nair et al., [56] reported that vitamin C, α -tocopherol, α - and β -carotenes, lutein and lycopene are exhausted in peptic ulcer patients irrespective of *H.pylori* status. Such micronutrient antioxidants are known to protect the gastroduodenal mucosa by scavenging ROS. Administration of nizatidine alone to *H.pylori*-infected duodenal ulcer patients failed to improve neither the levels of plasma MDA and blood GSH nor the activities of GPx and SOD. A similar result was reported by Oh et al., [75] who proved that H_2 antagonists act mainly as antisecretory agents with weak or no antioxidant activity. On the other hand, treatment of those patients with *H.pylori* eradication therapy or nizatidine in combination with either vitamin E, thioctic acid or a combination of both agents produced a significant reduction or even normalization of plasma MDA levels. As mentioned before *H.pylori* infection depletes gastroduodenal mucosa from many micronutrients leading to increased ROS and MDA levels [73,74]. Therefore, the administration of *H.pylori* eradication therapy (which remove *H.pylori* as a source of ROS) or the micronutrient antioxidants, (vitamin E and/or thioctic acid) may play a basic role in replenishing the depleted micronutrients thus, enhancing ROS removal and decreasing MDA level. Moreover, these findings support the antioxidant effect of these agents.

The activity of blood SOD was increased significantly to reach normal or about normal values following the administration of *H.pylori* eradication therapy or nizatidine in combination with either vitamin E, thioctic acid or a combination of both agents. The normalization and /or significant elevation of blood SOD activity in *H.pylori*-infected duodenal ulcer patients may be attributed to the capacity of antioxidant vitamins to scavenge *H.pylori*-generated H_2O_2 and superoxide anion radicals thus saving SOD activity [75].

Similar to SOD activity, the GPx activity was decreased in *H.pylori*-infected duodenal ulcer patients, an effect that could be explained by redox system imbalance in those patients [76]. The significant decrease in blood GPx activity stems from the massive oxidative stress to which *H.pylori*-infected peptic ulcer patients are continually exposed [77]. Accumulation of organic and inorganic peroxides in the blood of those patients plays a pivotal role in the depletion of erythrocyte GPx activity [78], which is further aggravated by deficiency of micronutrient antioxidants in the blood of those patients [56,57]. The abnormally low plasma selenium levels in peptic ulcer patients may be another major cause for the poor blood GPx activity in those patients [58]. Administration of *H.pylori* eradication therapy to *H.pylori*-infected peptic ulcer patients restored blood GPx activity back to normal levels probably by eradicating *H.pylori*, a major source of ROS production. Similar results were obtained following the administration of nizatidine in combination with either vitamin E, thioctic acid or a combination of both agents. The improvement or restoration of GPx activity can be ascribed to the potent antioxidant properties of both vitamin E [24] and thioctic acid [38].

Another mechanism by which antioxidant micronutrients can block *H.pylori*-induced FR production resides in their ability to augment the body's immune response against this pathogen either by enhancing the humoral and cell-mediated immunity [59], increasing blood B- and T-lymphocytes [60], promoting lymphocyte proliferation and neutrophil's phagocytic activity in addition to a significant reduction of macrophage-induced superoxide anion production [61].

In this study a course of triple drug regimen consisting of nizatidine (300mg once daily for 6 weeks), clarithromycin (500 mg twice daily for 10 days) and amoxicillin (1000mg twice daily for 10 days) was used to eradicate *H.pylori*. This regimen achieved 100% eradication rate as indicated by negative rapid urease tests. Various antibiotics used either singly or in combination with bismuth compounds, H₂ receptor antagonists and/or proton pump inhibitors (PPIs) have been tried for eradication of *HP*. Studies from various centers have revealed a wide diversity of eradication rates ranging from 15% at best when a single antibiotic is used to over 95% when antibiotic combinations are used [49]. Another important finding in the current study was that the use of *H.pylori* eradication therapy or combined use of nizatidine with either vitamin E, thioctic acid or a combination of both resulted in remarkably higher ulcer healing rates in duodenal ulcer patients (100, 86.67, 73.34, 93.34%) compared to the regular use of nizatidine alone (60%). These results are in accordance with those of previous studies that emphasize the role of *H.pylori* eradication [62] and exogenous antioxidants in the prevention of gastroduodenal injury, promotion of healing and prevention of relapse [63,64,65]. These results support the importance of opposing ROS and prevention of lipid peroxidation in the course of ulcer healing besides the traditional acid suppression approach.

Patients treated with nizatidine in combination with both vitamin E and thioctic acid showed higher ulcer healing rates compared to those treated with nizatidine alone or in combination of either agents. This may be ascribed to the synergistic antioxidant effects of vitamin E and thioctic acid besides the additional ability of vitamin E to suppress acid production in those patients [66].

In conclusion, the results of the present study indicate that rapid and complete duodenal ulcer healing requires an effective collaboration between gastric acid suppression, eradication of *H.pylori* and elimination of oxidative stress. This can be efficiently achieved by the use of a triple therapy consisting of nizatidine, clarithromycin and amoxicillin in combination with both vitamin E and thioctic acid.

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