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Therapeutic Effects of Vitamin E in Gastric Stress Ulcers and Obesity in Rats

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Abstract: Stress – related disturbances are classified among the health problems that have global interest, especially which are related to gastrointestinal system. The consequent oxidative stress is confirmed to be responsible for the development of gastric mucosal ulcers and necrosis. That is accompanied with the release of different inflammatory mediators. On the other hand, the ratio of obesity is gradually increasing in the world. It impairs the quality of life because of its associated serious complications. Vitamin E is a fat-soluble, free radicals scavenger antioxidant. It has anti-inflammatory and cell membrane-stabilizing properties. Besides, it has anti – obesity effects. The present study highlights the potential therapeutic effects of vitamin E in the treatment of stress-induced gastric ulcers in rats. Stomach injury was induced in fasted rats by cold- restraint stress (CRS) method. Vitamin E was administered orally at a dose of 100 mg/kg for 7 consecutive days. Rats were sacrificed on the 8th day. Assessment of the stomach injury was by studying body weight changes, macroscopic examinations, histological study, and determination of oxidative stress markers (MDA stomach content and SOD enzyme activity). Vitamin E administration alleviated the stomach injury degree, and caused a remarkable body weight decrease, with a statistical significance in comparison with the stressed group. Vitamin exerted good effects in reducing body weight, and healing of stress-induced gastric ulcers in rats.

Keywords: stomach, Vitamin E, antioxidant, body weight, stress-induced ulcers.

Introduction:

Gastric ulcer is an open sore in the stomach mucosal barrier that can be formed by Helicobacter pylori infections, nonsteroidal anti-inflammatory drugs (NSAIDs), or exposure to stressful events (Elshazly et al., 2018). Over the last few years, the implication of psychologic agents in the pathogenesis of peptic ulcers received much attention (Stephen et al., 2008). Stress is evolved as a response to severe conditions. It leads to stomach ulceration by irritating the secretion of acid and pepsin, and impairing the mucosal defense (Chandranath et al., 2011). Cold restraint stress method is widely used and is found to cause certain gastric lesions in experimental animals (Rashad et al., 2019). In addition, obesity is associated with the development of a large number of health disorders, like erosive gastritis and gastric ulcers (Camilleri et al., 2017). The main contributors to oxidative stress in obesity include vitamin deficiencies, chronic inflammation, and type of diet (Manna et al., 2015). Furthermore, reactive oxygen species (ROS), accumulated then, have been reported to be involved in regulation of body weight by affecting hypothalamic neurons, which control satiety and hunger behavior (Manna et al., 2015). Vitamin E is known as a potent non-enzymatic antioxidant which plays a role in radicals scavenging, and attenuates the oxidative stress caused by (ROS) in our living tissues. Furthermore, it attenuates factors of aggression causing ulceration (Kamisah et al., 2014). It is a lipophilic vitamin, classified into two major forms: tocopherols and tocotrienols (Ibrahim et al., 2012). It has been pointed out that the mechanism of anti-obesity effect of vitamin E depends on leptin and adiponectin levels (Fukui et al., 2019). In light of that, the intention was to further evaluate the therapeutic effects of vitamin E on experimentally- induced gastric injury and oxidative damage.

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Materials and Methods:

Animals

Female and male wistar albino rats weighing 119-303 g were used in this study. The animals were purchased from the Scientific Research Center, Damascus, Syria. The animals were kept at controlled environmental conditions (temperature 23±2 ℃, humidity 55±15%, under a 12 h light/dark cycle). They were left for one week for adaptation before any experimental procedures. They had free access to diet and water ad libitum. All methods performed in this study were in accordance with regulatory guidance of the care and use of experimental animals**.**

Drugs

Vitamin E acetate SD 50 (BASF) was used in this study. This product can produce a milky suspension in water. Stock solutions were freshly prepared daily and used for feeding.

Experimental design

Induction of Ulcer

Rats were fasted for 24 hours prior to the experiment in mesh-bottomed cages to minimize coprophagia with free access to water (Turkyilmaz et al., 2019). All animals were put into individual tubular restraint cages of wire mesh. They were placed in a refrigerator at (4 C°±1) for 2 hours (Arsić et al., 2010). The door of the refrigerator was opened every 0.5 hours for inspection and follow-up (Turkyilmaz et al., 2019).

All experiments were performed during the same time of the day to avoid variations due to diurnal rhythms of putative regulators of gastric functions. All groups received either the drug or vehicle after they were subjected to CRS. Vitamin E was orally administered once daily for seven consecutive days, except for the normal control group, (they were kept in room temperature and were not exposed to any stress).

Treatment Groups

Animals were randomly divided into 3 groups:

Group1. Normal control (NC): (9 rats in this group) received oral vehicle (water).

Group 2. Cold restraint stress (CRS): (8 rats in this group) received oral vehicle (water).

Group 3. Vitamin E (E100): (9 rats in this group) received Vitamin E (100 mg/kg) (Turkyilmaz et al., 2019).

Tissue collection and preparation

After seven days of treatment, animals were sacrificed under deep ether anesthesia, and a midline incision was made. The stomach was isolated, then opened along the greater curvature, and stretched moderately on a cork board after washing with saline. Gastric mucosa was examined by naked eye and magnifying lens. A precise evaluation of the lesions was made, then each specimen was fixed in 13% formalin. Stomach tissue samples were stored at −80 °C for further analysis.

Clinical findings

Rats were checked daily for body weight, behavioral changes, and food intake. body weight was measured at regular time intervals from day 0 to 7. Changes of body weight (%) were calculated.

Macroscopic scoring

Number and severity of gastric lesions were scored according to the following criteria (Arsić et al., 2010):

0: no lesion, 1: mucosal edema and petechiae, 2: (1–5) small lesions (1–2 mm), 3: > 5 small lesions or 1 intermediate lesion (3–4 mm), 4: ≥ 2 intermediate lesions or 1 gross lesion (> 4 mm), and 5: perforated ulcers.

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Each lesion was considered as an ulcer to calculate the following parameters:

Degree of ulceration= total ulcer score/ No. of animals ulcerated (Nwafor et al., 2000).

ulcer index (UI)= total ulcer score / No. of animals in group.

It was expressed as: UI ± SD (standard deviation) (Arsić et al., 2010).

Curative ratio = [1- (UI treated/UI ulcerated) × 100] (Nwafor et al., 2000).

Biochemical assays

Accurately weighed tissues of stomach were homogenized in cold phosphate-buffered saline (pH 7.4, 50 mmol) to prepare 10 % tissue homogenate. The suspension was divided into two portions. The first one was used for the measurement of malondialdehyde (MDA). The second part was centrifuged for 20 min at 4 °C. The resultant supernatant was used for SOD activity determination**.**

Estimation of lipid peroxidation

Detection of malondialdehyde (MDA) (the end resultant of lipid peroxidation) by a colorimetric reaction with thiobarbituric acid (TBA), is a highly sensitive indicator for assessment of the mucosal injury in gastric tissue exposed to oxidative stress (Zemmouri et al., 2017). In brief, 0.5 ml of stomach tissue homogenates were mixed with 2 ml of TBA reagent containing 0.375% TBA, 15% trichloroacetic acid and 0.25 N HCl. Samples were boiled for 15 min, cooled and centrifuged. The absorbance of the supernatant was spectrophotometrically read at 532 nm, using an extinction coefficient of 1.56×10^5 /M cm.

Final Concentration of unknown sample/gram tissue = 100 x µM LPO equivalent/gram tissue (Patil et al., 2014).

Estimation of superoxide dismutase (SOD) activity

The method is as follows: First, a certain amount of pyrogallol solution (60 mmol in 1 mmol HCl, 37 °C) was thoroughly mixed with pH 7.4 Tris-HCl buffer (0.05 M, pH 7.4) containing 1 mM Na2EDTA. The volume was adjusted to 3000 µl using the buffer. The A325 nm value of the mixture without a sample was measured every 30 s for 5 min at 37 °C. Afterward, we repeat the exact previous step with the addition of the sample. Enzyme activity, which matches the amount of enzyme that suppresses the auto-oxidation of pyrogallol by 50%, was calculated and expressed per mg of protein (Li X et al., 2012).

Histopathological observations

A portion of the stomach of each rat was fixed in 13% formalin. The specimens were embedded in paraffin wax and cut into sections of 5 mm thickness. The sections were stained with hematoxylin and eosin (H and E) dye for the histopathological study. The histological sections were examined by an experienced pathologist who was blinded to the treatment for: grade and type of inflammation (score: 0-4) presence of epithelial erosions (score: 0-2), presence of epithelial ulcers (score: 0-1), edema in the lamina propria (score: 0-1), blood vessels congestion (score: 0-5), epithelial atrophy (score: 0-1), and epithelial hyperplasia (score: 0-1), yielding a maximum total score of 15 (Li WF et al., 2014).

Statistical analysis

Data analyses were performed using Prism (version 8) statistical package. Data were expressed as means ± SEM. Different groups were compared using one-way analysis of variance (ANOVA) followed by Sidak test for multiple comparisons of parametric data, and Kruskal–Wallis test followed by Dunn test for multiple comparisons of non-parametric data. P values < 0.05 were considered statistically significant**.**

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RESULTS

Clinical findings, general observation, and body weight changes

After 24 h of induction of ulcer, most animals developed soft feces and progressively body weight loss, with weakness and decreased food intake. At the end of the experiment, body weight of CRS group was reduced by (-1.51%) compared with that of the NC group, which revealed body weight increase by (+2.82%) with no statistical significance (p=0.1654). Group (E100) revealed a significant decrease in body weight by (-10.76%) compared to CRS group (p=0.0078). (Table. 1).

Some rats died during the experiment. That is likely due to haemorrhagic or perforated stomach lesions.

Table. 1: Effect of vitamin E on the body weight in stress - induced ulcer in rats

Parameter / Group	Initial body weight	Final body weight	Body weight change %
NC	170.7±17.52	173.9±15.11	2.82±1.57
CRS	160±13.82	158±14.63	-1.51±1.9
E100	181.8±16.24	172±18.03	-10.76±2.43 **

** P<0.01 compared to CRS group.

Macroscopic scoring

NC group stomachs obviously revealed healthy mucosa in most samples. Two samples showed some tiny pinpoint lesions. In contrast, darkened reddish hemorrhagic lesions were observed in CRS group samples, with variant forms and sizes. Also, there was a generalized hyperemia in the gastric mucosa. CRS extensively increased the ulcer index (P<0.0001) compared to NC group.

Vitamin E significantly decreased the ulcer index compared to CRS group (P=0.004). (66.68 %) curative ratio was recorded. (Table. 2, Figure. 1, Figure. 2).

Table.2: Effect of vitamin E on gastric parameters in stress - induced ulcer in rats

Parameter / Group	Ulcer severity	Ulceration Degree	Curative ratio
NC	3	1.5	-
CRS	32	4	-
E100	12	3	66.68%

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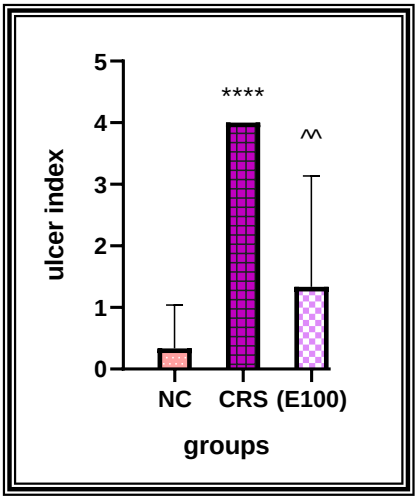

Figure.1: Effect of vitamin E on ulcer index in stress - induced ulcer in rats

**** p<0.0001 compared to NC group; ^^ p<0.01 compared to CRS group

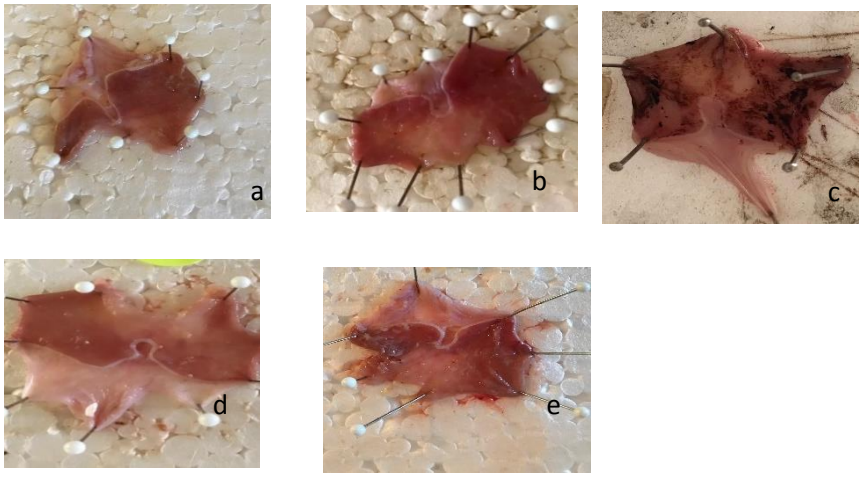

Figure.2: Macroscopic morphology changes in the gastric mucosa. a, NC group (grade0); b, NC group (grade1); c, CRS group (grade 4); d, (E100) group (grade 0); e, (E100) group (grade 4).

Histopathological study

Histological evaluation of gastric specimens in stress - induced ulcerated rats had manifested an extensive damage to the gastric mucosa (deep erosions and ulcers), severe blood vessels congestion, edema in the lamina propria, inflammatory cells infiltration in the submucosal layer, with high scores of microscopic damage when compared to NC group (P<0.0001).

Significant histologic improvement was observed in (E100) group when compared to CRS group (P=0.0005). (Table. 2, Figure. 3).

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Table.2: Effect of vitamin E on the microscopic score in stress - induced gastric ulcer in rats

group	Edema (score 0-1)	epithelial Hyperplasia (score 0-1)	Blood vessels congestion (score 0-5)	Epithelial erosion (score 0-2)	Epithelial ulceration (score 0-1)	Inflammation infiltration (score 0-4)	epithelial Atrophy (score 0-1)	Total Scores 15
NC	0 (0-1)	0 (0-1)	4 (4-4)	0 (0-1)	0 (0-0)	1(0-3)	0(0-0)	6 (5-9)
CRS	1(0-1) ###	1 (0-1)	5(5-5) ##	2(0-2)	0(0-1) ##	3(0-4)	0(0-1) #	11(11-12) ####
E100	1(0-1)	0(0-0) **	4(2-4) ****	1(0-1)	0(0-0) **	1.5(0-4)	0(0-1) ***	7(2-8) ***

The table shows median values followed by minimum and maximum scores (in brackets). #### p<0.0001 ### p<0.001 ## P<0.01 # P<0.05 compared to NC group. **** p<0.0001 *** P<0.001 ** P<0.01

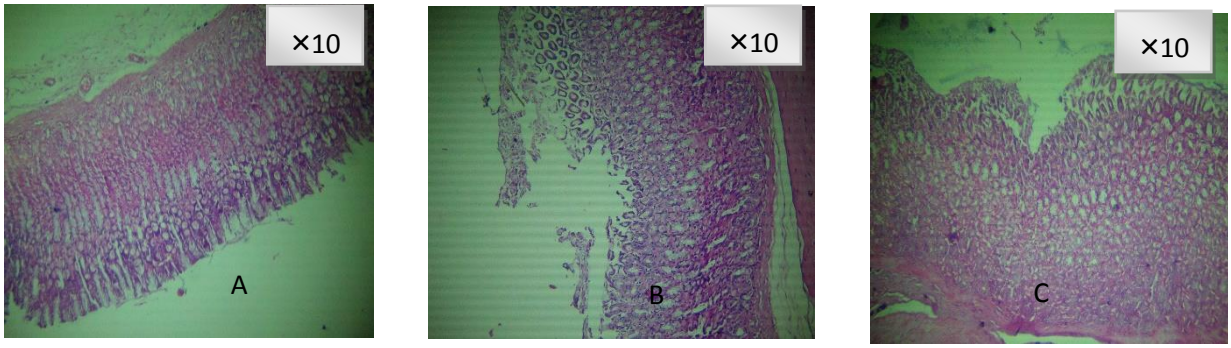

Figure. 3: Histological appearance of stomach tissue sections. A, NC group (grade 6); B, CRS group (grade 10); C, (E100) group (grade 4).

Biochemical investigations

CRS caused oxidative cascade in rats' stomachs; it was assessed by lipid peroxides (LPO) levels and SOD activity measurement.

Stomach MDA content:

CRS group showed a significant elevation in MDA levels compared to NC group (P<0.0001). Meanwhile, rats received vitamin E (E100 group) revealed decreased MDA levels compared to CRS group, but with no statistical significance (P=0.0558). (Figure. 4).

Stomach superoxide dismutase (SOD) activity

Rats exposed to stress substantially showed decreased SOD activity in stomach tissues compared to NC group (P<0.0001). Administration of vitamin E noticeably enhanced the enzyme activity compared to CRS group (P<0.0001). (Figure. 4).

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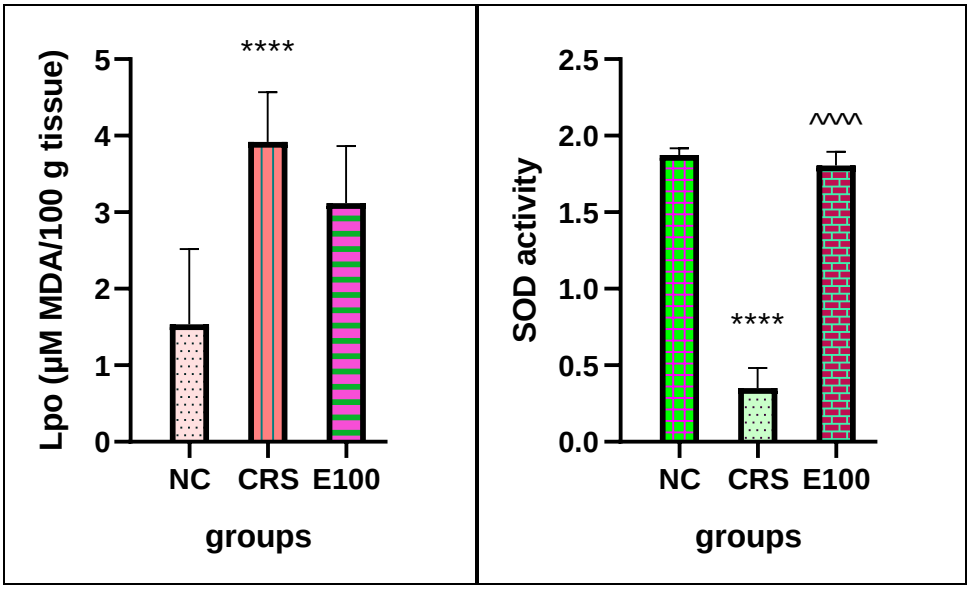

Figure.4: Effect of vitamin E on lipid peroxides levels and SOD activity in stress - induced ulcer in rats

**** p<0.0001 compared to NC group; ^^^^ p<0.0001 compared to CRS group

Discussion

stress ulcers, one of the most common health problems that affect people of all ages around the world (Azlina MF et al., 2019). Previous studies declared that oxidative stress and cells hypoxemia are the causes of gastric ulcer pathogenesis, secondary to the accumulation of ROS. It leads to injuries in different cellular structures including proteins, lipids, and DNA (Khoshnoud et al., 2020).

Many experimental models of ulceration mimicking the disease in human have been recommended to test the potential effects of different drugs. CRS synergistically with fasting in rats is one of the most common employed methods (Rashad et al., 2019). Therefore, we applied this technique in this study.

The present results confirmed that CRS produces an oxidative stress status accompanied with severe inflammatory response in rats. That was illustrated by body weight loss, reduction in food intake, and gastric mucosal damage. Macroscopic results revealed characteristic acute lesions such as mucosal edema, patchy hemorrhagic ulcers and erosions. That appeared by a significant increase in ulceration parameters, which is in agreement with many studies (Bahadir et al., 2016). Moreover, that was associated with changes in biomarkers which include a significant elevation in MDA levels by 155%, and a notable depletion in SOD activity by 81% compared to NC group. That was in harmony with the findings of Elsaed et al. They attributed that to the overproduction of ROS which mediate the cascade of stress-induced mucosal harm (Elsaed et al., 2018).

Administration of vitamin E caused a considerable loss of body weight by 613% compared to NC group. That can be elucidated by the study of Alcalá et al. They declared that vitamin E reduces triglycerides levels, and causes a significant enlargement in the adipocyte size. Therefore, circulating free fatty acids will not be then redirected to other tissues (Alcalá et al., 2015). Zhao et al. pointed out that treatment with tocotrienol attenuates high-fat diet-induced obesity and inflammatory cytokines production in mice (Fukui et al., 2019). Furthermore, treatment with vitamin E markedly diminished gastric mucosal damage that stress induced. That gets along with the findings of Nur Azlina et al (Nur Azlina et al., 2009). They imputed that to the capacity of vitamin E to inhibit lipid peroxidation by trapping free radicals and interrupting their work. As an anti-oxidant, vitamin E decreased gastric MDA content by 20%, and significantly improved the activity of SOD by 414% compared to CRS group. That is in concordance with the study of Shah et al (Shah et al., 2009).

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Conclusion:

Taken together, these data support that antioxidant supplementation might play an important positive role in the oxidative damage induced by stress. Also, it is able to be helpful in the management of obesity. In light of that, vitamin E is recommended to be explored in clinical trials having obese patient with gastric ulcers.

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