



Protective effect of Apple Cider Vinegar on Stress Induced Gastric Ulcer

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ABSTRACT

A gastric ulcer is a break in the normal gastric mucosa integrity that extends through the muscularis mucosa into the sub-mucosa or deeper. This work aimed to study the effect of chronic administration of vinegar on gastric acid secretion, and if this action has any effect on stress-induced ulcer in male albino rats. Study groups included 24 rats divided into 2 main groups (12 rats/group): pyloric ligation group (A) and water immersion-induced gastric ulceration group (as a form of stress) (B). We evaluated; free and total HCL secretion in group A, ulcer score, ulcer index, percentage ulcer protection, , eNOS enzyme activity, COX-2 enzyme activity, glutathione reductase gene expression, CCK gene expression and proton pump (H^+/K^+ ATPase) gene expression in group B. We revealed significant decrease in free and total acidity, ulcer score and index, COX-2 enzyme activity and Proton pump (H^+/K^+ ATPase) gene expression among vinegar fed rats compared with the results of the control group. On the other hand there was significant elevation in the percentage ulcer protection, eNOS enzyme activity, glutathione reductase gene expression and CCK gene expression among vinegar fed rats compared to control group. In conclusion, the present study revealed protective effect of vinegar on stress-induced gastric ulceration.

Key words: Gastric ulcer, HCL, H^+/K^+ ATPase pump, H_2 -receptor blockers, vinegar.

INTRODUCTION

Vinegar, from the French *vinaigre*, meaning “sour wine,” can be made from almost any fermentable carbohydrate source¹. The chemical and organoleptic properties of vinegars are a function of the starting material and the fermentation method. Acetic acid, the volatile organic acid that identifies the product as vinegar, is responsible for the tart flavor and pungent, biting odor of vinegars. However, acetic acid should not be considered synonymous with vinegar. The US Food and Drug Administration (FDA) states that diluted acetic acid is not vinegar and should not be added to food products customarily expected to contain vinegar². Other constituents of vinegar include vitamins, mineral salts, amino acids, polyphenolic compounds (eg, gallic acid, catechin, caffeic acid, ferulic acid), and nonvolatile organic acids (eg, tartaric, citric, malic, lactic)³.

Numerous terms have been used to describe stress ulcer in critically ill patients, including stress ulcer/ulceration, stress erosions, stress gastritis, hemorrhagic gastritis, erosive gastritis, and stress-related mucosal disease⁴.

A major factor in the development of stress ulcer is splanchnic hypo-perfusion, which results from a number of stress-related effects such as sympathetic nervous system activation, increased catecholamine release, vasoconstriction, hypovolemia, decreased cardiac output, and release of pro-inflammatory cytokines⁵. The inflammation induced in the gastric mucosa is accompanied by increased TNF- α production⁶, which augments neutrophil-derived superoxide generation⁷ and stimulates IL-1 production, leading to neutrophil accumulation⁸. Neutrophils produce superoxide radical anion ($O_2^{\bullet-}$), which belongs to group of ROS. Superoxide radical anion reacts with cellular lipids, leading to the formation of lipid peroxides that are metabolized to MDA (Malondialdehyde) and 4-hydroxynonenal (4-HNE)⁹.

During stress, the imbalance of aggressive and defensive factors in the stomach plays a pivotal role in gastric hemorrhage and ulcer formation¹⁰. Thus, the aim of the work was to the possible effect of chronic administration of vinegar on gastric acid secretion, and to study if this action has any effect on stress-induced ulcers in male albino rats.

MATERIALS & METHODS

***Experimental animals:** A total of 24 male albino rats weighing 150-175 gm were used. The rats were housed in wire mesh cages at room temperature, veterinary care were provided by laboratory animal house unit of Kasr Al-Ainy faculty of medicine, Cairo University. Before the day of experiments all rats were starved for 24 h, with free access to water until the beginning of experimental protocol.

Rats were divided into two groups:

Group A: experimental design for pyloric ligation induced gastric ulcer: 12 rats were divided into 2 subgroups, each consists of 6 animals:

Subgroup i : fed for 6 weeks on a standard laboratory rat diet offered *ad lib*.

Subgroup ii: fed for 6 weeks on a standard laboratory rat diet enriched with 15% of natural vinegar¹¹ (5% acetic acid, Gallettis.n.c., via Faverzani, 13. 26046 San Danelepo. Cremona-Italia-Lic.) offered *ad lib*.

On the 35th day, the 2 groups of rats were fasted 24 h prior to induction of gastric ulcer. Pyloric ligation was done by ligating the pyloric end of the stomach of rats¹¹. Animals was allowed to recover and stabilized in individual cage and deprived of water during post-operative period. After 3 h of surgery, rats were sacrificed by cervical dislocation. Both free and total HCl secretions were measured in this group(A).

Group B: experimental design for water immersion restraint-induced gastric ulcer: 12 rats were divided into two groups, each consists of 6 animals:

Subgroup i: fed for 6 weeks on a standard laboratory rat diet offered *ad lib*.

Subgroup ii: fed for 6 weeks on a standard laboratory rat diet enriched with 15% of natural vinegar (5% acetic acid) offered *ad lib*

The rats fasted 24 hrs prior to stress induction. Rats were immobilized in a stress cage and then immersed to the level of the xiphoid in a water bath at $23 \pm 0.2^\circ\text{C}$ for 4 hours¹². Then they were removed from the cage and sacrificed by cervical dislocation. Ulcer score, ulcer index, percentage ulcer protection, histology of the Stomach, eNOS enzyme gene expression, COX-2 enzyme activity, glutathione reductase enzyme gene expression, CCK gene expression and proton-pump(H^+/K^+ ATPase enzyme) gene expression were measured in this group (B).

***Determination of ulcer index:** Stomachs of group B rats (water immersion rats) were opened at the greater curvature, fixed on cork for determination of ulcer index. The ulcer index was calculated according

to the method of Suzuki *et al.*, 1976¹³. The lesions were counted with the aid of hand lens (10X) and each given a severity rating as follows:

- 1 Less than 1mm (Pin point)
- 2 1-2 mm
- 3 Greater than 2 mm and above

The ulcer score was divided by a factor of 10 to get the ulcer index.

***Determination of percentage ulcer protection:** Percentage ulcer protection was calculated by the formula¹⁴:

$$\text{Ulcer protection (\%)} = (U_c - U_t / U_c) \times 100$$

Where:

U_c = ulcer index of control group.

U_t = ulcer index of test group.

***Pyloric ligation:** An aseptic surgical procedure was employed for group (A) animals. The rats were anesthetized by intra-peritoneal injection with thiopental sodium (30mg/kg)¹², after that the abdomen was opened by a small midline incision of approximately 3 cm, at one cm below the xiphoid process. Stomach was exposed and a tight knot was applied around the pyloric sphincter using 4-0 silk ligature. The stomach was placed carefully and abdomen wall closed by interrupted sutures. Immediately after suturing the incision, the wound was cleaned and was covered by local antibiotic ointment (Terramycin). The rats were returned to their cages and left for 3 hours¹².

***Animal sacrifice and samples collection:** Animals were sacrificed by cervical dislocation, the thorax and abdomen were opened, esophagus was ligated and the stomachs were dissected and removed quickly. The contents of the stomach were collected.

***Measurement of gastric acid secretion:** After collection of gastric fluid, the total volume of gastric content was measured. The gastric contents were centrifuged at 1000 rpm for 10 min. One ml of the supernatant liquid was pipetted out and diluted to 10 ml with distilled water. The solution was titrated against 0.01N NaOH using Topfer's reagent as indicator, to the endpoint when the solution turned to orange color. The volume of NaOH needed was taken as corresponding to the free acidity. Titration was further continued till the solution regained pink color. The volume of NaOH required was noted and taken as corresponding to the total acidity¹⁵.

***Measurement of cox-2 enzyme activity:** Cayman's COX Activity Assay Kit (Cayman Chemical Company, Michigan, USA) measures the peroxidase activity of COX. The peroxidase activity is assayed colorimetrically by monitoring the appearance of oxidized N,N,N',N'-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm¹⁶. It can be used with both crude (cell lysates/tissue homogenates) and purified enzyme preparations. The kit includes isozyme-specific inhibitors for distinguishing COX-2 activity from COX-1 activity.

***Detection of eNOS, CCK, glutathione reductase and h+/k+ atpase gene expression:** By real time quantitative polymerase chain reaction (qPCR) (chemicals purchased from Cayman Chemical Company, Michigan, USA).

qPCR differs from regular PCR by including in the reaction fluorescent reporter molecules that increase proportionally with the increase of DNA amplification in thermo cycler. There are two types of fluorescent chemistries for this purpose: double-strand DNA-binding dyes and fluorescently labeled sequence specific probe/primer. SYBR Green I dye and TaqMan® hydrolysis probe are the common examples for these two, respectively. The SYBR Green method doesn't need fluorescently labelled probe/primer and costs much less than the TaqMan® method. The key equipment for qPCR is a specialized thermo cycler with fluorescence detection modules which is used to monitor and record the fluorescence in real time as amplification occurs.

A typical workflow of qPCR for gene expression measurement involves RNA isolation, reverse transcription, qPCR assay development, qPCR experiment and data analysis.

RESULTS

The results of present work showed a significant (P -value < 0.05) decrease in the mean values of both total and free acidity between subgroup Aii (Vinegar fed) and control subgroup Ai. As shown in Table 1 and Figure 1, the mean value of total acidity of subgroup Ai was $71.683 \text{ mEq/L} \pm 1.076$ and that of free acidity was $29.733 \text{ mEq/L} \pm 0.628$, while in subgroup Aii, the mean value of total acidity was $65.8 \text{ mEq/L} \pm 0.672$ and that of free acidity was $25.567 \text{ mEq/L} \pm 0.612$. The results of the present work also showed a significant (P -value < 0.05) decrease in ulcer index between Vinegar fed rats (subgroups Bii) with a mean value of 0.767 ± 0.082 and control subgroup Bi in which the mean value of the ulcer indices was 1.117 ± 0.075 (Table 2 and Figure 2). The mean value of percentage ulcer protection among vinegar fed rats (subgroup Bii) was $25.942\% \pm 10.395$.

The results of present work – as shown in Table 3 - also showed a significant (P -value < 0.05) increase in eNOS enzyme gene expression between subgroups Bii (vinegar fed) with a mean value of 0.63 ± 0.054 and control subgroup Bi with a mean value of 0.283 ± 0.039 (Figure 3).

Furthermore, the results of present work also showed a significant (P -value < 0.05) decrease in COX-2 enzyme activity between subgroup Bii with a mean value of $6.687 \text{ mmol/min/ml} \pm 0.335$ and subgroup Bi with a mean value of $11.647 \text{ nmol/min/ml} \pm 1.156$ (Figure 4).

In addition, we showed a significant (P -value < 0.05) increase in glutathione reductase enzyme gene expression between subgroup Bii with a mean value of 56.967 ± 4.869 and subgroup Bi with a mean value of 30.733 ± 5.854 (Figure 5).

There was also a significant (P -value < 0.05) decrease in H^+/K^+ ATPase enzyme gene expression between subgroups Bii with a mean value of 6.953 ± 0.71 and subgroups Bi with a mean value of 11.167 ± 0.794 (Figure 6). In addition, the results of present work have shown a significant (P -value < 0.05) increase in CCK gene expression between subgroups Bii with a mean value of 0.643 ± 0.036 and subgroups Bi with a mean value of 0.385 ± 0.079 (Figure 7).

Moreover we demonstrated from macroscopic and microscopic (histo-pathological) examination more significant protection against stress-induced gastric ulcer in vinegar fed rats in subgroup Bii (Figures 9 and 11) as compared to control subgroup Bi (Figures 8 and 10).

Table (1): Comparison of total acidity and free acidity between subgroups Ai (control) and Aii (Vinegar fed).

		Subgroup Ai	Subgroup Aii
Total acidity	Mean [#]	71.683	65.8
	S.D. \pm	1.076	0.672
Free acidity	Mean [#]	29.733	25.567
	S.D. \pm	0.628	0.612
P Value			$<0.05^*$

[#] Mean is in mEq/L

*: statistically significant compared to corresponding value in subgroups Ai ($P < 0.05$).

Table (2): Comparison of ulcer index between subgroups Ai (control) and Aii (vinegar fed).

		Subgroup Bi	Subgroup Bii
Ulcer index	Mean	1.117	0.767
	S.D. \pm	0.075	0.082
P Value			$<0.05^*$

*: statistically significant compared to corresponding value in subgroups Bi ($P < 0.05$).

Table (3): Comparison of eNOS enzyme gene expression, COX-2 enzyme activity, glutathione reductase enzyme gene expression and H^+/K^+ ATPase enzyme gene expression and between subgroups Bi (control) and Bii (vinegar fed).

		Subgroup Bi	Subgroup Bii
eNOS enzyme gene expression	Mean	0.283	0.63
	S.D. \pm	0.039	0.054
COX-2 enzyme activity	Mean [#]	11.647	6.687
	S.D. \pm	1.156	0.335
glutathione reductase enzyme gene expression	Mean	30.733	56.967
	S.D. \pm	5.854	4.869
H^+/K^+ ATPase enzyme gene expression	Mean	11.167	6.953
	S.D. \pm	0.794	0.710
CCK gene expression	Mean	0.385	0.643
	S.D. \pm	0.079	0.036
P Value			<0.05*

[#]Mean is in nmol/min/ml

*: statistically significant compared to corresponding value in subgroup Bi ($P < 0.05$)

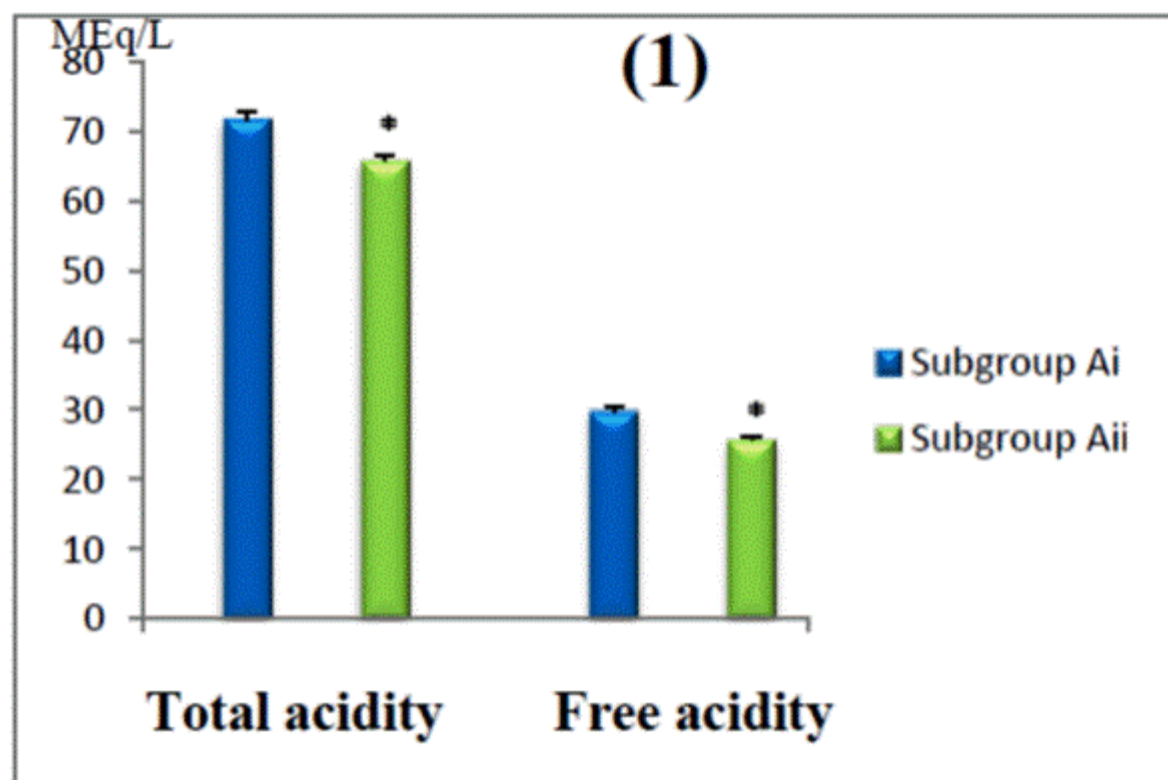


Figure (1): Comparison of total and free acidity between subgroups Ai (control) and Aii (vinegar fed). Values are represented as mean \pm SD. *: statistically significant compared to corresponding value in subgroup Bi ($P < 0.05$).

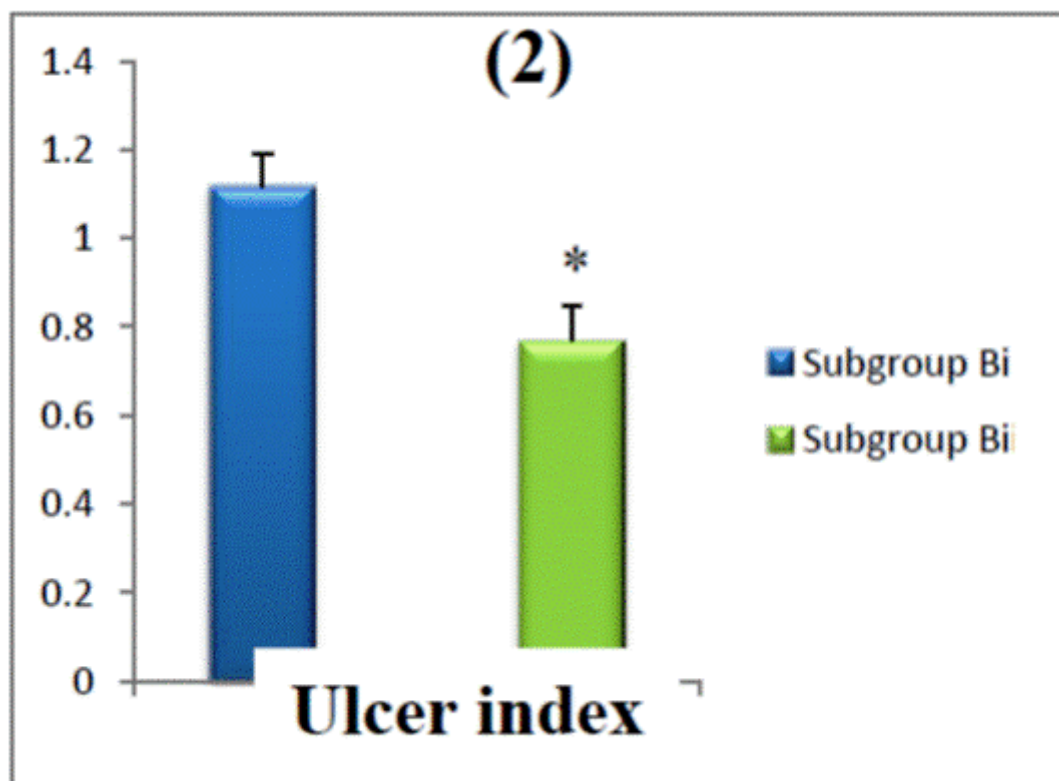


Figure (2): Comparison of ulcer indices between subgroups Bi (control) and Bii (vinegar fed). Values are represented as mean \pm SD. *: statistically significant compared to corresponding value in subgroup Bi ($P < 0.05$).

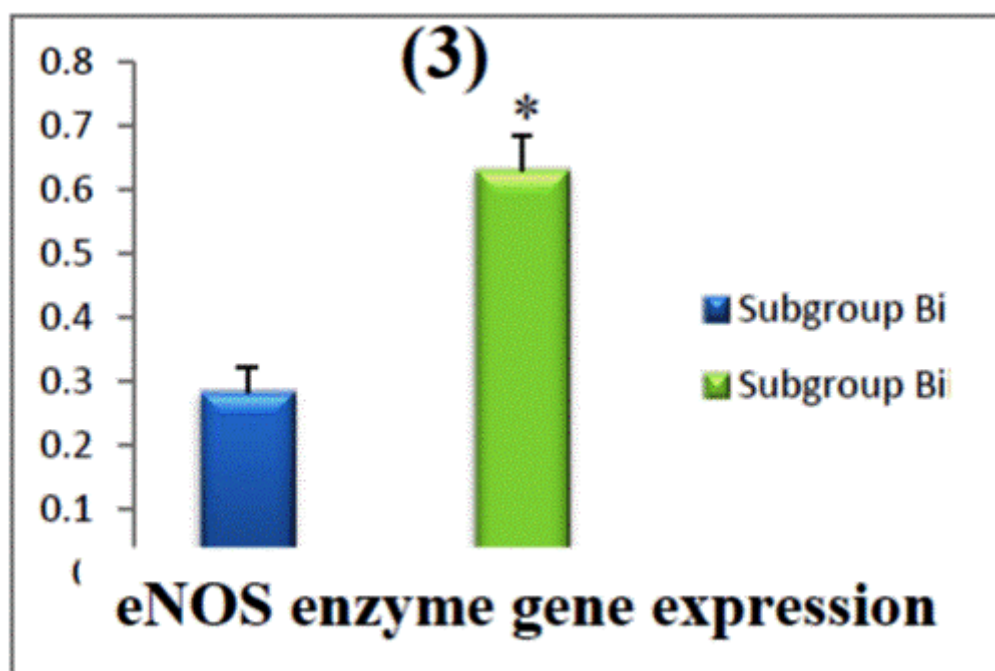


Figure (3): Comparison of eNOS enzymes gene expression between subgroups Bi (control) and Bii (vinegar fed).

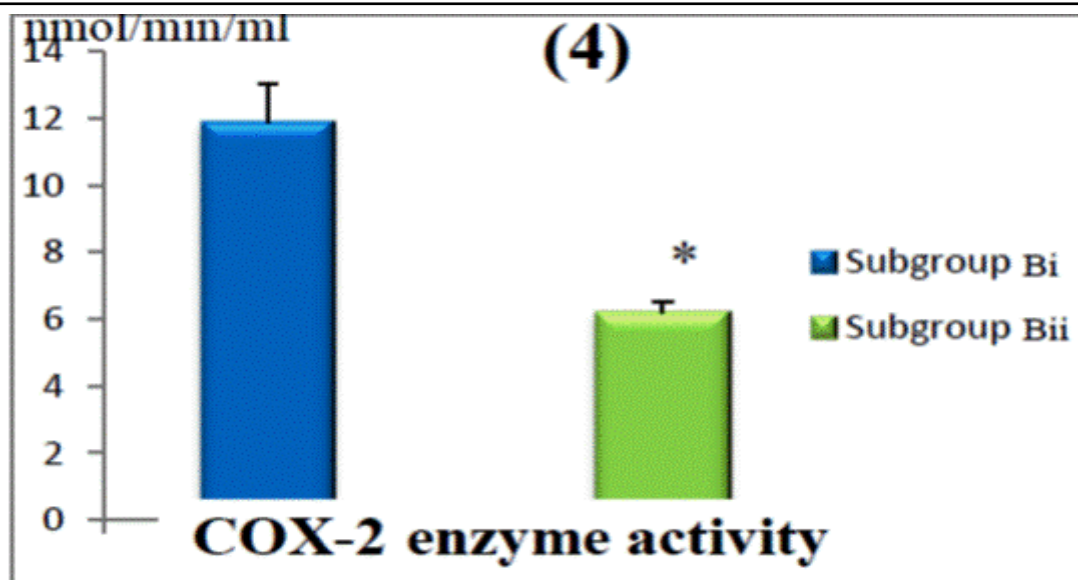


Figure (4): Comparison of COX-2 enzyme activity between subgroups Bi and Bii.

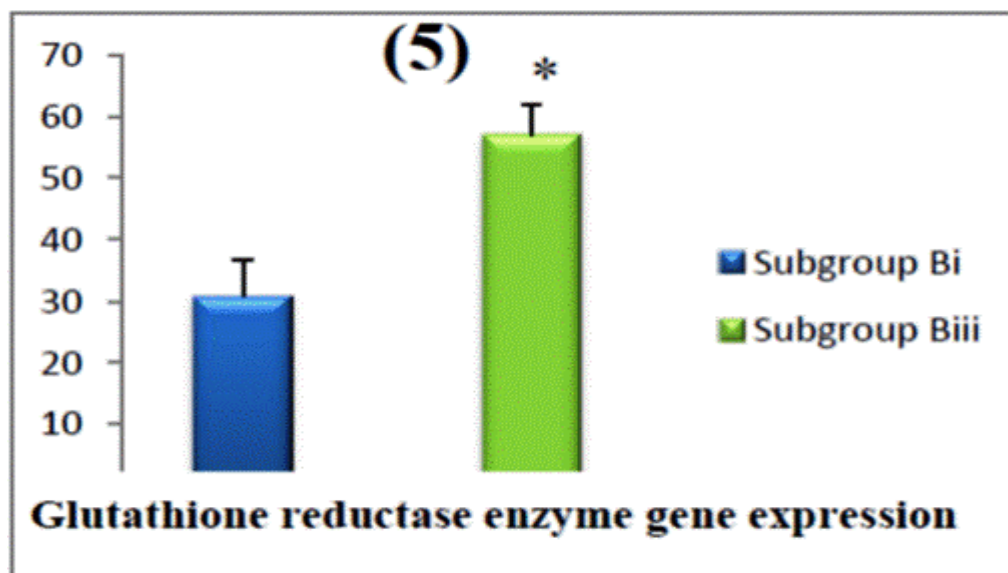


Figure (5): Comparison of glutathione reductase enzyme gene expression between subgroups Bi and Bii.

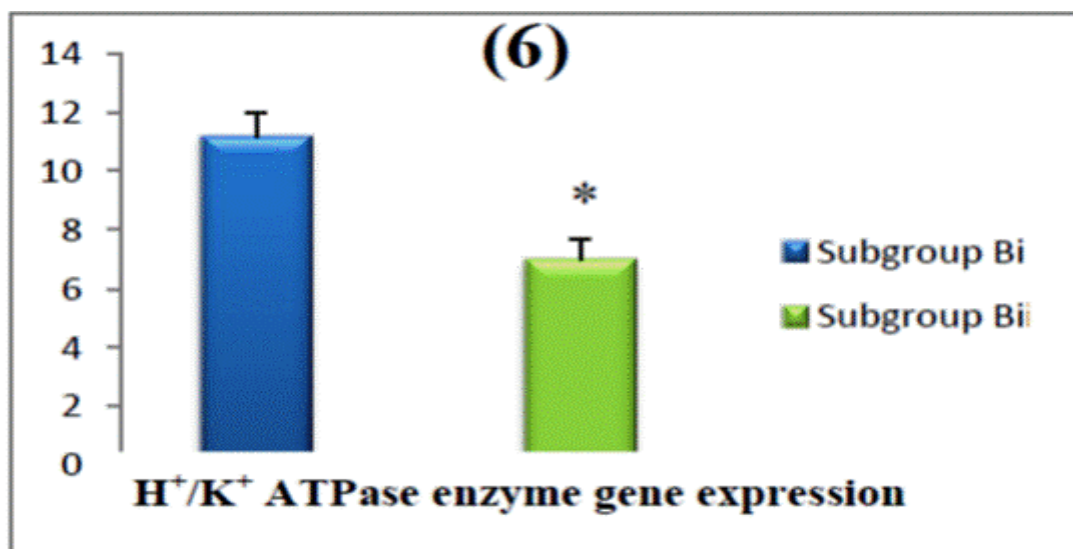


Figure (6): Comparison of H⁺/K⁺ ATPase enzyme gene expression between subgroups Bi and Bii.

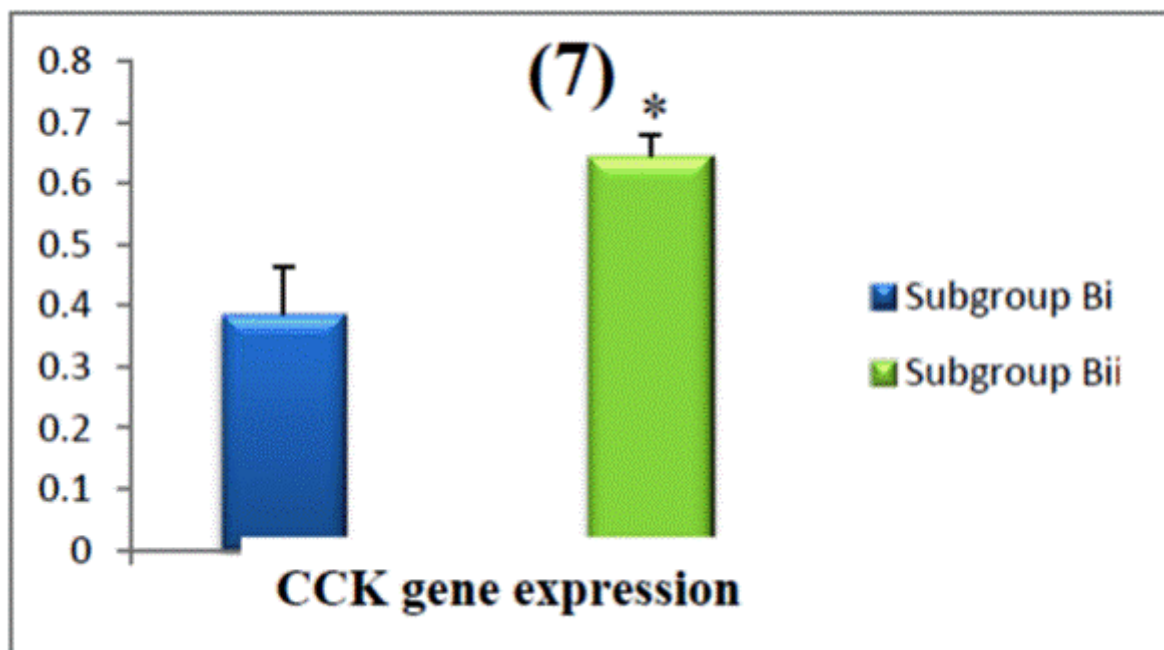


Figure (7): Comparison of CCK gene expression between subgroups Bi and Bii.

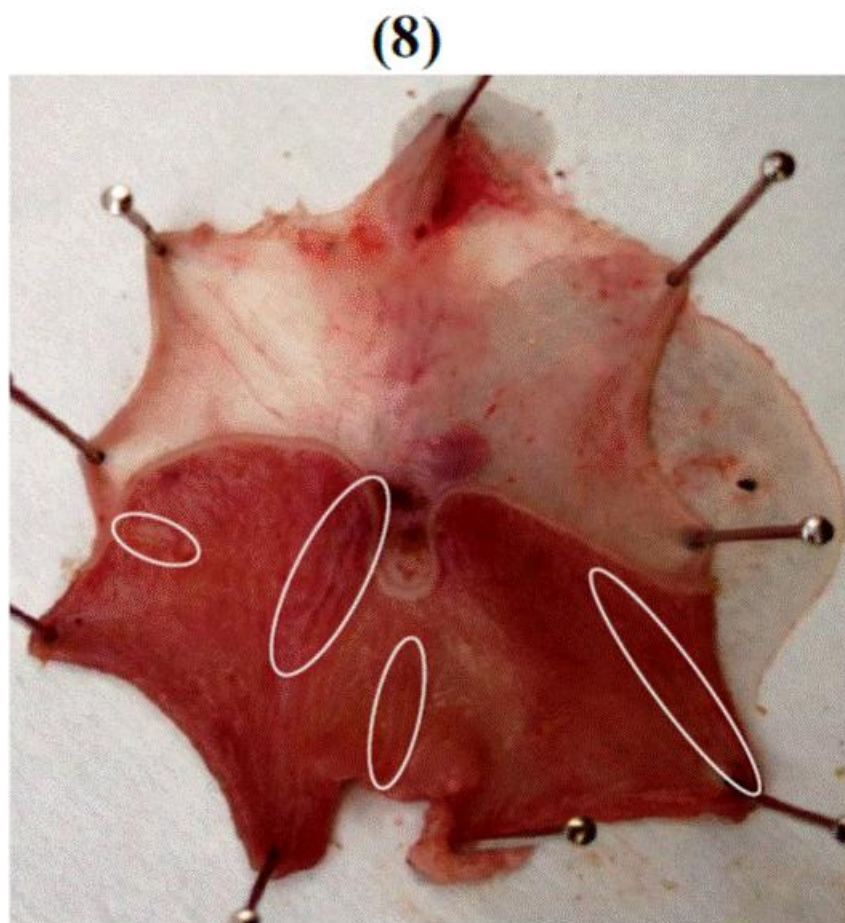


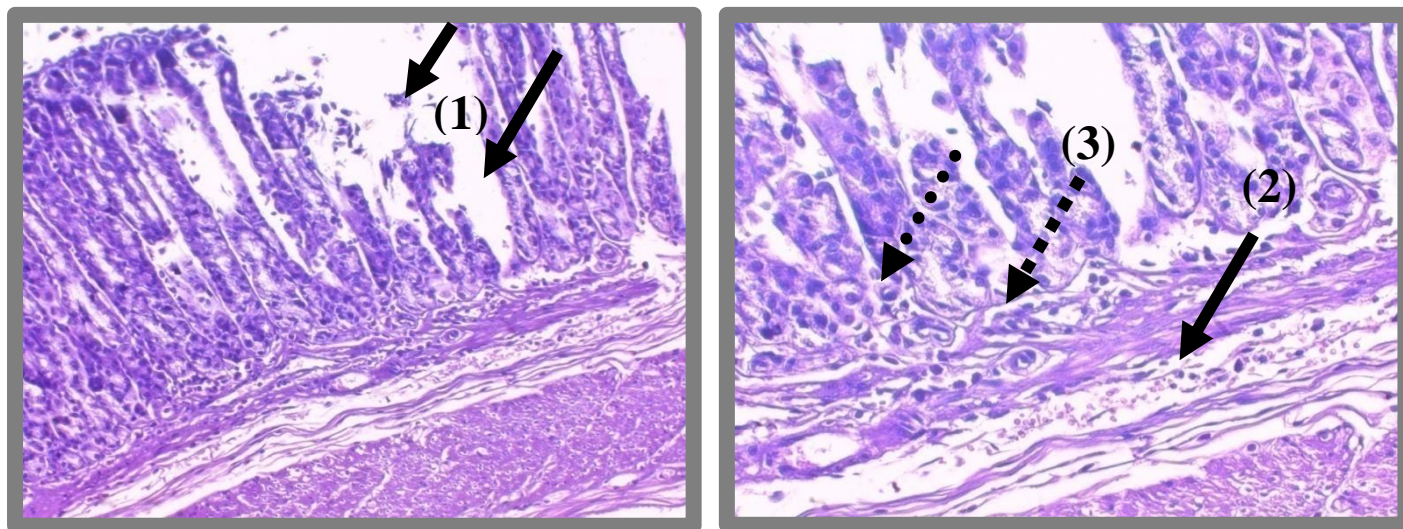
Figure (8): Macroscopic representation of gastric mucosa of Subgroup Bi (control group).

(9)



Figure (9): Macroscopic representation of gastric mucosa of Subgroup Bii (vinegar fed group).

figure (10)

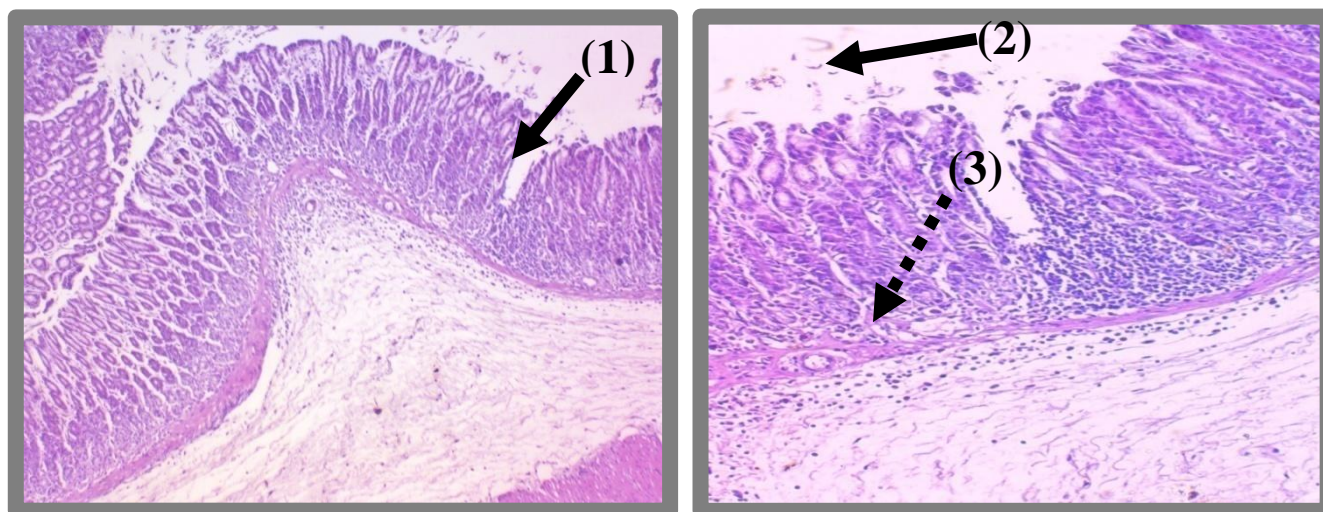


Serial sections of gastric excision biopsies revealing wide ulceration of the gastric mucosa (1), with edematous lamina propria (2) with severe lymphoplasmic cell infiltrate with excess neutrophils (3 dashed arrows).

(A picture is consistent with severe acute ulcerative gastritis.)

Figure (10): Microscopic representation (10X and 20X) of gastric mucosa of subgroup Bi (control group) stained with Hx & E.

Figure (11)



Sections of gastric excision biopsies revealing focal superficial ulceration of the gastric mucosa above the muscularis mucosa (1), accompanied by extrusion of a fibrin purulent exudates into the lumen (2), with moderately edematous lamina propria with engorged capillaries, moderate lymphoplasmic cell infiltrate with neutrophils (3).

(A picture is consistent with moderate acute gastritis with focal superficial ulceration)

Figure (11): Microscopic representation (10X and 20X) of gastric mucosa of Subgroup Bii (vinegar fed group) stained with Hx & E.

DISCUSSION

The results of the present work illustrated that feeding rats with vinegar (subgroups Bii) produced significant reduction in stress-induced gastric ulcer.

On measuring total and free acidity, the results of present work demonstrated that vinegar fed rats (subgroup Aii) had a highly significant decrease in the mean value of both total and free acidity compared to control subgroup Ai. Vinegar fed rats had also a highly significant increase in the mean value of CCK gene expression compared to control subgroup Ai.

The results of present work demonstrated that vinegar fed rats (subgroups Bii) had a highly significant decrease in the mean value of gastric H^+/K^+ ATPase enzyme activity and the mean value of ulcer index compared to the control group (subgroups Bi). Hyperacidity is a pathological condition due to uncontrolled hypersecretion of hydrochloric acid from the parietal cells (PC) of gastric mucosa through the proton pumping by H^+/K^+ ATPase harboured on the plasma membrane of PC¹⁷. H^+/K^+ ATPase is a regulatory enzyme found in the plasma membrane of parietal cells involved in passage of protons into the lumen of stomach causing acidity¹⁸.

Health benefits of apple have been reported¹⁹, and one of important category of apple active constituents is phenols. Phenols are secondary plant metabolites and act as antioxidant, antibacterial²⁰, and antiviral agents²¹. Variety of substances, such as carotenoids, flavonoids, polyphenols, phenolic acid and uric acid are very famous antioxidants²². Among these substances, polyphenols and their by-products are the most important antioxidants in plants. Several studies in humans or in animals have demonstrated that polyphenols possess significant chemo preventive properties due to their antioxidant capacity²³. Polyphenols are present in fruits, wines and their by-products like vinegars. Although the antioxidant role of many fruits and different wines *in vitro* and *in vivo* upon healthy and different kind of disease subjects has been extensively studied²⁴, there are fewer studies to examine the antioxidant activity of vinegars.

Vinegars contain different antioxidant compounds, and their final quality depends to the raw material used as substrate, the acetification system used, and the ageing procedure used during their production²⁵. Due to the chemical diversity of antioxidant compounds present in vinegars, and perhaps, the additive, synergistic and antagonistic interactions among these molecules and also the other nutrients present in them, do not necessary reflect their total antioxidant capacity (TAC)²⁶.

Antioxidant capacity was found to be positively correlated with both, sugar and phenol content of vinegars. Regular apple vinegar (RAV), exhibited improved health benefits with the presence of residual phenols in addition to acetic acid²⁷.

Specific non-enzymatic scavengers, such as tocopherols, ascorbic acid, flavonoids, and phenols, can prevent biological damages by trapping radical oxidants such as SOD²⁸. Therefore, SOD active constituents in vinegar would also have effects on maintaining normal vascular function²⁹. Phenolic acids and their derivatives are widely spread in plants³⁰ and a number of phenolic acids are linked to various cell wall components³¹. Free and bound phenolics are known to play a crucial role in the defence mechanism, offering protection against oxidative stress (OS) caused by both biotic and abiotic factors. Phenolics have been shown to possess antiulcer activity³². The antioxidant activity of phenolics may be an important contributing antiulcer factor since free radicals (FR)/reactive oxygen species (ROS) are related to the occurrence of ulcers³³.

This is supported by the results of present work that have shown a highly significant increase in the mean value of eNOS enzyme gene expression, a highly significant increase in the mean value of glutathione reductase enzyme gene expression and a highly significant decrease in the mean value of COX-2 enzyme activity in vinegar subgroup (Bii) compared to control subgroup (Bi).

SUMMARY AND CONCLUSION

In conclusion, the present study revealed that vinegar offered protective effect on the stomach of mediated by anti-ulcer, antioxidant and gastric protective mechanisms on stress-induced gastric ulceration. For future research work, it's recommended to study the effect of vinegar on other modules of gastric ulceration and their possible underlying mechanisms.

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CONFLICT OF INTEREST

There is no conflict of interest.

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