

The Protective Effect of Vanadium Sulphate on Ethanol-induced Gastric Ulcer

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Objective: To evaluate the protective effect of vanadium sulphate on ethanol-induced gastric ulcer in rats and its mechanism of action.

Setting: Department of Anatomy, King Khalid University, Saudi Arabia.

Design: Experimental animals study.

Method: Except for the control group (group I), gastric ulcer was induced in five groups of rats (II to VI), each consists of six. Ethanol was fed into rats which were pre-treated with distilled water, cimetidine, vanadium, selenium and a combination of vanadium and selenium (group II to VI respectively). The ulcer indices were determined in all these groups. Following macroscopic observations, specimens of the stomachs were taken and processed for histologic examination. Stomachs were then homogenized separately for each group and the supernatants were assayed for the activities of superoxide dismutase (SOD), catalase (CAT), levels of reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS). Levels of these compounds from all groups were statistically analyzed for comparison.

Result: Rats pre-treated with the reference drug cimetidine showed more or less normal gastric mucosa. However, mild disruption of the surface mucosa was observed in rats receiving selenium and vanadium. Those receiving combination of selenium and vanadium showed almost normal mucosa. Furthermore, vanadium alone or in combination with selenium demonstrated a significant reduction of tissue lipid peroxidation levels, and potent ameliorative effect of the enzymatic and non-enzymatic components of the endogenous antioxidant systems.

Conclusion: Vanadium sulphate significantly inhibits lipid peroxidation and enhances the effects of enzymes that scavenge free radicals that are implicated in the pathogenesis of ethanol-induced ulcers in rats. Selenium seems to enhance its action and exerts a synergistic effect.

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Vanadium is a trace element found in water, rocks and coal at various concentrations¹. In a small study performed by the University of Texas Health center, vanadyle sulphate was found to reduce hyperglycemia, improve liver and muscle insulin sensitivity and significantly

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improved glycemic control in Type II diabetes². Recent experimental studies in mice have also shown that a complex of vanadium improved diabetes, obesity and hypertension in mice and improved spatial learning and memory in diabetic mice^{3,4}. Compounds of vanadium have also been shown to possess antioxidant and antibacterial effects⁵.

In a short communication by Suther et al, the effect of sodium metavanadate was studied in ethanol and pylorus ligation induced ulcer models in rats⁶. The complex has shown a significant reduction in the total acid output and a rise in carbohydrates and mucin; thus, the authors concluded that sodium metavanadate possessed a significant antiulcer effect. Selenium has also been shown to possess a gastro-protective effect against ethanol-induced gastric mucosal lesions through prevention of lipid peroxidation and activation of radical scavenging enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase⁷.

Reactive oxygen species (ROS or pro-oxidants) present as free radicals generated by gastric mucosal cells in some physiological and pathological circumstances. Any derangement between pro-oxidants and antioxidants, in which pro-oxidants prevail is known as oxidative stress, which is believed to initiate stomach ulcers and gastric carcinoma⁸. Ethanol induced gastric damage has been suggested to be mediated by the generation of free radicals⁹. It is worth mentioning that ethanol is metabolized in the body and releases superoxide anion and hydroxy free radicals thus leading to the prevalence of these radicals and ethanol-induced gastric lesions and gastrointestinal damage¹⁰. Ethanol is known to produce severe gastric hemorrhagic lesions when fed to rats via gavage. It is believed that the lesions include depletion of gastric mucus content, damage to mucosal blood flow and mucosal cell injury.

Furthermore, ethanol-induced gastric mucosal damage is the result of overproduction of free radicals which leads to increase in lipid peroxidation¹¹. Increase in peroxide and oxygen-derived free radicals result in significant changes in cellular levels and leads to membrane damage, exfoliation, epithelial erosion and cell death¹².

The aim is to evaluate the protective effect of vanadium sulphate and its combination with selenium in ethanol-induced gastric ulcer in rats.

METHOD

The study was conducted in accordance with the national institute of health's guide for the care and use of laboratory animals¹³.

The animals used in this study were weighting 200±10 gm and were divided into six groups, each consists of six rats, housed in a cage in an air-conditioned room and kept in standard laboratory conditions under natural light and dark cycle (12 hour light/dark) maintained at an ambient temperature 25±2°C. The animals were fed standard pellet diet and water ad libitum.

Group I received one ml of distilled water for 7 days. The rats from group II to VI received distilled water, cimetidine (20 mg/kg), vanadium (0.5 ppm), selenium (100 µg/kg) and a combined treatment of vanadium (0.5 ppm) and selenium (100 µg/kg) respectively for one week¹⁴⁻¹⁶. On the last day of treatment, ulceration was induced in all of these groups by administering a single dose of 2 ml/kg 70% ethanol¹⁷.

All treatments except vanadium which was added in the drinking water to its final concentration (0.5 ppm) were administered orally using stainless steel cannulae. At the end of each experiment, rats were sacrificed after one hour using diethyl ether anesthetization and their stomachs were inspected, photographed and removed to measure gastric ulcer index (UI). Subsequently, sections of the stomachs were removed for histopathological examination and determination of the levels of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), to measure the activities of superoxide dismutase (SOD) and catalase (CAT).

To quantitatively measure the protective effect of each treatment on gastric induced ulcers, UI were calculated as described by Nwafor et al¹⁸. The stomach was cut open along the greater curvature. The lesions were examined macroscopically using hand lens and UI was calculated by using the following equation:

$$\text{UI} = \frac{\text{Total ulcer score (mm}^2\text{) in a group of rats}}{\text{Number of ulcerated animals in the same group}}$$

Values were expressed as Mean \pm SD for 6 rats in each group according to the parameters investigated. Data were then fed in graphpad prism version 5 and analysis was done by a one way analysis of variance (ANOVA) and Tukey's t-test. Values were considered significantly different at $P < 0.05$.

Cimetidine (Cat no. SKF-92334), vanadium (vanadium oxide sulfate, Cat no. MKBH3141V) and selenium (Cat no. 7782-49-2) were purchased from Sigma-Aldrich (UK). Selenium and cimetidine were prepared to final concentration by dissolving in distilled water whereas vanadium was added to the desired concentration in drinking distilled water.

RESULT

The ethanol administered group (group II) showed high UI which was significantly higher ($P=0.0001$) than all other groups of rats. Administration of the positive drug (cimetidine, group III) resulted in significant decrease ($P=0.0001$) of UI ($P=0.010$). Administration of all drugs to rats resulted in significant decrease ($P < 0.05$). The effect was most profound in group VI which had combined dose of selenium and vanadium and the effect was significantly lower than that of all other tested drugs (group IV and V).

The ANOVA test revealed that UI of group IV which received vanadium alone was significantly higher than the rats that received the positive drug (group III, $P=0.006$), selenium (group V, $P=0.034$) or a combined doses selenium and vanadium (group VI, $P=0.001$). UI of group V which received selenium alone was significantly lower than that of group III ($P=0.002$) and group IV ($P=0.001$) but still significantly higher than the UI in group VI ($P=0.001$), see figure 1 and 2.

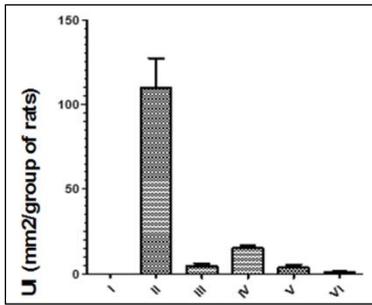


Figure 1: Gastric Ulcer Index (UI) in All Groups of Rats, Values Are Significantly Different Compared to I-VI

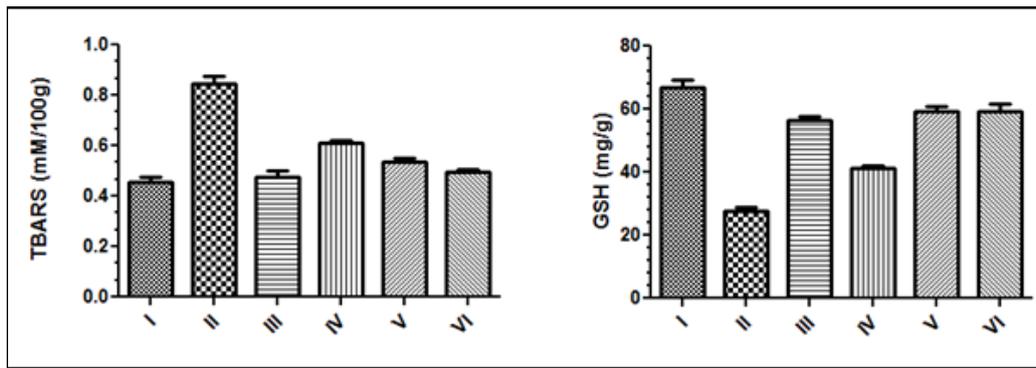


Figure 2: Levels of Thiobarbituric Acid Reactive Substances (TBARS) and Reduced Glutathione (GSH) in the Stomach Homogenate of All Experimental Groups of Rats. Values Are Significantly Different Compared to Group I-VI

The gastric mucosa of rats received distilled water (group I) had a normal macroscopic appearance where the rats given distilled water and ethanol showed severe injuries in the gastric mucosa, see figure 3A. The gastric mucosa of rats pretreated with cimetidine, vanadium, selenium and combination of vanadium and selenium showed normal gastric mucosa compared to those received distilled water and ethanol, see figures 3B-3E.

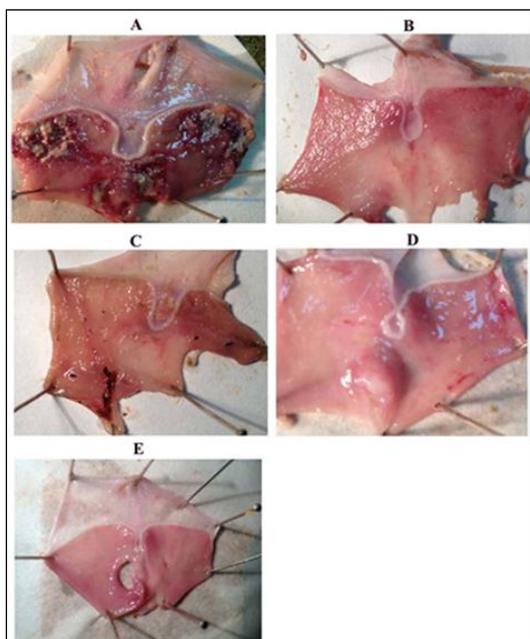


Figure 3(A-E): Gross Appearance of Gastric Mucosa of Rats Administred Ethanol and Pretreated (A-Distilled Water, B-Cimetidine, C-Vanadium, D-Selenium and E-Combination of Vanadium and Selenium. Severe Erosions are Seen in the Gastric Mucosa of A and B-E Shows Normal of Gastric Mucosa)

Figure 4 (A: i and ii) showed normal architecture of the gastric mucosa, muscularis mucosa and submucosa. Stomachs of rats which received ethanol and distilled water showed severe disruption and detachment of the surface mucosa, erosion of the fundic glands, interstitial bleeding and the submucosa showed edema and inflammatory cell infiltration, see figure 4 (B: i and ii). Rats pretreated with the reference drug cimetidine showed more or less normal gastric mucosa, see figure 4 (C: i and ii). However, mild disruption of the surface mucosa was observed in rats receiving selenium and vanadium, see figure 4 (D&E: i and ii). Those receiving a combination of selenium and vanadium showed almost normal mucosa, see figure 4 (F: i and ii).

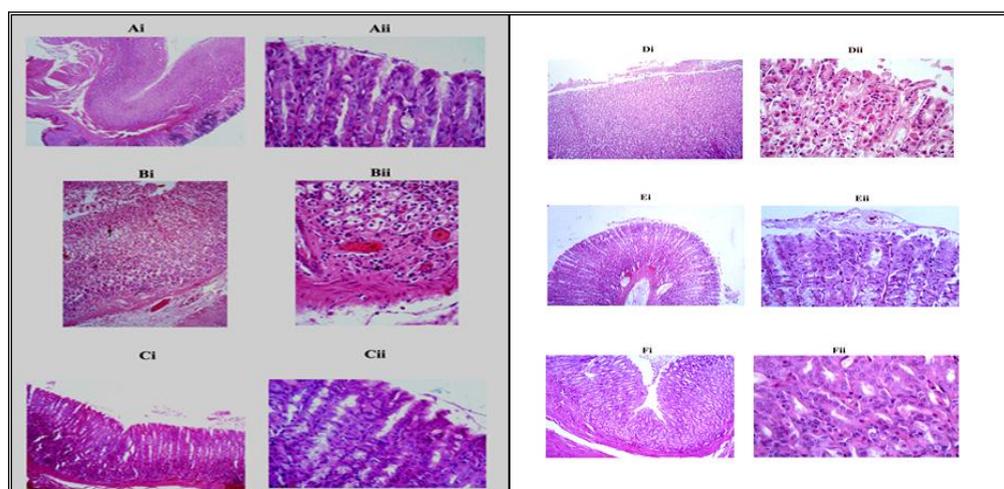


Figure 4 (A-F): Light Microscopy Examination of Rat Gastric Mucosa (A-Control, B-Distilled Water, C-Cimetidine D-Vanadium, E-Selenium and F-Combination of

Vanadium and Selenium; (i) Showing Normal Mucosa and Slight Separation in the Upper Part of the Mucosa (ii) Showing Normal Fundic Glands)

Oral administration of ethanol to rats (group II, distilled water) resulted in a significant increase in TBARS levels ($P=0.0001$), significant decrease in SOD ($P=0.0001$) and CAT ($P=0.0001$) accompanied by a significant decrease in GSH levels ($P=0.0001$). Groups (III-VI) had reduced levels of the above parameter. The maximum decrease in TBARS levels occurred in group III (cimetidine), which was not significantly different compared to group I (control), group V (selenium) or group VI (selenium+vanadium). In spite its decrease, the ANOVA test revealed that the levels of TBARS in group V and VI remained insignificantly higher than that of control group. Except for group V (selenium), the ANOVA test showed that the levels of TBARS in group IV received vanadium alone was not significantly different compared to group V, but was significantly higher than group III ($P=0.0016$) and VI ($P=0.0067$).

However, there was a significant decrease in GSH levels in group II (administered ethanol alone) compared to control group ($P=0.0001$). Compared to group II, oral administration of all compounds resulted in significant increase in the level of this parameter in all groups of rats with maximum increase in group V (selenium) ($P=0.0001$) and VI ($P=0.0001$) (selenium +vanadium). The levels of this parameter groups III or IV administered cimetidine or vanadium respectively were significantly lower than that of the control group ($P=0.0066$ and $P=0.0001$ respectively). On the other hand, group V and VI (Selenium or vanadium and selenium respectively) showed no significant difference in TBARS levels compared to control group. The ANOVA test showed that the levels of GSH in group IV administered vanadium resulted in the minimum improvement in GSH level as its level remained significantly lower ($P=0.0001$) than that of group III, V and VI, see figure 1.

The activities of SOD and CAT were significantly raised in group II, which received ethanol alone compared to control group ($P=0.0001$). The activities of these two enzymes were significantly decreased with all treated groups III-VI. Group II (distilled water), group III (cimetidine) and group VI (selenium+vanadium) showed profound decrease ($P=0.0001$ for both SOD and CAT) in the activities of the two enzymes which were not significantly different when compared to each other; but were significantly lower when compared to their levels in groups IV (vanadium) ($P=0.0015$ and $P=0.0012$, SOD; $P=0.0037$ and $P=0.0038$, CAT respectively) or V (selenium).

The ANOVA test revealed that the maximum decrease in SOD levels in groups III and VI were not significantly different compared to control group, but the maximum decrease in CAT activity which occurred in these two groups were significantly higher than its levels compared to control group ($P=0.0082$ and 0.0079 respectively), see figure 5.

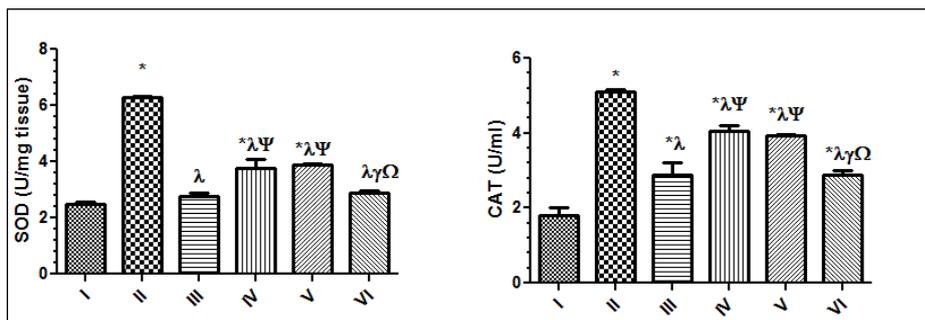


Figure 5: Activities of Superoxide Dismutase (SOD) and Catalase (CAT) in the Stomach Homogenate of All Experimental Groups of Rats. Values Are Significantly Different Compared to Group I-V

DISCUSSION

The present study demonstrated that administration of vanadium sulphate alone or in combination with selenium has significant protective effect on the gastric mucosa against ethanol challenge as shown by significant reductions in gastric ulcer index in rats, improvement in histopathological changes, reduction of tissue lipid peroxidation levels, as well as its potent ameliorative effect of the enzymatic and non-enzymatic components of the endogenous antioxidant systems.

Suther et al found a similar protective effect when they studied sodium metavanadate alone, in contradistinction to the present study, where a combination of selenium and vanadium sulphate was investigated⁶. Suther et al postulated that the protective role of vanadium was mediated by suppressing aggressive factors such as gastric acid secretion and enhanced mucin activity⁶.

In addition to their findings, we have documented for the first time, the protective effect of vanadium sulphate when the activities of SOD and CAT enzymes were determined in all groups of rats and compared to their controls. Ethanol induces depletion of gastric mucus, damage to mucosal blood flow and mucosal cell injury. One limitation in our current study is that we did not investigate the effect of vanadium on gastric content and blood flow.

The antioxidant effect of vanadium complex has also been recently documented by Francik et al and Vatsala et al^{18,19}. The protective effects of selenium demonstrated in this study were similar to those reported by Kim et al⁷.

In addition, the data presented seem to indicate a synergistic protective effect when vanadium was co-administered with selenium. This observation may require further comparative studies of these two compounds since they may present a potential novel approach in the prevention and treatment of gastric ulcers in humans.

CONCLUSION

We conclude that vanadium sulphate significantly inhibits lipid peroxidation and enhances the effects of enzymes that scavenge free radicals that are implicated in the pathogenesis of ethanol-induced ulcers in rats.

Author contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

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Competing interest: None. **Sponsorship:** None.

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Ethical approval: The Research Ethical Committee, College of Medicine, King Khalid University, KSA.

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