Amelioration of gastric mucosal lesions induced by multiple stressors in rats treated with garlic (*Allium sativum* L.) suspension

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**ABSTRACT**

Garlic (*Allium sativum* L.), renowned for its pleiotropic medicinal value in traditional medicine, is a popular culinary item consumed worldwide. The present study aimed to justify the use of garlic in Arab traditional medicine for the treatment of acute gastric ulcer. The plethora of its beneficial effects has been ascribed to its multiple bioactive constituents including organosulfur compounds. Nutraceutical products containing garlic-derived compounds are known to possess a myriad of pharmacological effects such as hepatoprotective, nephroprotective, cardioprotective, hypolipidemic, anti-microbial, anti-tumor effects. Studies in animal models and humans have shown a beneficial effect of garlic on the gastrointestinal system. Herein, using a rat model of gastric mucosal injury, simulating gastric ulcer disease of humans, we show that garlic suspension (250 and 500 mg/kg) dose-dependently mitigates gastric ulceration (evaluated as ulcer index) triggered by hypothermic restraint, pyloric ligation (Shay rats), indomethacin and necrotizing agents (80% ethanol, 0.2 mol/L NaOH and 25% NaCl). The results of this study show that the acute administration of garlic suspension significantly thwarted gastric acid secretion in pylorus-ligated rats and enhanced mucus secretion following the administration of 80% ethanol. Mechanistically, rats pretreated with garlic suspension showed a slight alteration in non-protein sulfhydryl and malondialdehyde levels of gastric tissue following treatment with 80% ethanol; this data points to a subtle antioxidant activity of the suspension. Taken together, these findings unravel gastroprotective effects of garlic suspension in rats subjected to multiple stress conditions. Dietary consumption of garlic and garlic-based supplements may have a beneficial impact on digestive health by alleviating the development and/or progression of gastric mucosal damage.

**Keywords:** Garlic, antioxidant, antiulcer, rats, nutraceutical.

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**INTRODUCTION**

Dietary garlic (*Allium sativum* L., family Alliaceae) is widely consumed in different cuisines and has been an integral part of folk medicine practices since time immemorial (Rana et al., 2011). Traditionally, garlic has been used to treat a wide array of disorders such as arthritis, asthma, toothaches, cancer, and cardiovascular disease; in modern times garlic supplements are available as over-the-counter products (Rana et al., 2011; Khatua et al., 2013; Bayan et al., 2014). The health benefits of garlic are attributed to its diverse range of pharmacological properties such as anti-inflammatory, nephroprotective, hepatoprotective, cardioprotective, hypolipidemic, antihypertensive, antimicrobial, antifungal, antiviral, antiparasitic, antithrombotic, and antitumor effects which has been confirmed by numerous animal and human studies (Omar and Al-Wabel, 2010; Kim et al., 2011; Rana et al., 2011; Alkreathy et al., 2012; Li et al., 2013; Shiju et al., 2013; Bayan et al., 2014; Bagul et al., 2015; De Gianni and Fimognari, 2015; Ebrahimi et al., 2015; Fratianni et al., 2016; Jeong et al., 2016; Ried, 2016).

Several studies have promulgated that the beneficial
In the present study, we examined the effects of acutely administered garlic suspension on gastric ulcers in rats triggered by hypothermic restraint stress, indomethacin, necrotizing agents (80% ethanol, 0.2 M NaCl) or pylorl ligation and further explored the possible mechanism(s) involved.

MATERIALS AND METHODS

Plant material and preparation of aqueous suspension

Fresh garlic for use in this experiment was procured from a local vegetable shop in Riyadh. It was then identified and authenticated by an expert taxonomist Dr. Mohammed Yousef at the College of Pharmacy, King Saud University. An aqueous suspension of the garlic was prepared by slicing it, followed by shade drying and then pulverizing it to a very fine powder (Mesh # 70 micron). A known amount of powder was then suspended in distilled water to attain the aqueous suspension.

Experimental animals

Wistar albino rats of both sexes were obtained from the Experimental Animal Care Center (EACC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. They were approximately the same age and weighed 150 to 200 g. Standard conditions of temperature, humidity and light (12 h light-dark cycle) were maintained. The rodents were fed with a Purina Chow diet and free access to water. 36 hours prior to the testing, the animals were fasted and only had access to water ad libitum.

The Ethics Committee of the Experimental Animal Care Society, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia approved all experimental procedures.

Gastric wall mucus determination

In order to determine the gastric wall mucus, the rats received 80% ethanol only or ethanol plus garlic suspension. After this, a glandular segment of the animal’s stomach was removed and weighed. Each segment was then treated immediately with 1% Alcian blue solution (in sucrose solution, buffered with sodium acetate at pH 5). The additional dye was then removed by rinsing with sucrose solution. Magnesium chloride solution was used to extract the dye complexed with gastric wall mucus. Next, a 4 ml aliquot of blue extract was shaken with an equal volume of diethyl ether. The emulsion obtained was then centrifuged and the absorbance of the aqueous layer was recorded at 580 nm. Calculation of the quantity of Alcian blue extracted per gram of the glandular tissue (net) was then done.

Determination of anti-secretory activity

In order to determine the anti-secretory activity, the rats which were put under 36 h fasting condition were anesthetized under light ether and their pylorus was ligated. Precaution was taken to avoid bleeding of the blood vessels. Soon after this step, aqueous suspension of garlic was administered intraperitoneally and 6 h later the animals were sacrificed. Next, the stomachs were removed, their contents collected, measured, centrifuged and then subjected to analysis for titratable acidity against 0.01 NaOH at pH 7 and the total acid output was calculated (Shay, 1949).

Indomethacin-induced gastric mucosal ulceration

Oral administration of Indomethacin which has been suspended in 1.0% carboxymethyl cellulose in water (6 mg/ml) was done to the fasted rats at 30 mg/kg body weight (0.5 ml/100 g). In the same way, the control rats were treated with an equivalent amount of the vehicle. 6 hours after this treatment the animals were sacrificed after which their stomachs were removed, rinsed with normal saline and ulcers were scored (Bhargava et al., 1973).

Gastric lesions induced by necrotizing agents

1 ml of different necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl) were used to produce gastric lesions in each rat present in the test group. 30 minutes prior to the administration of necrotizing agents, the rats were orally given the garlic suspension. 1 hour after this treatment, the animals were sacrificed, their stomachs excised and opened along the greater curvature. After washing with normal saline, the gastric lesions were quantified using biomolecular magnifier and ulcers were scored (Robert et al., 1983). The lesions were further assessed by two observers blinded to the experimental protocol.

Hypothermic restraint stress-induced ulcers

The method used by Senay and Levine (1967) has been followed with minor adjustment. The animals were first put on fast for 36 h with access only to water ad libitum. Next, the rats received a treatment of 1 to 2 g/kg body weight of oral garlic suspension. One hour later, the rats were immobilized in restraint cages and then placed inside a ventilated refrigerator maintained at a temperature of 2 to 4°C. After 3 h, they were taken out, sacrificed, their stomach excised and then examined for the severity of intraluminal bleeding according to the following arbitrary scale: 0- no blood detected; 1- thin blood follows the rugae; 2-thick blood follows the rugae; 3- thick blood follows the rugae with blood clots in certain areas; 4- thick
blood (Chiu et al., 1983). After wiping the blood off, the total area of lesions in each stomach was scored as described above.

**Estimation of non-protein sulphydryl (NP-SH) groups**

In order to analyze the oxidant antioxidant balance the gastric mucosal NP-SH was measured (Sedlak and Lindsay, 1968). After removal of glandular stomach, it was homogenized in ice-cold 0.02 M ethylene-diamine-tetraacetic acid. Next, the homogenate was mixed with distilled water and 50% (w/v) aqueous trichloroacetic acid and then centrifuged. The supernatants were mixed with phosphate buffer (pH 8), 5,5’ dithiobis (2-nitrobenzoic acid) (DTNB) was added and the sample was shaken. Within 5 minutes of addition of DTNB, the absorbance was read at 1/12 nm, against a reagent blank with no homogenate.

**Determination of malondialdehyde (MDA)**

The method used by Utley et al. (1967) was followed. After removal of stomach tissues, each tissue sample was homogenized in 0.15 M KCl (at 4°C, Potter-Elvehjem type C homogenizer) to give a 10% w/v homogenate. Aliquots of the homogenate (1 ml) were then incubated at 37°C for 3 h in a metabolic shaker. Next, 1 ml of 10% aqueous trichloroacetic acid (TCA) was added and mixed followed by centrifuging the mixture at 800 g for 10 min. One millimeter of the suspension was removed and mixed with 1 ml of 0.67% thiobarbituric acid in water and then placed in a boiling water bath for 10 min. The mixture was then cooled and diluted with 1 ml distilled water. The absorbance of the solution was then measured at 535 nm. The content of MDA (nmol/g wet tissue) was then calculated by reference to a standard curve of the MDA solution.

**Statistical analysis**

The values have been given as arithmetic means ± standard error of the mean (SEM). Statistical Analysis of the data was done by using one-way analysis of variance (ANOVA). This was followed by Dunnett’s t-test and Student’s t-test.

**RESULTS**

Firstly, we explored the effect of garlic suspension pretreatment on gastric ulceration in rats subjected to hypothermic restraint stress. To this end, hypothermic restraint stress potentiated bleeding in the gastric lumen and caused ulceration. These effects were significantly curtailed by pretreatment with garlic suspension (250 and 500 mg/kg) in a dose-dependent manner (Table 1).

Next, we explored the impact of garlic suspension pretreatment on gastric mucosal damage triggered by indomethacin. To this end, administration of garlic suspension dose-dependently attenuated the ulcer index in indomethacin-treated rats concomitantly pretreated with garlic suspension (250 and 500 mg/kg) as compared to rats administered indomethacin alone (Table 2).

**Table 1. Effect of Allium sativum suspension on Hypothermic Restraint Stress- induced intraluminal bleeding and gastric lesion in rats.**

<table>
<thead>
<tr>
<th>Treatments, n = 6</th>
<th>Dose (mg/kg)</th>
<th>Intraluminal bleeding score</th>
<th>Gastric lesion ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (distilled water)</td>
<td>-</td>
<td>1.83 ± 0.30</td>
<td>20.16 ± 1.07</td>
</tr>
<tr>
<td>Allium sativum suspension</td>
<td>250</td>
<td>1.50 ± 0.22</td>
<td>18.66 ± 0.95</td>
</tr>
<tr>
<td>Allium sativum suspension</td>
<td>500</td>
<td>0.66 ± 0.21*</td>
<td>13.33 ± 0.80**</td>
</tr>
</tbody>
</table>

(Mean ± SE). Six rats were used in each group. *p < 0.05, *** p < 0.001 vs control (distilled water) group, student’s t-test.

**Table 2. Effect of Allium sativum suspension on indomethacin-induced gastric lesions.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Indomethacin only)</td>
<td>-</td>
<td>30.033 ± ??</td>
</tr>
<tr>
<td>Allium sativum suspension</td>
<td>250</td>
<td>18.17 ± 2.67*</td>
</tr>
<tr>
<td>Allium sativum suspension</td>
<td>500</td>
<td>13.67 ± 5.52</td>
</tr>
</tbody>
</table>

(Mean ± SE). Six rats were used in each group. *p < 0.05 vs control (indomethacin only) group, student’s t-test.

We further examined whether pretreatment with garlic suspension could similarly inhibit gastric mucosal damage triggered by other agents that mediate cellular damage. As shown in Table 3, treatment of rats with necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl) elicited gastric ulceration which was significantly and dose-dependently reduced by pretreatment with garlic suspension (250 and 500 mg/kg).

We then evaluated whether garlic suspension pretreatment affected gastric mucus secretion in conferring its protective effect against gastric mucosal damage. To this end, administration of 80% ethanol in rats resulted in a significant decline in gastric wall mucus which was slightly but significantly thwarted by administration of 250 and 500 mg/kg garlic mucus in a dose-dependent manner (Table 4).
Table 3. Effect of *Allium sativum* suspension on gastric lesions induced by necrotizing agents.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>80% EtOH (mean ± SE)</th>
<th>0.2 mol/L NaOH (mean ± SE)</th>
<th>25% NaCl (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>6.66 ± 0.61</td>
<td>7.16 ± 0.40</td>
<td>6.50 ± 0.84</td>
</tr>
<tr>
<td><em>Allium sativum</em> suspension</td>
<td>250</td>
<td>3.16 ± 0.98**</td>
<td>5.00 ± 0.25</td>
<td>1.50 ± 0.34***</td>
</tr>
<tr>
<td><em>Allium sativum</em> suspension</td>
<td>500</td>
<td>1.16 ± 0.40***</td>
<td>1.66 ± 0.66***</td>
<td>1.16 ± 0.16***</td>
</tr>
</tbody>
</table>

(Mean ± SE). Six rats were used in each group. ** p < 0.01, *** p < 0.001 vs control group, student's t-test.

Table 4. Effect of *Allium sativum* suspension on the change in gastric wall mucus induced by 80% ethanol.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Gastric wall mucus (mean ± SE, µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>401.36 ± 28.66</td>
</tr>
<tr>
<td>80% Ethanol only</td>
<td>-</td>
<td>275.23 ± 30.51**</td>
</tr>
<tr>
<td><em>Allium sativum</em> suspension</td>
<td>250</td>
<td>286.48 ± 19.42</td>
</tr>
<tr>
<td><em>Allium sativum</em> suspension</td>
<td>500</td>
<td>294.80 ± 19.45</td>
</tr>
</tbody>
</table>

(Mean ± SE). Six rats were used in each group. ** p < 0.01 vs control (80% Ethanol only) group, student's t-test.

Table 5. Effect of *Allium sativum* suspension on gastric secretion, acidity and gastric lesion index in pylorus-ligated shay rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Volume of gastric content (ml)</th>
<th>Titratable acidity (mEq/L)</th>
<th>Ulcer index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Distilled water)</td>
<td>-</td>
<td>8.75 ± 1.08</td>
<td>88.33 ± 2.82</td>
<td>0.83 ± 0.31</td>
</tr>
<tr>
<td><em>Allium sativum</em> suspension</td>
<td>250</td>
<td>3.58 ± 0.55***</td>
<td>72.22 ± 5.42**</td>
<td>Undetectable</td>
</tr>
<tr>
<td><em>Allium sativum</em> suspension</td>
<td>500</td>
<td>2.83 ± 0.40***</td>
<td>56.67 ± 3.80***</td>
<td>Undetectable</td>
</tr>
</tbody>
</table>

(Mean ± SE). Six rats were used in each group. ** p < 0.01, *** p < 0.001 vs control (distilled water) group, student's t-test.

To elucidate whether the gastroprotective effects of garlic suspension were due to alterations in gastric acid secretion we used the pylorus-ligated shay rat model and quantified volume of gastric content and titrable acidity. As shown in Table 5, treatment with garlic suspension (250 and 500 mg/kg) significantly and dose-dependently reduced gastric content and titrable acidity in pylorus-ligated shay rats suggesting that gastroprotective effects of garlic suspension are fostered by its inhibitory effect on gastric acid secretion.

Furthermore, we evaluated whether the administration of garlic suspension could blunt gastric mucosal damage in pylorus-ligated shay rats. As shown in Table 5, pretreatment with garlic suspension significantly and dose-dependently decreased the ulcer index in pylorus-ligated shay rats pointing to its anti-ulcer effects in *vivo*.

To disclose the underlying mechanisms mediating the gastroprotective effects of garlic suspension pretreatment we determined tissue NP-SH levels in rats administered 80% ethanol to induce gastric mucosal damage. To this end, gastric NP-SH levels in gastric tissue of rats treated with 80% ethanol was significantly lower than untreated rats (Table 6). Pretreatment with garlic suspension did not significantly counteract NP-SH depletion following 80% ethanol administration (Table 6).

To further characterize the redox-sensitive gastroprotective effect of garlic suspension, we analyzed MDA concentration in gastric tissue samples. As depicted in Table 7, treatment of rats with 80% ethanol significantly enhanced tissue MDA concentration, an effect that was significantly and dose-dependently inhibited by treatment with 250 and 500 mg/kg onion suspension. These data point to the participation of redox-sensitive mechanisms in the amelioration of gastric mucosal damage conferred by garlic suspension pretreatment.

**DISCUSSION**

Pathogenesis of gastric ulceration *in vivo* is regulated by a fine balance between cytoprotective mucus on the gastric mucosal surface and acid secretion into the gastric lumen. An imbalance in any of these factors fosters the pathogenesis of a breakdown in the integrity of the mucosal lining leading to the development of ulcers and hemorrhage. Here, we disclose the inhibitory effect of acute garlic suspension pretreatment in rats on gastric ulceration triggered by necrotizing agents (80% ethanol, 0.2 M NaOH and 25% NaCl), indomethacin, hypothermic restraint and pylorus ligation. The present study underpins the modulation of redox balance by garlic suspension as a crucial mechanism in mediating its anti-ulcerogenic effect.

Our data show that garlic suspension pretreatment...
increased the abundance of cytoprotective mucus on the gastric lining, an effect that could directly mediate its gastroprotective effects in rats subjected to acute gastric injury elicited by multiple stressors in vivo. Our observation that garlic suspension attenuated indomethacin-induced gastric lesions underlines the possibility of garlic suspension in interfering with cyclooxygenase (COX) and prostaglandin metabolism. Interestingly, administration of aged garlic extract was recently shown to reduce indomethacin-induced gastric ulcers in rats by increasing prostaglandin E2 levels in the gastric tissue (El-Ashmawy et al., 2016). The organosulfur constituent of garlic S-allyl cysteine has been reported to alleviate gastric mucosal damage induced by non-steroidal anti-inflammatory drugs by the inhibition of COX-2 (Park et al., 2014). In another study, PMK-S005, a synthetic s-allyl-L-cysteine, was reported to confer protection against gastric damage by downregulating COX-2 expression (Choi et al., 2014). Intriguingly, multiple compounds in garlic were shown to have an inhibitory effect on prostaglandin E2 levels that mediates the contraction of rat gastric fundus (Gaffen et al., 1984). Strikingly, organosulfur compounds in garlic were shown to influence COX-2 expression and prostaglandin E2 generation in RAW264.7 cells (You et al., 2013). In the same study, the authors have also shown that the garlic constituent diallyl trisulfide potently inhibited COX-2 and inducible nitric oxide synthase as well as mediating a reduction in proinflammatory cytokines (You et al., 2013). Notably, aqueous garlic extract were shown to mitigate ischemia-reperfusion injury in rat forebrain via reduction of the arachidonic acid metabolites prostaglandin E2 and leukotriene C4 (Batirel et al., 1996).

Gastric tissue redox balance is a decisive element in the pathogenesis of gastric ulcers. Mounting evidence suggests that garlic bioconstituents exert antioxidant effects which influence the pathophysiology of different cellular functions studied in humans and animal models. Our data show that garlic suspension pretreatment had a modest antioxidant effect in terms of modulation of gastric tissue malondialdehyde and NP-SH levels. Aged garlic capsules were shown to reduce oxidative stress-associated alterations in malondialdehyde and total glutathione levels as well as catalase and superoxide dismutase activities in animal models of indomethacin-induced ulceration (Badr and Al-Mulhim, 2014). Pretreatment with garlic oil prior to ethanol administration in rats was shown to thwart ulcer index and ameliorate oxidative stress as well as lipid peroxidation (Khosla et al., 2004). The chemical s-allyl cysteine from garlic was documented to increase total anti-oxidant concentrations and trigger a significant induction of heme oxygenase-1 in parallel to enhancing mucus secretion (Park et al., 2014). Diallyl disulfide, a secondary organosulfur compound derived from garlic, was reported to curtail ethanol-induce gastric mucosal damage by modulating the activities of antioxidant enzymes including catalase, glutathione peroxidase, and glutathione reductase (Lee et al., 2015).

The gaseous mediator hydrogen sulfide is essential in the maintenance of gastrointestinal integrity and ulcer healing (Magierowski et al., 2015). Recent studies have shown that hydrogen sulfide mediates protection against gastric injury by reducing oxidative stress (Liu et al., 2012). Intriguingly, hydrogen sulfide has been shown to mediate the pharmacological activity of garlic extracts in vivo (Benavides et al., 2007). It is tempting to speculate that the gastrointestinal protective effects of garlic and its constituents may be mediated, at least in part, via hydrogen sulfide-dependent signaling mechanisms.

### Table 6. Effect of *Allium sativum* suspension on the NP-SH concentration in gastric ulcer induced by 80% ethanol.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>NP-SH concentration (µmol /100 mg wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>11.97 ± 0.88</td>
</tr>
<tr>
<td>80% Ethanol only</td>
<td></td>
<td>5.92 ± 0.22***</td>
</tr>
<tr>
<td><em>Allium sativum</em> suspension</td>
<td>250</td>
<td>5.45 ± 0.12</td>
</tr>
<tr>
<td><em>Allium sativum</em> suspension</td>
<td>500</td>
<td>5.27 ± 0.30</td>
</tr>
</tbody>
</table>

(Mean ± SE). Six rats were used in each group. *** p < 0.001 vs control (80% Ethanol only) group, student's t-test.

### Table 7. Effect of *Allium sativum* on malondialdehyde concentration in gastric ulcer induced by 80% ethanol.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>MDA (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>1.02 ± 0.037</td>
</tr>
<tr>
<td>80% Ethanol only</td>
<td></td>
<td>5.42 ± 0.25***</td>
</tr>
<tr>
<td><em>Allium sativum</em> suspension + 80% Ethanol</td>
<td>250</td>
<td>5.26 ± 0.23</td>
</tr>
<tr>
<td><em>Allium sativum</em> suspension + 80% Ethanol</td>
<td>500</td>
<td>4.38 ± 0.19*</td>
</tr>
</tbody>
</table>

(Mean ±SE) Six rats were used in each group. *p < 0.05, *** p < 0.001 vs control (80% Ethanol only) group, student's t-test.
In conclusion, the present study provides biological evidence that garlic suspension pretreatment alleviates gastric mucosal injury induced by multiple stressors in rats, an effect mediated, at least in part, by its antioxidant potential. Regular dietary consumption of garlic may thus have beneficial effects on maintaining the integrity of the digestive tract.

REFERENCES


