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Mechanisms of Antiulcerogenic Effect of Garlic (*Allium sativum)* in Albino Rats

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This work was carried out in collaboration between all authors. Author SAT designed the study, performed the statistical analysis, wrote the protocol. Authors OFS and ATG managed the analyses of the study. Author ATG managed the literature searches and wrote the first and subsequent drafts of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The aim of the present study was to investigate the possible effects of garlic juice, as well as feed supplemented with *Allium sativum*, on gastric ulceration, antioxidant activity and gastric mucus cell count in wistar rats.
Study Design: The albino rats were divided into six groups each group containing eight animals and treated for 30 days. A low dose (250mg/kg body weight) and high dose (500mg/kg) of garlic juice was orally administered to two of the experimental groups, while two other groups were fed with 5% and 10% *Allium sativum*/standard feed mix. The control group was fed on the standard rats' feed and water only, while a positive control group was given Misoprostol ($10\mu gkg^{-1}$) orally as a standard drug.
Place and Duration of Study: Department of Physiology, University of Ibadan between September 2011 and June 2012.
Methodology: Forty eight adult albino rats were divided equally into six groups. Groups I, II, III, IV, V and VI received fed standard, 250 mg *Allium sativum* /kg body weight, 500mg of *Allium sativum*/kg body weight, 5% supplement of *Allium sativum*, 10% supplement of *Allium sativum* and 10 ug of Misoprostol / kg body weight, respectively for 30 days. At the end of the study period, experimental ulceration was induced by administering $40mg/kg^{-1}$ body weight Indomethacin and six hours later the animals were sacrificed. The stomachs were excised and macroscopically examined for ulcers. Gastric mucous cell count (MCC) and antioxidant activity were subsequently assayed for.

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Results: The result showed that pre-treatment with garlic was significantly effective in reducing gastric ulceration incidence in animals ($p < 0.05$) as mean ulcer score decreased significantly in all groups treated with garlic. Superoxide dismutase (SOD) and catalase (CAT) increased significantly, especially in animals in the high dose group. No significant variation in the lipid per oxidation in all groups, while gastric mucus cell count was also significantly increased in most treatment groups.
Conclusion: These results suggest that garlic decreases ulcerogenesis in experimental animals. This can be attributed to its effects of increasing antioxidant activity and gastric mucous cell count.

Keywords: Allium sativum; antioxidant; anti-ulcer activity; gastric mucus cell.

1. INTRODUCTION

Peptic Ulcers are produced when there is an imbalance between protective factors and aggressive factors in the stomach [1]. Such factors could range from natural causes like gastric cancer, infections (Helicobacter pylori) and lifestyle factors like drugs e.g. non steroidal anti-inflammatory agents, alcohol, stress and cigarette smoking [2]. The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent recurrence. PUD is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy. Today, there are two main approaches to treating peptic ulcer. The first is by reducing or inhibiting acid secretion and the second is by reinforcing the protective factors [3]. The types of drugs normally used include H2 receptor antagonists (e.g. cimetidine), proton pump inhibitors (e.g. omeprazole) and cytoprotective agents (e.g. sucralfate). However, most of these drugs show side effects like arrhythmias, gynaecomastia, enterochromaffin-like cell hyperplasia and hematopoietic changes [4]. Thus, there is an urgent need for alternative treatment for peptic ulcer. In this context, extensive studies and research have been undertaken which mainly focus on search of anti-ulcer agents of plant & marine origin [5]. Herbal medicines are now used by up to 50% of the western population, in a number of instances (~10%) for the treatment or prevention of digestive disorders [6]. The focus of most research work on herbal therapies for peptic ulcer is on their ability to increase the protective factors or decrease the aggressive factors mentioned above. Apart from these, considerable attention is now being placed on the possibility of these therapies having a positive effect on antioxidant activity in the body. Antioxidants are substances that can lessen or combat the cellular damage done by free radicals. This definition explains the positive physiological role of many substances regarded as antioxidants such as superoxide dismutase, catalase, among others. Lipid peroxidation is one of the biochemically measurable processes by which free radicals cause membrane damage, cell damage and tissue injury [7]. The level of antioxidant defense systems has been found to greatly decrease in disease states [8]. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells.

Garlic commonly known as *Allium sativum* belongs to the family Liliaceace. Garlic extracts have been reported to be used in the treatment of a wide range of disorders in the past such as in hypertension, maintenance of body electrolytes; and also as an antibacterial, antiviral and antifungal [9]. Also, Adeniyi et al. [10] reported that all the strains of Helicobacter pylori were inhibited by the final concentration of garlic extract at a dose of 6mg/ml. This study is

however focused on the evaluation of the possible positive therapeutic effects of garlic pre-treatment in gastric ulceration, as well as an investigation into the probable mechanisms of action, in this case via gastric mucus cell and its antioxidant effects.

2. MATERIAL AND METHODS

2.1 Animals

Forty-eight healthy wistar (48) wistar rats, weighing between 160-220g were used for this study. The rats were obtained from the central animal house of the Faculty of Basic Medical Sciences, University of Ibadan. They were acclimatized for two weeks after which they were divided into six groups. Each study was made up of eight rats and they were treated for 30 days.

2.1.1 Experimental groups

Group I : Control; Rats in this group were fed with standard rats' feed and water only.
Group II: Low dose (250mg/kg body weight) of *Allium sativum* juice was administered orally.
Group III: High dose (500mg/kg body weight) of *Allium sativum* juice was administered orally.
Group IV: Prefed on 5% supplement of *Allium sativum* powder mixed with feed and pelletised.
Group V: Prefed on 10% supplement of *Allium sativum* powder mixed with feed and pelletised.
Group VI: Standard drug, Misoprostol (10µg/kg body weight) administered orally as part of daily water intake.

Each group was kept in a separate cage. All animals were fed with commercially-formulated rats' feed bought from Ladokun Livestock Feed Limited, Ibadan. Water was given ad libitum. Their cages were cleaned daily. Feed and water was also changed on a daily basis.

2.2 Preparation of Garlic Juice

Garlic bulbs, *Allium sativum*, were purchased from Bodija market, Ibadan, Nigeria. They were peeled and then pounded. The juice was then squeezed out and sieved into a very clean container. The extracted garlic juice was prepared fresh daily and was administered orally to the animals. The doses given were already reported to be safe and not toxic, and LD50 had been reported to be 0.87g/100g [10].

2.3 Preparation of Feed/Garlic Mix

Garlic bulbs were dried and ground to a fine powder. Standard feed was also ground into powder form, and then garlic powder was mixed in proportions of 5% and 10% of the total feed. The mixture was then re-pelletized in order to ascertain that the mix was evenly-distributed and the entire preparation was consumed by the animals. After re-pelletization, the feeds were spread to dry and fed to the animals in the specified groups only.

2.4 Experimental Induction of Ulceration

Indomethacin was administered via the oral route at a dosage of 40mg per kg body weight to the animals after pre-treatment with garlic and the standard drug for 30 days. The animals were fasted 24 hours before commencement of Indomethacin administration. They were however allowed free access to water.

2.5 Gastric Mucous Cell Count (MCC)

The stomachs were histologically prepared on a glass slide and the gastric mucous cells were counted using an improvised calibrated microscope using Motic 1000 photomicrograph softex at M × 400. Equal sections of different sections of the stomachs were stained with Hematoxylin and Eosin (H and E) and Periodic Acid Schiff (P.A.S) for differential staining of carbohydrates produced distinctly by the mucus cells.

2.6 Assay of Antioxidant Enzymes

The rats were fasted 24 hrs prior to the commencement of the experiment. On the day of the experiment, animals were sacrificed and their stomachs excised and washed in potassium chloride solution in ice. The stomachs were then weighed. Homogenizing phosphate buffer (K_2HPO_4 and KH_2PO_4) prepared with pH adjusted to 7.4 was used to homogenize stomachs in a homogenizer. The homogenized tissues were later centrifuged using a cold centrifuge at the Central Laboratory, University of Ibadan, at a speed of 10,000 rmp for 10 minutes at 4ºC. The supernatant was collected and then placed back in ice.

2.6.1 Catalase assay

Catalase activity was determined according to the method of Sinha [11]. This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate then produced is measured colorimetrically at 570 - 610 nm.

2.6.2 Superoxide dismutase assay

The level of Superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich [12]. The ability of superoxide dismutase to inhibit the auto-oxidation of adrenaline (epinephrine) at pH 10.2 makes this reaction a basis for a simple assay for this dismutase.

2.6.3 Lipid peroxidation assay

Lipid peroxidation levels were measured by the thiobarbituric acid (TBA) reaction using the method of Ohkawa et al. [13]. Under acidic condition, malondialdehyde (MDA) produced from the per oxidation of membrane fatty acid and food products react with the chromogenic reagent, 2-thiobarbituric acid (TBA), to yield a pink coloured complex with maximum absorbance at 532 nm and fluorescence at 553 nm. The pink chromophore is readily extractable into organic solvents such as butanol.

2.7 Statistical Analysis

All results were subjected to statistical analysis using Computer Software Graph Pad Prism. The values were expressed as Mean ± Standard error of mean (X ± S.E). Differences within groups were assessed using analysis of variance. Student "t" test was used to assess the differences between two groups and these were regarded as significant at $p<0.05$.

3. RESULTS AND DISCUSSION

This experiment investigated the effects of *Allium sativum* juice and powder on ulcerogenesis. It also assessed the possible effects on antioxidant activity, and its effect on mucus cell count in experimental rats. As can be observed in Table 1 above, all of the treatment groups exhibited a significant decrease in mean ulcer score. This proves that garlic has protective effects on the gastric mucosa against ulcer formation. The level of ulceration in the group fed with 10% supplemented *Allium sativum* feed was the most decreased with a preventive index of 84.17%. This is in agreement with results described by Tilmissany et al. [14] who observed that pre-treatment of animals with garlic extracts lead to decrease in both number and extent of ulceration. They also observed that there is a significant increase in plasma level of NO in ulcerated animals and significant decrease in PGE2 level. This suggests a pathway by which garlic reduces ulceration in animals, by possibly nitric-oxide releasing effects as showed by Tilmissany et al. [14]. The Mean ulcer score was also significantly reduced in animals prefed on the 5% *Allium sativum* powder-supplemented diet when compared with control. In the groups pretreated with *Allium sativum* juice, the low dose group showed percentage inhibition of 35% while the higher dose had 76.67% Table 1. These results show that the observed effects of garlic in these two groups are probably dose-dependent. The mix also seemed to be more active than the juice in preventing ulcer formation. The mean ulcer score in animals pretreated with Misoprostol was higher than that observed in all the treatment groups. This suggests that garlic has a more potent effect in prevention and healing of ulcers than the standard drug used. Misoprostol and its cytoprotective effects are well-known [15].

Table 1. Effects of *Allium Sativum* on Ulcerogenesis in Rats

Group	Mean Ulcer Score	Ulcer Index	% Inhibition
I	19.92±1.89	1.20	-
II	13.04±0.65**	0.78	35%
III	4.58±0.35**	0.28	76.67%
IV	6.04±0.65**	0.36	70%
V	3.17±0.61**	0.19	84.17%
VI	13.33±0.63	0.86	28.33%

*Group I: Control given no treatment, Group II: Given 250mg/kg of Allium sativum juice orally for 15 days, Group III: Given 500mg/kg of Allium sativum juice orally for 15 days, Group IV: Given 5% Allium sativum supplemented feed for 15 days, Group V: Given 10% Allium sativum supplemented feed for 15 days, Group VI: Given 10µg/kg^{-1} Misoprostol orally **Value significantly less/higher than the control ($p<0.01$)*

As seen in Table 2 above, Mucous Cell Count (MCC) for the test groups was highest in group II which had 63.33±2.25 per area field, showing a significant increase when compared to 32.33±2.92 per area field ($p\leq0.05$) observed in the control. Also the Mucous cell count was increased significantly to (49.17±3.37, 46.33±4.53 and 48.17±2.01 per area field) in albino rats groups (III, IV and V, respectively when compared to the control group.

In the Misoprostol treated group, Mucus cell count was 48.83±4.64 per area field which was a significant increase. Misoprostol is a synthetic prostaglandin E_1 (PGE_1) analogue that has the ability to stimulate increased secretion of the protective mucus that lines the gastrointestinal tract and increase mucosal blood flow, thereby increasing mucosal integrity. The observed results in the treatment groups show that garlic utilises this same mechanism and might even be more active in stimulating the propagation of mucous cells and therefore increased secretion of the protective mucous lining of the gastric mucosa.

Table 2. Effects of *Allium Sativum* on Mucous Cell Count (MCC)

Group	Mucous Cell Count (MCC/field) Mean ± S.E.M	Significance Level (P<0.05)
I	32.33±2.92	--
II	63.33±2.25	*
III	49.17±3.37	*
IV	46.33±4.53	*
V	48.17±2.01	*
VI	48.83±4.64	*

*Group I: Control given no treatment, Group II: Given 250mg/kg of Allium sativum juice orally for 15 days, Group III: Given 500mg/kg of Allium sativum juice orally for 15 days, Group IV: Given 5% Allium sativum supplemented feed for 15 days, Group V: Given 10% Allium sativum supplemented feed for 15 days, Group VI: Given 10µg/kg^{-1} Misoprostol orally. *Value significantly higher than the control*

In Table 3, the effects of *Allium sativum* juice and powder on antioxidant activity in albino rats are shown. There was a significant increase in catalase activity in rats fed with 10% supplemented *Allium sativum* feed when compared with the control. Earlier reports have been made that the powder of garlic increases the antioxidant capacity in hamsters [16] and garlic oil and its component were found to enhance catalase activity in cells [17]. Similarly, garlic in fish farming enhances the activity of non-specific defense systems in Tilapia nilotica (O. niloticus) [18], while Catalase (CAT) activity in serum and liver tissue homogenates equally showed significant increase in fish fed on diets containing garlic compared to control group [19]. 500mg/kg of *Allium sativum* juice, 5% supplemented *Allium sativum* powder and Misoprostol (the standard drug) all produced an insignificant increase in catalase activity compared to control. This suggests that garlic might be more effectively used therapeutically as a free radical scavenger when the dose is equal to or higher than 10% of a supplemented diet-equivalent in humans. Misoprostol is well-known for its use in preventing NSAID-induced gastric ulcers, by inhibiting the secretion of gastric acid via its G-protein coupled receptor, this being mediated by inhibition of adenylate cyclase which leads to decreased intracellular cyclic AMP levels and decreased proton pump activity at the apical surface of the parietal cell [15]. It also exhibits a cytoprotective effect by stimulating increased secretion of protective mucus and increased mucosal blood flow, thereby enhancing mucosal integrity. The results of the experiment show that Misoprostol causes a slight increase in catalase activity. The level of lipid peroxidation in homogenates tissue of albino rats showed a non significant variation between groups fed with *Allium sativum* and the control group. This could be attributed to the short duration of feeding. An earlier study [20] showed that garlic when administered raw caused significant alterations in total cholesterol in subjects with raised plasma cholesterol in their subjects after a long period of feeding.

Superoxide dismutase activity in stomach homogenates of female rats pre-treated for 15 days with 500mg/kg of *Allium sativum* juice showed significant increase compared to the control. Also, the group fed with 10% supplemented *Allium sativum* feed had a significant increase in SOD activity compared to the control group. Results obtained in fish had earlier

showed that garlic extract was found to exert antioxidant action by scavenging reactive oxygen species, enhancing the cellular antioxidant enzymes SOD in the cells [18].

Table 3. Effects of *Allium sativum* on Activity of Antioxidant Enzymes

Groups	Catalase (µmole H_2O_2 consumed /min/mg protein	Lipid Per oxidation (unit/g tissue)	superoxide Dismutase (unit/mg protein)
I	117.60±3.66	0.75±0.08	46.58±6.70
II	115.52±5.11	0.79±0.08	50.72±6.00
III	122.49±4.61	0.64±0.03	80.70±6.57**
IV	124.37±7.55	0.75±0.06	48.38±5.27
V	129.68±8.33*	0.72±0.04	94.66±5.91**
VI	123.66±2.24	0.77±0.16	48.80±4.51

*Group I: Control. Group II: 250mg/kg of Allium sativum juice orally for 15 days. Group III: 500mg/kg of Allium sativum juice orally for 15 days. Group IV: 5% supplemented Allium sativum feed for 15 days. Group V: 10% supplemented Allium sativum feed for 15 days. Group VI: 10µg/kg^{-1} Misoprostol orally. *Value significantly less/higher than the control (p<0.01). *Value significantly less/higher than the control (p<0.05)*

4. CONCLUSION

The results of this research clearly confirm that garlic possesses gastro protective properties against ulceration, an effect that is similar to, and might even be better than, that of known drugs such as Misoprostol now used in the treatment of peptic ulcer. This is by virtue of the fact that it stimulates an increase in mucous cell count thereby increasing mucus secretion. Garlic also has a positive enhancing effect on the activity of antioxidant enzymes against oxidative substances in rats.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Institutional Review Board of College of Medicine, University of Ibadan.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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