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

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Abstract: 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. The animals used were divided into six groups and treated for 30 days. A low dose (250 mg kg^{-1} body weight) and high dose (500 mg kg^{-1}) 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 mg kg^{-1}) orally as a standard drug. 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. Malonyldialdehyde (MDA) concentration however decreased mainly in both the group given a high dose of garlic juice (500 mg kg^{-1}) and the group fed with 10% supplemented *Allium sativum* feed while gastric mucus cell count was also significantly increased in most treatment groups. 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.

Key words: *Allium sativum*, antioxidant, anti-ulcer activity, gastric mucus cell, groups

INTRODUCTION

Peptic ulcers are produced when any factor causes an imbalance between the protective factors and aggressive factors in the stomach (Hoogerwerf and Pasricha, 2001). 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 (Yuan *et al*., 2006). 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 for treating peptic ulcer. The first deals with reducing the production of gastric ulcer and second with reinforcing gastric mucosal protection (Valle, 2005). The types of drugs normally used include H_2 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 (Akhtar *et al*., 1992). 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 and marine origin (Singh *et al*., 2008). 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 (Langmead and Rampton, 2001). 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 earlier. 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 (Freeman and Crapo, 1982). The level of antioxidant defense systems has been found to greatly decrease in disease states (Maxwell, 1995). 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 E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of

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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 (Oluwole, 2001). Also, Adeniyi *et al.* (2006) reported that all the strains of *Helicobacter pylori* were inhibited by the final concentration of garlic extract at a dose of 6 mg mL^{-1}.

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.

MATERIALS AND METHODS

Animals: A total of forty eight wistar rats, weighing between 160-220 g 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 2 weeks after which they were divided into six groups. Each study was made up of eight rats and they were treated for 30 days.

Experimental groupings

Group I: Control; rats in this group were fed with standard rats' feed and water only.

Group II: Low dose (250 mg kg^{-1} body weight) of *Allium sativum* juice was administered orally.

Group III: High dose (500 mg kg^{-1} 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 (10 μg kg^{-1} body weight) administered orally.

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.

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 0.87 g/100 g (Adeniyi *et al*., 2006).

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.

Experimental induction of ulceration: Indomethacin was administered via the oral route at a dosage of 40 mg kg^{-1} body weight to the animals after pre-treatment for 30 days. The animals were fasted 24 h before commencement of Indomethacin administration. They were however allowed free access to water.

Gastric mucous cell count: The rats were fasted overnight and sacrificed. 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&E) and Periodic Acid Schiff (PAS) for differential staining of carbohydrates produced distinctly by the mucus cells.

Assay of antioxidant enzymes: The rats were fasted 24 h prior to the commencement of the experiment. On the day of the experiment, animals were sacrificed and their stomachs brought out 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 cut into bits in ice. 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 min at 4°C. The supernatant was collected and then placed back in ice.

Protein determination: Protein concentration of the homogenate was determined using Biuret Reaction Method as described by Gornall *et al.* (1949).

Catalase assay: Catalase activity was determined according to the method of Sinha (1972).

Superoxide dismutase assay: The level of Superoxide Dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972).

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, 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 consonance with results described by Mohamed *et al.* (1999) 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 Mohamed *et al.* (1999). 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%. 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 (Wilson *et al.*, 1986). As seen in Table 2, 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 ($p = 0.05$) observed in the control. Group III had a MCC of 49.17±3.37 per area field and also exhibited significant increase. Group IV had a MCC of 46.33±4.53 per area field and Group V had MCC of 19.17±2.01 per area field. In the Misoprostol treated group, Mucus cell count was 48.83±4.64 per area field which was a significant increase. Misoprostol has been known as 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.

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 (Yaoling *et al.*, 1998) and garlic oil and its component were found to enhance catalase activity in cells (Borek, 2001). Similarly, garlic in fish farming enhances the activity of non-specific defense systems in Tilapia nilotica (*O. niloticus*) (Diab *et al.*, 2002) 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 (Metwally, 2009). The 500 mg kg^{-1} of *Allium sativum* juice, 5% supplemented *Allium sativum* powder and Misoprostol (the standard drug) all produced an insignificant increase in catalase activity compared to

Table 1: Effects of *Allium sativum* on ulcerogenesis in rats

Groups	Mean ulcer score	Ulcer index	Inhibition (%)
I	19.92±1.89	1.20	-
II	13.04±0.65**	0.78	35.00
III	4.58±0.35**	0.28	76.67
IV	6.04±0.65**	0.36	70.00
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 250 mg kg^{-1} of *Allium sativum* juice orally for 15 days, group III: given 500 mg kg^{-1} g 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 10 μg kg^{-1} Misoprostol orally. *Value significantly less/higher than the control

Table 2: Effects of *Allium sativum* on Mucous Cell Count (MCC)

Groups	Mucous Cell Count (MCC/field) Mean±SEM	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	*

Table 3: Effects of *Allium sativum* on activity of antioxidant enzymes

Groups	Catalase (μmole H_2O_2 consumed/min/mg protein)	Lipid peroxidation (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: 250 mg kg^{-1} of *Allium sativum* juice orally for 15 days; group III: 500 mg kg^{-1} 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: 10 μg kg^{-1} Misoprostol orally. *Value significantly less/higher than the control

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 (Wilson *et al*., 1986). 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. Superoxide dismutase activity in stomach homogenates of female rats pre-treated for 15 days with 500 mg kg^{-1} 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 (Borek, 2001).

CONCLUSION

The results of this research clearly confirm that garlic possesses gastroprotective 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.

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REFERENCES

Adeniyi, B.A., F.S. Oluwole and F.M. Anyiam, 2006. Antimicrobial and antiulcer activities of methanol extract of *Allium sativum* on *Helicobacter pylori*. J. Boil. Sci., 6: 521-526.

Akhtar, M.S., A.H. Akhtar and M.A. Khan, 1992. Antiulcerogenic effects of *Ocimum basilicum* extracts, volatile oils and flavonoid glycosides in albino rats. Int. J. Pharm., 30: 97-104.

Borek, C., 2001. Antioxidant health effects of aged garlic extract. J. Nutr., 131: 1010S-1015S.

Diab, A.S., G.O. El-Nagar and Y.M. Abd-El-Hady, 2002. Evaluation of *Nigella sativa* L. (Black seeds: Baraka), *Allium sativum* (garlic) and Biogen as a feed additives on growth performance and immuostimulants of *Oreochromis niloticus* fingerlings. Suez Canal Vet. Med. J., 2002: 745-775.

Freeman, B.A. and J.D. Crapo, 1982. Biology of disease-free radicals and tissue injury. Lab. Invest., 47: 412-426.

Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177: 751-766.

Hoogerwerf, W.A. and P.J. Pasricha, 2001. Agents Used for Control of Gastric Acidity and Treatment of Peptic Ulcers and Gastroesophageal Reflux Disease. In: Goodman and Gilamans The Pharmacological Basis of Therapeutics, Hardman, J.G. and L.E. Limbird (Eds.). McGraw-Hill, New York, pp: 1005-1019.

Langmead, L. and D.S. Rampton, 2001. Herbal treatment in gastrointestinal and liver disease-benefits and dangers. Aliment Pharmacol. Ther., 15: 1239-1252.

Maxwell, S.R., 1995. Prospects for the use of antioxidant therapies. Drugs, 49: 345-361.

Metwally, M.A.A., 2009. Effects of garlic (*Allium sativum*) on some antioxidant activities in *Tilapia nilotica* (*Oreochromis niloticus*). World J. Fish Marine Sci., 1: 56-64.

Misra, H.P. and I. Fridovich, 1972. Determination of the Level of Superoxide Dismutase in whole Blood. Yale University Press, New Haven, pp: 101-109.

Mohamed, A.H., K.E.H. Eltahir, M.B. Ali, M. Galal, I.A. Ayeed, S.I. Adam and O.A. Hamid, 1999. Some pharmacological and toxicological studies on *Balanites aegyptiaca* bark. Phytother. Res., 13: 439-441.

Oluwole, F.S., 2001. Effects of Garlic on some haematological and biochemical parameters. Afr. J. Biomed. Res., 4: 139-141.

Singh, R., J. Madan and H.S. Rao, 2008. Anti-ulcer activity of black pepper against absolute ethanol induced gastric mucosal damage in mice. Pharmacognosy Magazine, 4: 232-235.

Sinha, A.K., 1972. Colorimetric assay of catalase. Anal. Biochem., 47: 389-394.

Valle, J.D., 2005. Peptic Ulcer Diseases and Related Disorders. In: Harrison`s Principles of Internal Medicine, Braunwald, A.S., D.L. Fauci, S.L. Kasper, D.L. Hauser, Longo and J.L. Jameson (Eds.). 16th Edn., McGraw-Hill, New York, pp: 1746-1762.

Wilson, D.E., E. Quadros, T. Rajapaksa, A. Adams and M. Noar, 1986. Effects of misoprostol on gastric acid and mucus secretion in man. Dig. Dis. Sci., 31: 126s-129s.

Yaoling, L.C., S. Jiunrong, S.M. Gsyh, Y.L. Mingler, J.R. Chen, M.S. Shien and J.M. Shien, 1998. The effects of garlic powder on the hypolipidemic function and antioxidative status in hamsters. Nutr. Sci. J., 23: 171-187.

Yuan, Y., I.T. Padol and R.H. Hunt, 2006. Peptic ulcer disease today. Nat. Clin. Pract. Gastroenterol. Hepatol., 3: 80-89.