

Histological Evaluation of the Jejunum and Ileum of Rats after Administration of High Dose Garlic Aqueous Extract

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Abstract:

Objectives: This study investigated the adverse effects of excessive consumption of garlic on the small intestine (jejunum and ileum) of adult male Wistar rats.

Methodology: Sixteen (16) Wistar rats with average weight of 181.5 g were grouped into two: Control Group A which received distilled water, and Treatment Group B which received 1000 mg/kg/ml aqueous extract of garlic, orally for 28 weeks. The aqueous extraction of raw garlic was done to obtain a concentration of 1000 mg/kg/ml. The animals were sacrificed by cervical dislocation after the last day of administration, and tissues for histological studies were fixed in buffered formalin, while those for enzyme studies were homogenised, and appropriate biochemical kits used to study the activities of acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).

Results: The histological sections of the jejunum of animals that received the high dose of aqueous garlic extract revealed the presence of vacuolations, cell death and loss of epithelium, and intact muscle layer; the Periodic-Acid Schiff (PAS) positivity also reduced, while the ileum also showed degeneration of the brush borders, loss of epithelial cells, reduction in the number of goblet cells, vacuolations, and a reduced intensity of PAS positivity. Activities of ACP, ALP and LDH in the jejunum and ileum were increased.

Conclusion: Consumption of excessive amount of garlic could cause structural changes to the intestinal tract, which are capable of affecting intestinal functions, such as decrease in glycogen activity in the small intestine, and impairment of the absorptive activities.

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Introduction

Garlic has been used severally for its medicinal potentials. ⁽¹⁾ It is seen by many as a multipurpose medicinal plant having found its way into the traditional management of many diseased conditions, including some gastrointestinal disorders. ^(2, 3) Garlic, when used in low quantities, has beneficial effects on the gastrointestinal tract. It aids gastrointestinal absorption, and protects against gastric erosion. ⁽⁴⁾ Horie and colleagues ⁽⁵⁾ observed in animal models that administration of aged garlic extract (AGE) protected the small intestine from drug-induced injury like ulceration and mucositis. Garlic increases healing of acetic acid induced chronic gastric ulcers and prevents the development of both gastric and duodenal ulcers. ⁽⁶⁾

Garlic also inhibits the growth of *Helicobacter pylori*, a micro-organism which plays a major role in the aetiology of gastric cancer. ⁽⁷⁾ The diallyl disulphide component of garlic has been shown to decrease the rate of development of oesophageal cancer in rats ^(8, 9), and its anti-cancer activity is due probably to the prebiotic fructo-oligosaccharide it contains. ⁽¹⁰⁾ Garlic is effective against many diseases in which the activity of reactive oxygen species (ROS) is a major cause, such as cancers, diabetes and atherosclerosis. ⁽¹¹⁾ Some of the constituents of garlic interact with the reactive oxygen species or toxic radicals to neutralise or minimise their negative impact on the body. ^(12, 13) Aged garlic extract has been reported to possess immune enhancing and antioxidant properties, ⁽¹¹⁾ and also may protect the small intestine of rats from anti-tumour drug-induced damage. ⁽²⁾

However, excessive consumption of garlic could result in gastric mucosal erosion, reddening of the mucosa, and loss of intestinal epithelial cells. ⁽³⁾ Studies by Sood *et al* ⁽¹⁴⁾ showed that garlic intake led to inhibition of intestinal absorption of glutamic acid, sucrose and glucose.

This research was aimed at studying the implications of excessive consumption of garlic on the histochemistry and functions of the small intestine.

Materials and Methods

The study was conducted in accordance with the Ethical Guidelines of the Ethical

Committee on Animal Care and Use of the College of Health Sciences, University of Ilorin.

Preparation of Aqueous Garlic Extract

The raw garlic-*Allium sativum* was collected from an area in Ilorin, Kwara State and was authenticated at the Department of Plant Biology, University of Ilorin, Nigeria. The raw garlic cloves were peeled, pounded with mortar and pestle and blended with blender. The extraction was done using distilled water at ambient temperature. The blended raw garlic was then dissolved in distilled water and kept in refrigerator for 12 hours. The solution was filtered and the filtrate was concentrated in a water bath at temperature of 40°C. From the garlic paste formed, a concentration of 1000 mg/kg/ml was prepared.

Experimental Animals

A total of sixteen adult male Wistar rats were used for this work; their average weight was 181.5 g. They were allowed to acclimatise for 2 weeks and randomly grouped according to their weights. They were housed in cages with adequate space to encourage free movement, under natural light and dark cycles (12hr light and 12hr dark) at room temperature, and were given standard rat pallet diet and water *ad libitum*.

There were 2 groups for this study. While the Control Group A was given distilled water, the Treatment Group B received 1000 mg/kg/ml aqueous extract of garlic. These were orally administered by means of a feeding tube for 28 weeks.

Animal Sacrifice

Twenty-four (24) hours after the last administration and an overnight fast, the animals were sacrificed by cervical dislocation. Abdominal incision was made and the jejunum and ileum identified and excised. Tissues for histological preparations were fixed in 10% buffered formalin solution, while those for tissue enzyme studies were homogenized using a homogeniser.

Tissue Homogenisation

After excising each tissue from the animal, it was immediately weighed and homogenized using homogenizer. 4 mls of cold sucrose solution of 0.25 Molar was used in the process.

When the tissue was completely meshed, it was centrifuged at 5000 rpm for 5 min, and then using the Pasteur's pipette, the supernatant was collected and put in plain bottles and immediately frozen at -20°C , prior to analysis. Appropriate biochemical kits were used to quantitatively determine the level of activities of acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).

Results

Observation in Weight Change

The animals that were treated with high dose aqueous garlic extract had a significant reduction in their weights from an initial 191 ± 9.61 g to 155 ± 9.18 g (with a difference of 36 g), while the Control Group had a significant increase of 44 g from an initial weight of 164 ± 12.18 g to a final weight of 208 ± 7.22 g (Table 1).

Enzyme Assay

The results of the enzymes study showed apparent increase in the levels of jejunal and ileal ACP, ALP and LDH, in animals treated with high concentration of garlic, compared with the control. The activities of ACP and LDH were higher in jejunum compared to ileum in both the Control and Treatment Groups (Table 2).

Histological Observation

The photomicrographs of the jejunum and ileum of the control animals revealed a well preserved cellular integrity. There was no evidence of cellular degeneration, necrosis, when viewed under a light microscope (Figures 1, 3). The epithelial lining of the mucosa was intact, and no vacuolations seen. There was evidence of glycogen activity as the slides appeared magenta in colour and the goblet cells appeared Periodic-Acid Schiff (PAS) positive (Figures 5, 7).

The jejunum of the animals treated with aqueous garlic extract showed evidence of cellular degeneration. There were vacuolations, cell death and loss of epithelium, but the muscular layer was still intact (Figures 2). Although it was PAS positive, there was a reduction in the intensity of the magenta colour, showing decrease in glycogen activity (Figures 6).

The photomicrograph of the ileum of animals treated with aqueous garlic extract also revealed deleterious effects, such as degeneration of the brush borders and loss of epithelial cells, reduction in the number of goblet cells due to cell death, and presence of vacuolations (Figures 4). Staining with PAS was less intense compared with the control group, showing evidence of reduced glycogen activity (Figures 8).

Discussion

Jejunum and ileum are, to a good extent, similar both in structure and function, and are both involved in digestion of food which also entails absorption of nutrients. These roles are greatly enhanced by the epithelial lining of the digestive tract whose main functions are to provide a selectively permeable barrier between the contents of the tract and the tissues of the body, to facilitate the transport and digestion of food, to promote the absorption of the products of this digestion and to produce hormones that affect the activity of the digestive system. Cells in this layer produce mucus for lubrication and protection.⁽¹⁵⁾ Many workers have stated that moderate consumption of garlic enhances some gastrointestinal functions, such as absorption, and has some mucosal protective ability against the activity of *H pylori* and the development of ulcers.^(8, 10) The current study however showed that when garlic is consumed excessively, it causes structural changes which are capable of affecting intestinal functions. Also, a significant weight loss was noticed in animals fed with garlic, compared with the weight gain in Control animals, and many authors have suggested the use of garlic in weight reduction therapy.

The intestinal mucosa of the animals given high dose aqueous extract of garlic showed degeneration of the epithelium, vacuolations and loss of cells, similar to the findings of Hoshino *et al*⁽³⁾ who reported gastric mucosa and loss of epithelial cells of the intestinal lining in adult Wistar rats on administration of different preparations of garlic. Also Sood *et al*⁽¹⁴⁾ reported alterations of brush border membrane, thinning and sloughing off of villous structures and vacuolization in jejunum of rats that were treated with garlic.

The loss of epithelial cells in both the jejunum and ileum were most likely due to

administration of high dose of garlic extract. Atrophy of epithelium results in defective absorption of nutrients, excessive loss and diarrhoea. This can also lead to Malabsorption Syndrome which is also characterized by greatly hindered nutrients absorption.⁽¹⁵⁾ In the presence of diminishing goblet cells, there is inadequate production of mucus, and hence the impairment of its functions. The high sulfurous nature of garlic, especially the allicin component, is probably responsible for the damage to the gastro-intestinal tract.⁽¹⁶⁾

According to Gupta and Sandhu,⁽¹⁷⁾ aqueous garlic extract enhances the level of activities of ACP, ALP and LDH in the small intestine. Similarly, the current study found that the activities of these enzymes at the tissue level were elevated. However, another study by Omotoso *et al*⁽¹⁸⁾ observed an elevated ALP level in the serum of rats when a lower dose of 500 mg/kg/d was administered, but a reduced serum value at a high dose of 1000 mg/kg/d, though not statistically significant difference from the Control animals. Abnormal increase in the levels, and consequently the activities, of these enzymes (ACP, ALP and LDH) could be detrimental to normal intestinal functions, leading to many gastrointestinal disorders, including diarrhoea.

Conclusively, in animals given high dose of garlic aqueous extract, there was evidence suggestive of cell death, such as cellular degeneration, disintegration of membranes, presence of vacuolations, and reduction in the number of goblet cells. Reduction in goblet cells and PAS positivity are suggestive of decrease in glycogen activity in the small intestine of animals exposed to garlic at a high concentration. Degeneration in the epithelial lining and brush borders are evidence to show possibility of impairment of the absorptive role of the small intestine in this group of Wistar rats.

Table (1). Difference in body weight

Groups	Initial weight (g)	Final weight (g)	Difference (g)
A	164 ±12.18	208 ±7.22	+44
B	191 ±9.61	155 ±9.18	-36

Table (2). Results of Enzyme Studies

	JEJUNUM		ILEUM	
	Control	Garlic	Control	Garlic
ACP (IUL)	16074.5	19702.5	13335.5	16820.0
ALP (IUL)	100.5	124.5	101.0	111.5
LDH (IUL)	19675.0	20083.5	18236.0	18860.5

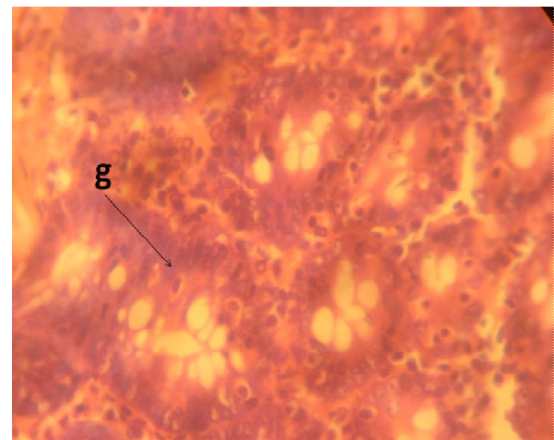


Fig. (1). Photomicrograph of the jejunum of the animals in the Control Group A which received distilled water showing a normal section; g= goblet cells (H&E).

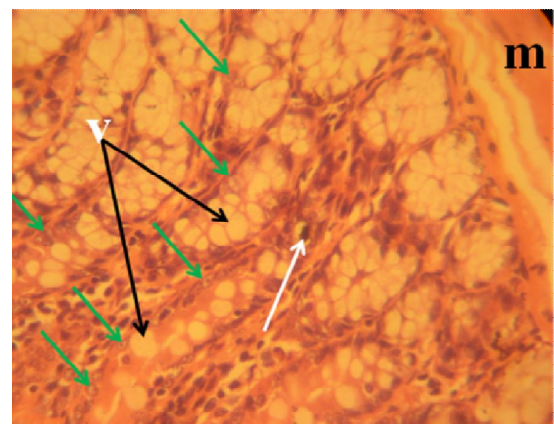


Fig. (2). Photomicrograph of the jejunum of the animals in the Treatment Group B which received 1000 mg/kg/d aqueous garlic extract showing an intact muscular layer (m), vacuolations (V), and dead and/or dying cells (green arrows). (H&E).

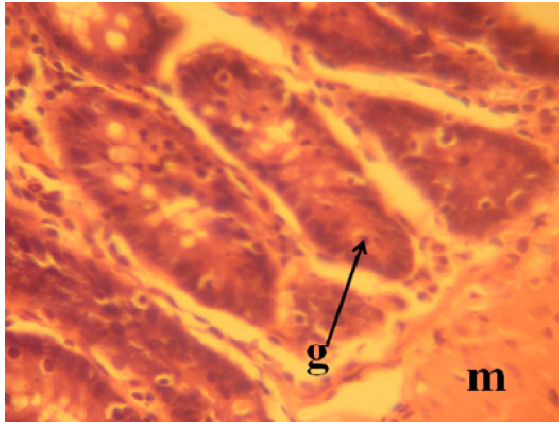


Fig. (3). Photomicrograph of the ileum of the animals in the Control Group A showing a normal architecture: (g= goblet cells, m= muscular layer) (H&E).

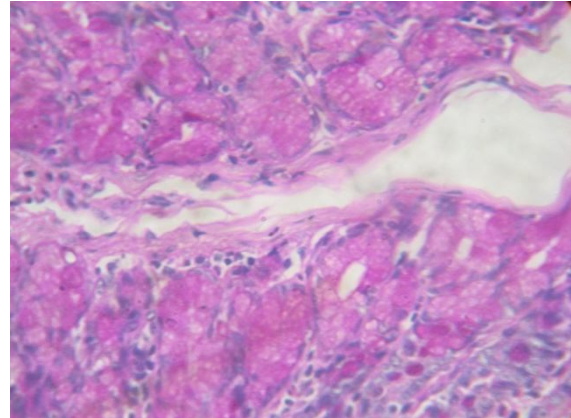


Fig. (6). Photomicrograph of jejunum of animals in Treatment Group showing reduced staining intensity for PAS (PAS X1920).

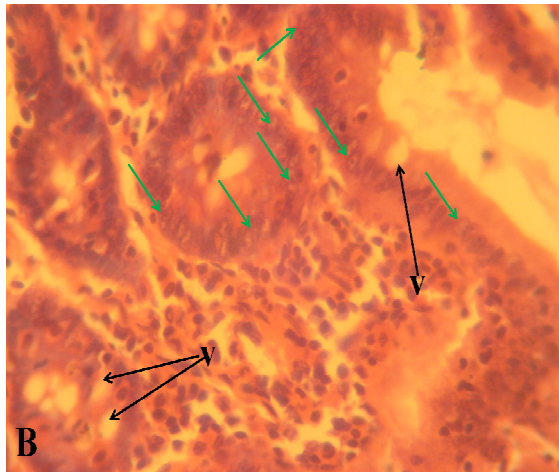


Fig. (4). Photomicrograph of the ileum of the animals in the Treatment Group B, showing numerous vacuolations (V) and dead and/or dying cells (green arrows). (H&E).

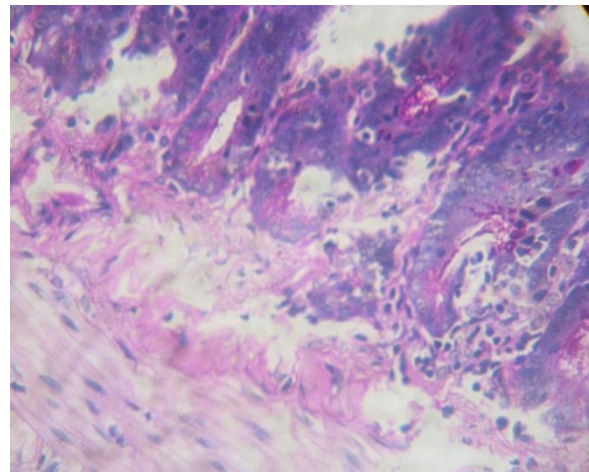


Fig. (7). Photomicrograph of ileum of animals in Control Group showing positive PAS staining (PAS X1920).

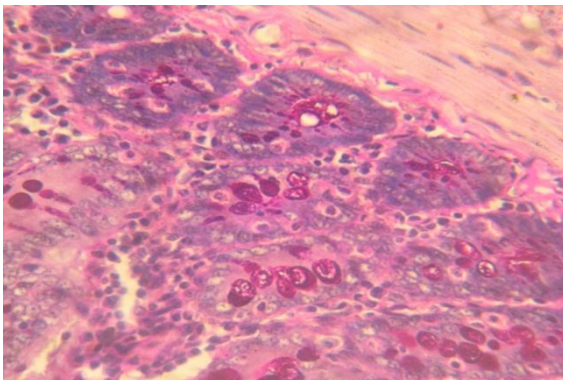


Fig. (5). Photomicrograph of jejunum of animals in Control Group showing positive PAS staining (PAS X1920).

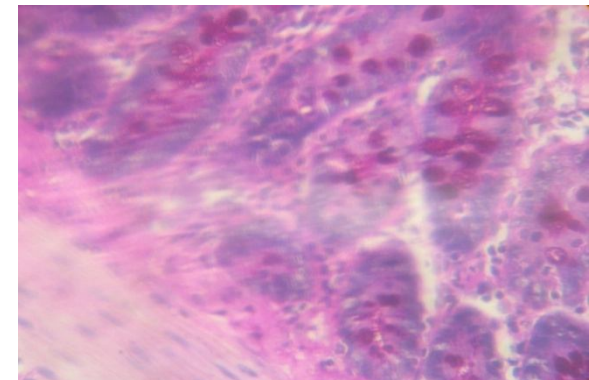


Fig. (8). Photomicrograph of ileum of animals in Treatment Group showing reduced staining intensity for PAS (PAS X1920).

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