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Effects of Garlic (*Allium sativum*) on Serum Biochemical Parameters and Histopathological Changes in Wistar Rats (*Rattus norvegicus*)

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ABSTRACT

Allium sativum is the most commonly used food additive with a good dietary role and medicinal potential. However, its indiscriminate use may result in either a high or low risk of side effects. This study was aimed at evaluating the effect of garlic powder on some biochemical parameters and histopathological changes in Wistar rats. Twenty-five Wistar rats were used in the study. Garlic was obtained and prepared into powder form. The animals received 50 g of normal feed/50 g of garlic powder (Group A); 40 g normal feed/60 g garlic powder (Group B); 30 g normal feed/70g garlic powder (Group C); 10 g normal feed/90 g garlic powder (Group D), or 100g normal feed (Group E) for four weeks. The serum biochemical enzymes; succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were analyzed. Histopathological examinations of the liver, small intestine, and kidney tissues were performed. The results indicated that significant ($P<0.05$) reductions in the AST and ALT activities were observed among the treatment groups compared to their baseline values. Meanwhile, SDH and LDH activities remained constant. Cytoplasmic vacuolation, congestion, and necrosis were observed in the liver tissues. Tubular atrophy and hypercellularity were observed in the kidney tissues with enlarged urinary space, while minor distortion of the crypts was observed in the small intestine of the rats. The findings of this study revealed that *Allium sativum* powder has hepatoprotective properties; however, it should be consumed with caution because uncontrolled use could have deleterious effects.

Keywords: *Allium sativum*, Garlic, Histology, Liver enzymes, Tissue changes, Wistar rats.

Introduction

Garlic (*Allium sativum*L.) is one of the edible plants consumed by individuals and has gained significant interest throughout human history as a medicinal panacea.[1] It belongs to the family Liliaceae and is a food additive that is useful in improving the health of humans and animals. The most important constituents of the plant are organosulfur compounds such as allicin, diallyl disulphide, s-allylcysteine, and diallyl trisulfide.[2] *Allium sativum* has been reported to control infection of animals caused by pathogenic bacteria and fungi, and consequently, improving animals' health.[3,4]Garlic has a long history of medicinal use, with numerous scientific findings supporting its health benefits. In a study, administration of garlic reduced marker enzymes of experimental rats. The extent of liver damage caused by toxic chemical substances can be evaluated by considering the liver enzyme markers such as AST, ALT, etc.[5] Garlic is utilized to stimulate the immune system, phagocytotic activity, and lymphocyte formation by releasing cytokines from natural killer cells.[4] Extracts have been prepared with ethanol and water as solvents from dried powdered garlic.[6]*Allium sativum* extracts enhanced glutathione peroxidase activity.[7] Generally, most plant products have antimicrobial and antioxidant properties. They also contain bioactive compounds and other synthetic molecules that have a lot of promise as antibiotic alternatives.[8]It's oral intake as tonic enhances rapid growth, stimulates appetite, and improves the immune system in animals. Pharmacological activities of *Allium sativum* have been reported. These include cardiovascular disease inhibition,[9,10] prevention of cancer,[11] stimulation of immune function,[12] neuroprotective functions,[13,14] hypoglycemic conditions,[15]hepatoprotective functions,[16-18] antioxidant potentials,[19] anti-clastogenic,[20] and antidiabetic activities.[21] Furthermore, the importance of garlic in the regulation of lipids and haematological parameters makes it a vital food ingredient.[16] The prophylactic administration of *Allium sativum* was reported to have hypocholesterolemic and hypoglycemic effects.[22] Increased body and liver weights on the Wistar rats exposed to 500 and 100 mg/kg of fresh garlic extract have also been reported.[8] A 2 g/kg garlic extract caused gastric, intestinal epithelial mucosal membrane damage, bleeding, ulcers, and sloughing of the villus structure in Wistar rats.[23] There is an increase in the self-prescribed intake of medicinal plants by consumers which violates World Health Organization (WHO) guidelines for botanical use. It is fascinating to know that the unregulated use of medicinal plants led to WHO recommendations for providing a safe dose to guide against side effects.[24] The safety regulation of global phytopharmaceutical products is essential and must be implemented to avoid toxicity or adverse effects in humans. Despite the frequent usage of herbs, scientific evidence verifying their safety and usefulness is required. Histopathology is considered an indicator of abnormal health conditions.[25] Possibilities of organ toxicity cannot be overruled on the intake of plant products. Blood regulation at a normal range enhances homeostatic mechanism, and changes in the blood parameters.[25] Liver enzymes are indicators of the deleterious effect of chemical products.[3] Garlic are important in the health of human, however, its uncontrolled use has some side effects such as diarrhea, breathing difficulties, and throat ulcers.[3]The present

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study was therefore conducted to evaluate the effects of garlic (*Allium sativum*) powder on some biochemical parameters and histopathological changes in Wister rats (*Rattusnorvegicus*).

Materials and Methods

Source of experimental animal

Twenty-five healthy male Wistar rats (101.67±3.76 g) were obtained from the breeding colony of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The animals were randomly divided into 5 groups (A-E) with 5 rats housed in a cage. The rats were maintained at standard laboratory conditions of temperature (25±2°C), humidity (50±5%), and 12 hours light and dark cycle with free access to water and poultry growers mesh (Vital feed, Jos, Nigeria). The animal study protocol was approved by the University of Nigeria, Animal Care Committee (UNN-ACC, Protocol No. 0764/2013).

Source of garlic

Garlic bulbs were obtained commercially from the Central Market, Nsukka, Enugu State, Nigeria. The garlic was authenticated by Mr. Onyeukwu Chijioke, of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The sample specimen of the garlic was deposited in the herbarium and allocated a voucher number (UNH/NO 214).

Preparation of garlic powder

To separate individual cloves, the epidermal skin was removed. The garlic cloves were split lengthwise and dried in a Heraeus oven LDO-300, Germany, which was preheated at 12°C for 20 minutes before increasing to 60°C for 6-8 hours to speed up the drying of the sliced garlic. They were allowed to cool and ground into fine powder. The powder was transferred into an airtight plastic container having a lid to prevent solidification.[23]

Experimental grouping and treatments

Garlic powder was supplemented into the commercial feed to achieve the following ratios: Group A: 50 g normal feed/50 g garlic powder; Group B: 40 g normal feed/60 g garlic powder; Group C: 30 g normal feed/70 g garlic powder; Group D: 10 g normal feed/90 g garlic powder; Group E: normal control, 100 g normal feed. The Wistar rats were fed a total weight of 200 g per day for four weeks at 9 am. At exactly 9 am, the feed remains were weighed after 24 hours of feeding.

Blood sample collection

Blood samples were collected from the medial canthus of the retrobulbar plexus of the eye and placed in Eppendorf tubes without anticoagulants. The serum biochemical parameters such as aspartate aminotransferase (AST),[26] alanine aminotransferase (ALT),[26] lactate dehydrogenase (LDH),[27] and succinate dehydrogenase (SDH).[28] were evaluated according to standard methods.

Histopathological analysis

The standard protocol for histopathological analysis was followed.[25] After a four-week treatment period, the rats' liver, small intestine, and kidney were removed. The tissues were preserved in Bouins fluid for 1 h and transferred to a 10% saline solution for further tissue processing. Sections of the organs were cut at 5µm using LEICA RM 2125 RTS rotary microtome section and were mounted on a glass microscope slide. The tissues were further stained with Mayer'shaematoxylin-eosin, mounted with Canada balsam, and examined by Olympus CH binocular microscope using X4, X10, and X40 objectives. Tissue sections were read under a binocular microscope (Olympus) and a cross-section was taken using a motic image with a 2.0 camera.

Statistical analysis

The Statistical Package for Social Sciences (SPSS version 16) was used to statistically analyze the data. Mean values were analyzed for significant differences ($P<0.05$) using the analysis of variance (ANOVA), while differences between means were partitioned using the Duncan Multiple range test at 0.05% probability.

Results and Discussion

Table 1 shows the effects of garlic powder on SDH, LDH, AST, and ALT. The activity of the ALT and AST enzymes were significantly reduced ($P<0.05$) in the rats fed with garlic powder. The reduction in the activities of ALT (8.54 ± 0.04I/U) and AST (22.00 ± 0.58I/U) in the liver was due to interference with protein metabolism in the cells.[29] Reduced ALT and AST activities in garlic supplementation studies using humans showed protection of the liver against damage and improved hepatic features.[29] There were concentration-and time-dependent changes in the AST and ALT enzymatic activities in the serum of the rats fed with garlic powder. Compared to the control, the SDH and LDH concentrations did not change significantly ($p>0.05$) at the end of the experiments. The elevated LDH level was due to a high lactate metabolism by pyruvate conversion.[15]

Normal liver morphology such as a sinusoid, central vein, and the parenchymal cells was present and arranged to form a lattice network. The thin strips interspaces of the sinusoids consisted of sparse connective tissues (Figure 1, Plate E). Hepatocyte cytoplasmic vacuolations appeared to be consistent with glycogen and macrovascular fatty change (Figure 1, Plates A and C). More so, necrosis of hepatocytes (Figure 1, Plate B), and degeneration of hepatic cords, the proliferation of inflammatory cells were observed (Figure 1, Plate D). The liver necrosis, congestion of the central vein, and vacuolations were due to the increased concentrations of the garlic powder. This was supported by the observed dilatation, cellular degeneration, blood vessel congestion, and engorgement of hepatic central veins in the liver of rats treated with high doses of garlic.[29,30]The hepatoprotective activity of *Allium sativum* was responsible for the normal liver architecture found in the treatment groups.

The histology of the normal small intestine demonstrated intact intestinal villi structure (Figure 2, Plate A), in contrast to the other groups administered with garlic powder, which had obvious degradation of villi and lacteals (a lymphatic capillary that absorbs fats in the villi of the small intestine) as shown in Figure 2, Plates A-D. However, distortion of the finger-like projection of the villi and lacteals was observed in the study. This could be due to slight toxicity caused by garlic powder in the small intestine. The study found crypt and lacteal distortion in the small intestine, which contradicted previous reports of increased villus and crypt depth and decreased epithelial thickness in the duodenum and jejunum of birds fed with garlic powder.[18]The proliferation of inflammatory cells and tubular atrophy in the kidney tissues showed enlarged urinary space. Renal corpuscles, which included the glomerulus and bowman capsules as well as the tubules, were visible in the kidney structure. The observed renal damage includes enlarged urinary spaces, atrophy of the glomerular tufts (Figure 3, Plate A), infiltrations of the inflammatory cells, predominantly mononuclear cells, cystic formation (Figure 3, Pates B-D), followed by an accumulation of eosinophilic proteinaceous materials in the lumen were all observed. Necrotic mutated cells with lesions of varying degrees in the liver and kidney in a study of anti-clastogenic effects of *Allium sativum* extract against lead-induced necrosis have been reported.[20] Garlic, however, as natural medicine should be administered moderately to reduce liver damage and side effects.

Conclusion

Despite the global reputation of garlic as healthy food, it should be emphasized that garlic had a 4-week harmful effect on Wistar rat tissues. Unregulated doses of garlic can alter the tissue architecture in the gastro-intestinal tract organs. Garlic did not cause any significant alterations in the liver enzymes. The study confirmed that a high dose of garlic can cause tissue alterations, compromising its antioxidant and preventive properties due to uncontrolled consumption. The findings of this study suggest that modest intake of *Allium sativum* as powder, raw, extracts, food additives, or tonic may help to improve the health advantages of garlic, especially when used as directed to minimize side effects.

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Table 1: Changes in the biochemical parameters in the control and treatment groups of albino rats

		DURATION OF EXPOSURE				
Experimental Groups	**Biochemical Parameters**	**Week 0 (baseline)**	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Group A	SDH (I/U)	0.86 ± 0.01^{a}	0.72 ± 0.0 1^{c}	0.82 ± 0.18^{c}	0.94 ± 0.01^{b}	0.96 ± 0.01^{c}
	LDH (I/U)	126.57 ± 37.68^{a}	126.75 ± 24.88^{bc}	132.57 ± 34.18^{bc}	126.57 ± 24.68^{bc}	140.10 ± 0.89^{ab}
	ALT (I/U)	60.67 ± 22.67^{b}	8.50 ± 0.11^{a}	9.39 ± 0.06^{c}	8.62 ± 0.01^{c}	9.91 ± 0.03^{b}
	AST (I/U)	80.10 ± 22.16^{a}	41.67 ± 9.88^{b}	39.00 ± 7.58^{a}	30.33 ± 10.88^{b}	22.00 ± 0.58^{d}
Group B	SDH (I/U)	0.85 ± 0.00^{a}	0.83 ± 0.03^{a}	0.86 ± 0.01^{b}	0.88 ± 0.01^{c}	0.97 ± 0.00^{a}
	LDH (I/U)	121.73 ± 0.87^{a}	133.18 ± 34.96^{b}	135.77 ± 29.63^{b}	121.73 ± 31.87^{b}	138.66 ± 1.40^{ab}
	ALT (I/U)	32.67 ± 9.67^{c}	12.76 ± 0.83^{a}	9.55 ± 0.33^{b}	8.54 ± 0.03^{c}	9.60 ± 0.02^{c}
	AST (I/U)	49.33 ± 7.33^{b}	37.67 ± 11.03^{b}	31.10 ± 5.58^{c}	35.10 ± 8.58^{b}	24.67 ± 0.87^{c}
Group C	SDH (I/U)	0.85 ± 0.00^{a}	0.77 ± 0.10^{b}	0.87 ± 0.10^{b}	0.97 ± 0.01^{a}	0.99 ± 0.10^{b}
	LDH (I/U)	125.42 ± 13.48^{a}	137.37 ± 34.30^{ab}	136.93 ± 31.98^{b}	125.42 ± 22.49^{cd}	137.44 ± 1.20b^{b}
	ALT (I/U)	66.10 ± 4.16^{c}	11.03 ± 0.38^{b}	8.62 ± 0.13^{e}	8.85 ± 0.07^{b}	9.83 ± 0.05^{b}
	AST (I/U)	45.33 ± 4.67^{b}	44.67 ± 5.88^{a}	35.10 ± 9.58^{b}	31.33 ± 4.88^{b}	30.00 ± 0.58^{a}
Group D	SDH (I/U)	0.35 ± 0.10^{b}	0.87 ± 0.10^{a}	0.92 ± 0.01^{a}	0.96 ± 0.01^{a}	0.99 ± 0.10^{b}
	LDH (I/U)	122.82 ± 24.34^{a}	144.17 ± 21.31^{a}	148.74 ± 21.68^{a}	122.82 ± 27.34^{a}	141.41 ± 0.56^{a}
	ALT (I/U)	35.33 ± 19.67^{c}	12.56 ± 0.44^{a}	8.88 ± 0.01^{d}	8.59 ± 0.03^{c}	8.54 ± 0.04^{d}
	AST (I/U)	57.33 ± 18.67^{b}	40.33 ± 13.38^{a}	34.67 ± 12.88^{b}	29.67 ± 6.88^{b}	27.33 ± 0.067^{b}
Group E (Control)	SDH (I/U)	0.86 ± 0.10^{a}	0.64 ± 0.01^{d}	0.92 ± 0.10^{a}	0.94 ± 0.10^{b}	1.09 ± 0.10^{a}
	LDH (I/U)	125.45 ± 22.34^{a}	124.29 ± 18.99^{c}	130.25 ± 13.77^{c}	125.45 ± 20.54^{d}	129.85 ± 0.26^{c}
	ALT (I/U)	70.67 ± 13.31^{a}	8.85 ± 2.43^{c}	10.25 ± 0.02^{a}	10.17 ± 0.03^{a}	11.36 ± 0.06^{a}
	AST (I/U)	93.33 ± 9.81^{a}	27.33 ± 3.76^{a}	33.33 ± 5.67^{b}	30.33 ± 4.88^{b}	22.10 ± 0.58^{d}

Values are mean ± SD of five individual observations; *: Means within the same column followed by different letters^{a,b,c,d} (Duncan multiple range test) are significantly different (P<0.05); A: 50 g normal feed/50 g garlic powder; B: 40 g normal feed/60 g garlic powder; C: 30 g normal feed/70 g garlic powder; D: 10 g normal feed/90 g garlic powder; E: Normal control, 100 g normal feed

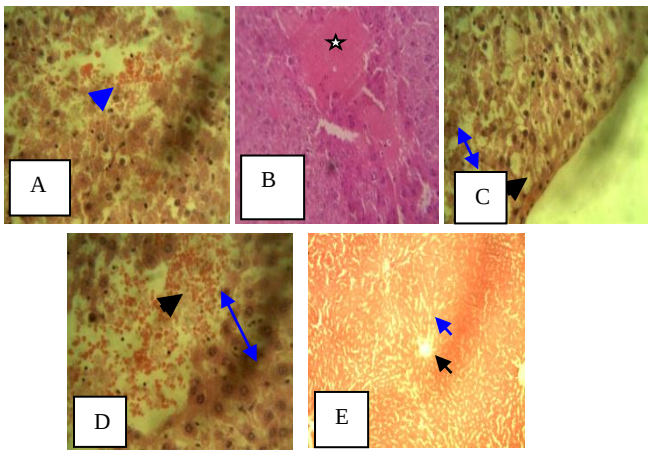

Figure 1: Cross section of the liver of rat fed with different doses of garlic powder.
A: Cytoplasmic vacuolation of the hepatocytes (black arrow) was observed in rat fed with 50 g normal feed/50 g garlic powder. H&E, x400 magnification; B:Congestion of the central vein (star) was observed in the group fed with 40 g normal feed/60 g garlic powder. H&E, x400 magnification;C:Cytoplasmicvacuolation of the hepatocytes (back arrow) and degeneration of hepatic cords (yellow arrow) in the group fed with 30 g normal feed/70 g garlic powder. H&E, x400 magnification; D:Loss of hepatic cords (black arrow) and proliferation of inflammatory cells (yellow arrow) was observed in the group fed with 10 g normal feed/90 g garlic powder. H&E, x400 magnification; E: Normal liver tissue showed intact sinsuoids (blue arrow) and central vein (black arrow). H&E, x100 magnification.

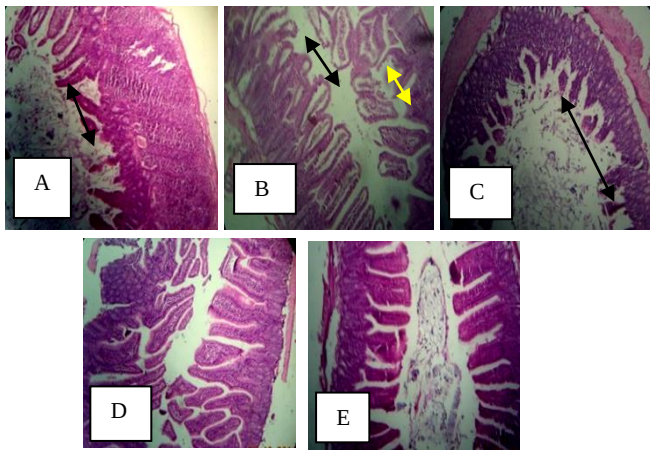

Figure 2: Cross section of the small intestine of rat fed with different doses of garlic powder.
A: Intact intestinal villi, crypts and lacteals (black arrow) H&E, x400 magnification; B: Normal villi observed with enlarged lacteal (yellow arrow). H&E, x400 magnification; C: Minor disintegration of the intestinal villi (black arrow). H&E,x100 magnification; D: No damage observed in the intestine. H&E, x400 magnification; E: No observed damage in the normal control of the small intestine (white arrow). H&E,x400 magnification.

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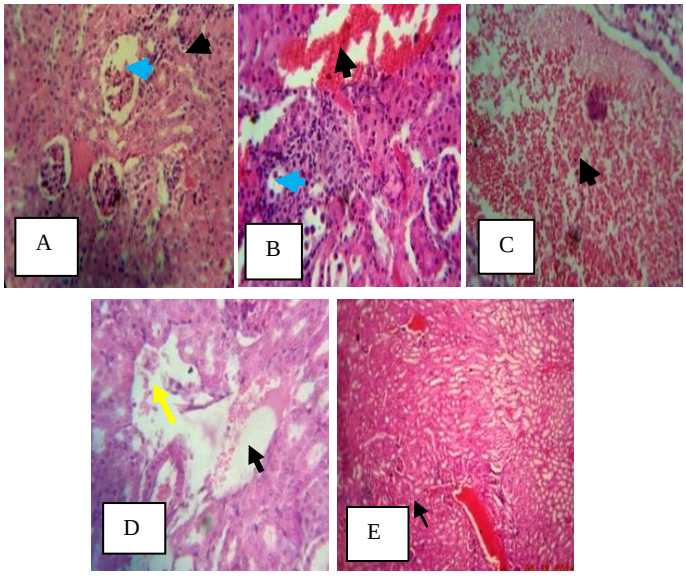

Figure 3: Cross section of the kidney of rat fed with different doses of garlic powder.
A: Enlarged urinary space (black arrow) and atrophy of glomerular tuft (blue arrow). H&E, x400 magnification; B: Moderate hyperplasia (hyper cellularity; blue arrow) infiltration of inflammatory cells (black arrow) was observed. H&E, x400 magnification; C:Infiltration of inflammatory cells (black arrow). H&E,x400 magnification; D:Cystic spaces (black arrow) and the lumen showed eosinophilicprotnacious materials (white arrow); E:Kidney structure showed that renal corpuscles comprised of glomerulus (black arrow) as well as the tubules and interlobulary artery, no damage was observed (yellow arrow).H&E,x100 magnification.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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