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Histological Effect of *Allium sativum* (Garlic) on the Liver of White Rabbits

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Authors' contributions

This work was carried out in collaboration among all authors. Author O. O. Olayemi designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author PA managed the analyses of the study. Author PA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Allium sativum commonly referred to as garlic has been known over the years for its medicinal and culinary purposes. It has also been reported to have several toxic effects when used excessively. However, the purpose of this study was to determine the histological effects of *Allium sativum* (garlic) powder on the liver of white rabbits at different dosages. Twenty rabbits were randomly divided int.o five groups with free access to food and water for a period of four weeks. Four groups B, C, D, and E were fed with garlic supplemented basal diet containing different concentrations of garlic powder i.e. 100mg, 200mg, 500mg, and 1000mg respectively. These groups were called the treated group. Group A was fed with basal diet only and was considered as the control group. The results obtained showed some histological changes such as the presence of cellular necrosis, vacuolations, lipofuscin pigments, pyknosis and nuclear hypertrophy which were as a result of liver glycogen depletion and hepatic cell damage which may be due to relatively high dosage of garlic used in some of the groups.

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1. INTRODUCTION

Allium sativum, commonly known as garlic, is a species in the onion family *Alliaceae*. Its close relatives include the onion, shallot, leek, chive [1]. The name "*allium sativum*" is derived from the Celtic word "all", meaning burning or stinging, and the Latin "sativum" meaning planted or cultivated [2]. Garlic has been used throughout history for both culinary and medicinal purposes. The garlic plant's bulb is the most commonly used part of the plant. With the exception of the single clove types, the bulb is divided into numerous fleshy sections called cloves. The cloves are used for consumption (raw or cooked), or for medicinal purposes, and have a characteristic pungent, spicy flavour that mellows and sweetens considerably with cooking, [3]. The English word, garlic, is derived from the Anglo-Saxon "gar-leac" or spear plant, referring to its flowering stalk [4].

Garlic belongs to the plant genus *Allium* and is known for its pungency and spiciness. Garlic is thought, in particular, to be linked to various beneficial health effects, from reducing blood pressure and cholesterol to treating cancer. Garlic may also possess antifungal, antibacterial, cardio-protective, and antioxidant and anti-cancer activities. Several compounds are involved in garlic's possible anticancer effects. There are two main medical ingredients which produce the garlic health benefits: allicin and diallyl sulphides [5]. Various garlic health benefits and medicinal properties have long been claimed and it has been used extensively in herbal medicine (phytotherapy). Garlic is a sulphurous compound and in general a stronger tasting clove has more sulphur content and hence more potential medicinal value. Modern science has shown that garlic is a powerful natural antibiotic, albeit broad-spectrum rather than targeted [6].

Garlic is a nature's wonder drug. Its medicinal value has been understood by herbalists for at least 2000 years. Garlic contains hundreds of minerals and nutrients and thus no one ingredient is the "active ingredient". Garlic's effectiveness and safety comes from these ingredients working together in concert. And if any ingredient should be found more potent than the others, and that ingredient were isolated and made into a medicine, it will probably have powerful negative side effects like virtually every other drug in use today [7].

Although health benefits of garlic are frequently reported, excessive intake can have harmful effects. In a rat study, allicin, the main pungent ingredient in garlic, was found to be an activator of Transient receptor potential cation channel subfamily A member 1 (TRPA1). The neurons release neurotransmitters in the spinal cord to generate pain signals and neuropeptides at the site of sensory nerve activation, resulting in vasodilation as well as inflammation [8].

The liver is the organ where nutrients absorbed in the digestive tract are processed and stored for use by other parts of the body. It is thus an interface between the digestive system and the blood. With the exception of the skin, the liver is the largest organ of the body and largest glands weighing about 1.5kg (1500g). It is situated in the abdominal cavity beneath the diaphragm. Most of it blood comes from the portal vein. The smaller percentage is supplied by the hepatic artery. The position of the liver in the circulatory system is optimal for gathering, transforming and accumulating metabolites and for neutralizing and eliminating toxic substances. The elimination occurs in the bile, an exocrine secretion of the liver that is important in lipid digestion [9, 6].

Garlic has been used widely as flavour due to its pungent smell and also for therapeutic measures. Many works have been done to confirm these therapeutic effects but its effects on liver at a very high concentration are the main focus in this work.

1.1 Research Hypothesis

It is likely that garlic affects the liver adversely especially when ingested at a very high concentration because liver actively participates in the metabolism.

1.2 Justification of Study

Garlic is a nature's wonder drug. Its medicinal value has been understood by herbalists for at least 2000 years (Paul, 1996). Various works have been done on the effectiveness of this herbal product with little work on its toxic effect.

Therefore, the purpose of this research work is to investigate the toxic effect of garlic on the architecture of the liver of rabbits and determine if this effect is dosage dependent.

1.3 Aim and Objectives

- To determine the histological effects of garlic on the liver of rabbits.
- To establish if the observed effect is dosage dependent.
- To determine the toxic dose of garlic on the liver of rabbits.

2. MATERIALS AND METHODS

2.1 Experimental Animal

Twenty (20) White rabbits supplied from Obafemi Awolowo University animal house were used in this study, having a body weight range in between 0.5-2.0 Kg, irrespective of sex. They were housed in labelled cages. The animals were fed with basal diet containing pellet feeds and cabbage leaves and tap water. They were allowed to acclimatize for two weeks.

2.2 Research Design

2.2.1 Animal grouping

Rabbits were divided into five groups each containing four rabbits with weight range between 0.5kg-2kg. The five groups which were tagged group A, group B, group C, group D, and group E were fed in the following order for four weeks;

Group A: This group which was the control group was fed with the basal diet and water only.

Group B: This group was fed with basal diet, water and 100mg of garlic powder.

Group C: This group was fed with basal diet, water and 200mg of garlic powder.

Group D: This group was fed with basal diet, water and 500mg of garlic powder.

Group E: This group was fed with basal diet, water and 1000mg of garlic powder.

In order to ensure that each animal took in the accurate milligram of garlic powder, the garlic powder was made into aliquot with distilled water and with aid of a sterile pasture pipette, it was administered orally to the rabbits.

2.3 Sample Collection

At the end of the four weeks feeding, the animals were sacrificed and their livers collected into different containers with 10% formal saline appropriately labelled for proper fixation. They were processed and stained with haematoxylin and eosin after which it was examined microscopically.

2.4 Materials/Eqiupments

Dissecting set, small plastic containers, Tissue caskets, tissue basket, Automatic tissue processor (LEICA TP 1020), Hot plate, Paraffin Wax, Metallic embedding mould, hot air oven, rotary microtome, and water bath.

2.5 Reagents

10% Formal saline, Ehrlich haematoxylin, 1% acid alcohol, Eosin, and different grades of alcohol (60%, 70%, 80%, 90% and absolute alcohol)

2.6 Processing

The liver tissues were processed with 18 hours automatic tissue processor (LEICA TP 1020) and the procedure in the machine is as follows:

- Beaker 1 10%formal saline 1hr 30mins
- Beaker 2 70% alcohol 1hr
- Beaker 3 80% alcohol 1hr
- Beaker 4 90% alcohol 1hr
- Beaker 5 95% alcohol 1hr
- Beaker 6 95% alcohol 1hr 30mins
- Beaker 7 Absolute alcohol I 2hr
- Beaker 8 Absolute alcohol II 2hr
- Beaker 9 Xylene I 1hr 30mins
- Beaker 10 Xylene II 1hr 30mins
- Wax bath I 2hr
- Wax bath II 2hr

After the liver tissues were processed, they are embedded in molten paraffin wax with the use of metallic embedding mould.

The embedded tissues were placed inside the refrigerator to effectively solidify it. They were trimmed after removing them from the embedding mould to expose the surface of the tissues. The tissue was then placed on the tissue-tek machine to cool the surface of the tissue so as to allow easy and effective cutting of ribbon of sections from the tissues. The tissue sections were cut at 5um with the use of rotary microtome. The tissues were placed on a clean grease free-frosted end slide and floated in the water bath (40^{o}c) with the use of 20% alcohol.

The sections were picked from the water bath, well labelled and was placed on the dryer to allow the section to stick properly to the slide so as to avoid lifting during staining.

2.7 Staining Procedure

Haematoxylin and Eosin technique was used to stain the tissue sections to demonstrate general tissue structure. The procedure is as follows:

- Dewax in xylene -15mins
- Hydrate in descending grades of alcohol - Absolute, 90%, 80%, 70%, 60%.
- Rinse in water
- Cover with Ehrlich's haematoxylin - 15mins
- Rinse in water
- Differentiate in 1% acid alcohol- 10secs
- Blue in tap water - 10mins
- Counter stain with aqueous eosin – 5mins
- Dehydrate in ascending grades of alcohol- 60%, 70%, 80%, 90%, and absolute.
- Clear in xylene
- Mount in DPX using a cover slip

2.8 Microscopic Examination

The mounted slides were examined using x10 and x40 objectives of a binocular light microscope. Microscopy was done for all the sections and the results were compared with that of the control sections before photomicrograph was taken.

3. RESULT

Table 1 shows the histological effect of *Allium sativum* (garlic) on the liver of white rabbits. These effects include Vacuolation, lipofuscin pigment, Pyknotic nuclei, and necrosis.

In group A (control group) there was no Vacuolation, lipofuscin pigment, Pyknotic nuclei, and necrosis.

In group B there was no Vacuolation, lipofuscin pigment, Pyknotic nuclei, and necrosis.

In group C there was no Vacuolation, but there was a mild grade of lipofuscin pigment, Pyknotic nuclei, and necrosis.
In group D there was a mild grade of Vacuolation and severe grades of lipofuscin pigment, Pyknotic nuclei, and necrosis.

In group E there were severe grades of Vacuolation, lipofuscin pigment, Pyknotic nuclei, and necrosis.

4. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

In this study the histological structure of the liver of the untreated group differed from that of the groups treated with garlic powder. These differences include the presence of cellular necrosis, Vacuolation, lipofuscin pigments, pyknosis and nuclear hypertrophy. These differences were due to liver glycogen depletion and hepatic cell damage; a similar result recorded by Mohamed and Abd Allah (2003) in the treatment of *Chrysichthys auratus* fish with garlic juice.

The liver of rabbits administered 1000mg of garlic powder (i.e. group E) repeatedly for 28 days showed the highest grade of cellular necrosis. There was a high variation in the sizes of the nuclei, a marked reduction in the number of the hepatocytes and the presence of numerous Pyknotic and binucleated (B) cells and vacuoles (Fig. 5). Vacuolation and hypertrophy of the hepatocytes maybe due to fatty degeneration of accumulation of lipid droplets, something also recorded by Egen-Schwind *et al*. (1992) and Banerjee *et al*. (2001) in the liver of rats treated with high doses of garlic.

In liver of rabbits administered 500mg of garlic powder (i.e. group D) repeatedly for 28 days, considerable numbers of hepatocytes were characterized by the absence of nuclei, and others exhibit Pyknotic nuclei. In other regions of liver sections, the hepatocytes were damaged (Fig. 4). There was marked degeneration of the hepatocytes and sinusoidal cells leading to Vacuolation (V) thereby giving a scattered architectural appearance.

Liver of rabbits administered 200mg of garlic powder (i.e. group C) repeatedly for 28 days; show a mild grade of necrosis. There are few degenerated hepatocytes some of which appear with Pyknotic nuclei. There is also the presence of lipofuscin pigments around the hepatocytes.

Table 1. Table showing the histological effect of *allium sativum* (garlic) on the liver of white rabbits

Group		Dosage of Garlic powder (mg)	Gross findings	Microscopic examination			
				Vacuolation	Lipofuscin pigment	Pyknotic nuclei	Necrosis
	A1		Normal	-	-	-	-
A	A2	0		-	-	-	-
	A3			-	-	-	-
	A4			-	-	-	-
	B1			-	-	-	-
B	B2	100	Normal	-	-	-	-
	B3			-	-	-	-
	B4			-	-	-	-
	C1			-	+	+	+
C	C2	200	Normal	-	+	+	+
	C3			-	+	+	+
	C4			-	+	+	+
	D1			+	++	++	++
D	D2	500	Normal	+	++	++	++
	D3			+	++	++	++
	D4			+	++	++	++
	E1			++	++	++	++
E	E2	1000	Normal	++	++	++	++
	E3			++	++	++	++
	E4			++	++	++	++

KEY

Mg ---------- Milligram

- ---------- Absent

+ ---------- Mild

++ ---------- Severe

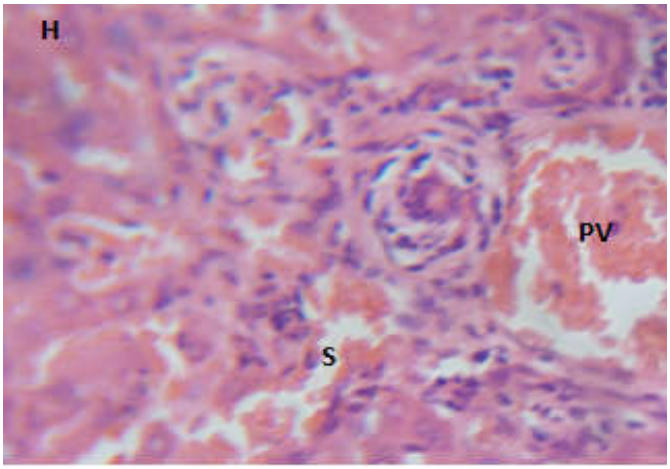

Fig. 1. Micrograph shows a well-preserved architectural arrangement having a well-arranged portal triad, limiting plates and portal vein (PV). The polygonal hepatocytes (H) spread out with their rounded nuclei. The sinusoids (S) lined with endothelial cells are also present (H & E, X400)

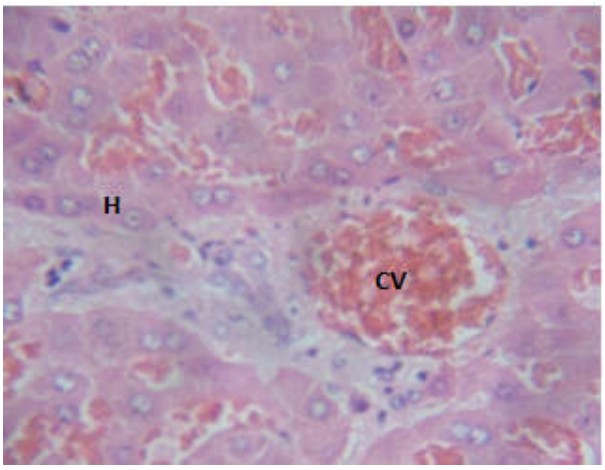

Fig. 2. Micrograph shows no histological difference compared to that of group A. The hepatocytes (H) and central vein (CV) are well positioned (H & E, X400)

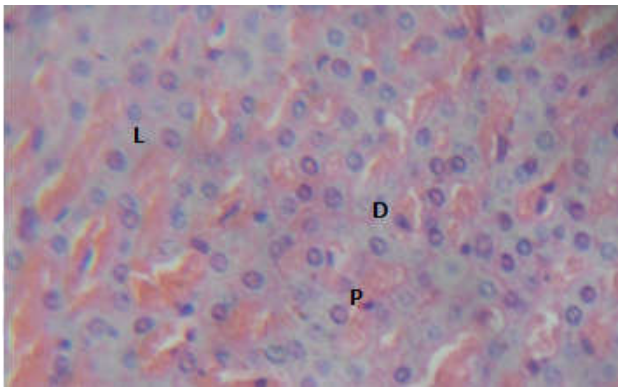

Fig. 3. Micrograph shows a mild grade of necrosis. There are few degenerated (D) hepatocytes some of which appear with Pyknotic nuclei (P). There is also the presence of lipofuscin (L) pigments around the hepatocytes (H & E, X400)

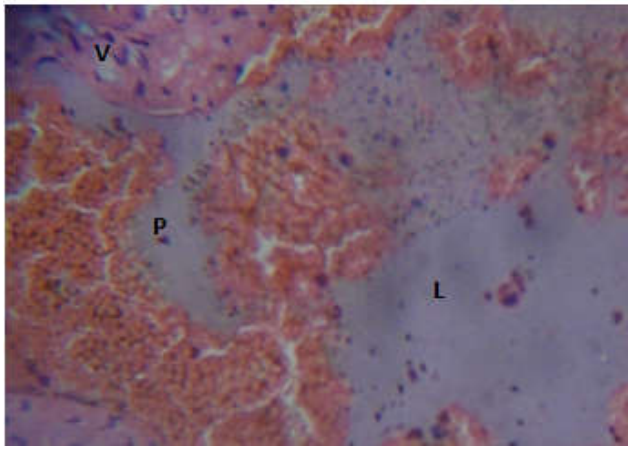

Fig. 4. Micrograph shows severe necrotic invasion. There is marked degeneration of the hepatocytes and sinusoidal cells leading to Vacuolation (V). Some hepatocytes exhibit Pyknotic nuclei (P) while some other hepatocytes are damaged

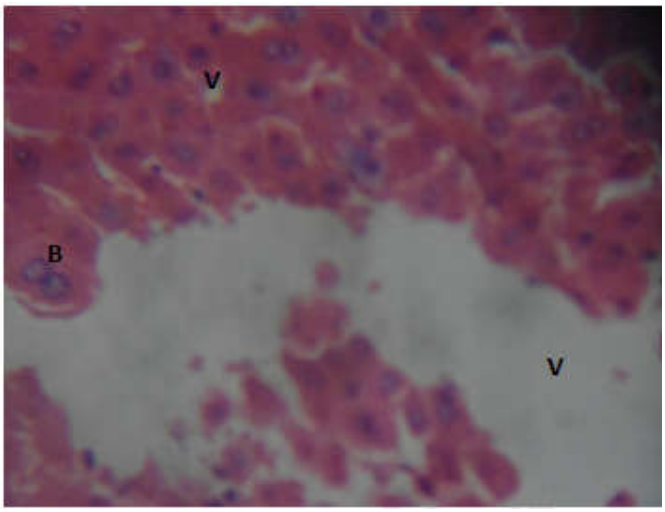

Fig. 5. Micrograph shows a massive invasion of the necrotic features into the liver tissue. There is a high variation in the sizes of the nuclei, a marked reduction in the number of the hepatocytes and the presence of numerous Pyknotic and nucleated (B) cells and vacuoles (H & E, X400)

The liver of rabbits administered with 100mg of garlic powder (i.e. group B) showed no histological differences when compared to the control sections (Group A).

According to Allegy.nte and Alnaqeeb (2010) hepatic injury is usually initiated by bio activation of drugs to chemically reactive metabolites, which have the ability to interact with cellular macromolecules such as proteins, lipids, and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA damage, and oxidative stress.

The liver tissues showed no gross abnormalities before they were processed.

4.2 Conclusion

Allium sativum (garlic) is a plant that has several health benefits however; in excess it could present some adverse and toxic effects. In this study repeated doses of garlic powder administered over a long period showed severe histological changes in the liver of the rabbits especially those with high doses.

In conclusion the histological changes such as the presence of cellular necrosis, Vacuolation, lipofuscin pigments, pyknosis and nuclear hypertrophy observed may be correlated to liver glycogen depletion and hepatic cell damage. In addition, the presence of lipofuscin pigment in some of the liver tissue shows that garlic enhances lipid metabolism.

4.3 Recommendations

It is clearly shown in this study that *Allium sativum* powder can have severe histological effects on the liver of rabbits, moderate administration of garlic is therefore strongly recommended in order to prevent its toxic effects. It is also suggested that more research should be done so as to ascertain the optimum dosage of garlic powder for humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written animal ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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