

Biochemical and Histopathological Assessment of Hepatic Response to *Allium sativum* Ethanolic Extract in Experimental Rats

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ABSTRACT

Garlic has shown to be of so much benefits to our health which comes from its bioactive compounds which have antioxidant, anti-inflammatory, anti-malaria and hepatoprotective effects. However, there is need to assess the potential effects of garlic extract on liver tissue. This study was aimed to ascertain the biochemical and histological effects of ethanolic extract of *Allium sativum* on the liver of adult male wistar rats. This study was carried out in the animal house of the Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli and JoyManuel Medical Diagnostic Laboratories, Nnewi, Anambra State. A total of twenty (20) healthy male Wistar rats were obtained from Kennedy's Animal Farm, Nnamdi Azikiwe University Awka, Campus. The rats were acclimatized for a period of two weeks (14 days) before they were grouped into four (4) for the administration of garlic extract. Group A (Control) received only feed and water, Group B received low dosage (0.1ml), Group C received medium dosage (0.15ml) and Group D received high dosage (0.2ml) of garlic extract for twenty-one (21) days. At the end of twenty-one (21) days, the animals were anaesthetized by chloroform in a closed jar. Blood samples were collected through ocular puncture for biochemical analysis using the method of Blaxhall and Daisley (2006). The rats were sacrificed and livers were harvested for histopathological analysis. The obtained data were subjected to statistical analysis using Microsoft excel. The result of the study revealed that Group B showed no significant mean difference in the initial and final weight of the rats ($p < 0.05$). However, there was a significant mean difference in weight of subjects in Group A, C and D before and after feeding with garlic extract respectively at ($p < 0.05$). The findings of this study also demonstrated no statistically significant difference in liver parameters (AST, ALT, ALP, CB and TB) among the groups (A, B, C and D) of the subjects ($p > 0.05$). The histological findings of this study also revealed focal areas of inflammatory background with mild vascular congestion when compared with the control group which showed normal morphology consistent with normal liver histology with no obvious sign of injury. Further studies on the exact mechanism(s) of action of the garlic extract which is not well understood presently are recommended in this study.

Keywords: Albino Wistar rat, *Allium sativum*, Biochemical effects, Histological effects Inflammatory, Liver

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INTRODUCTION

Garlic scientifically known as *Allium sativum* is a small, white, round bulb related to the onion bulb (*Collins English dictionary*). *Allium sativum* can also be seen as an edible bulb from a plant in the Lily family. Garlic has been used as a food and as a medicinal herb for decades now. Based on current trend, garlic use can be expected to continue to expand. Garlic has proven to be very effective in the treatment of a number of human diseases. It plays an important dietary and medicinal role worldwide (Dokubo and Uba 2023; Ezenwata et al. 2022a; 2022b). Currently, *Allium sativum* is the most commonly promoted as a dietary supplement for conditions related to the heart and blood vessels including high blood pressure and high cholesterol level. It was traditionally used for health purposes by people in many parts of the world including Egyptians, Greeks, Romans, Chinese and Japanese. Fresh garlic, garlic powder, and garlic oil are used to flavor foods. Garlic may be used topically (applied to the skin). Researches has it that garlic can be used to increase stamina (Dannester, 2003; Igbodika et al., 2014; Ubani et al. 2024a; 2024b; 2025). It is also recommendable to help respiration and digestion as well as cure parasitic infections such as the tapeworm infection. Garlic is probably safe for most people in the amounts usually eaten in foods. Side effects include breath and body odor, heartburn, and upset stomach. These side effects can be more noticeable with raw garlic. Some people have allergic reactions to garlic thus taking garlic supplements may increase the risk of bleeding. Garlic exhibits antioxidants action

by increasing the levels of cellular antioxidants, enzymes, catalase and glutathione peroxidase and scavenging reactive oxygen (Borek, 2006; Uba and Anidu, 2023; Uba and Obiefuna 2023; Uba et al., 2018a; 2018b; 2019a; 2019b).

The liver, the largest gland in the body weighs approximately 1500g and receives about 1500mL of blood per minute. It is a wedge-shaped organ located in the right upper quadrant of the abdomen and occupies most of the right hypochondrium and epigastrium (Moore *et al.*, 2018; Egrefa *et al.*, 2020a; Uba 2018; 2019a; 2019b). The liver has 2 surfaces namely, the diaphragmatic and visceral surfaces. The liver was customarily divided by anatomists into 4 lobes; the right and left lobes, the quadrate and caudate lobes (Sinnatamby, 1998; Ekwenze *et al.*, 2025; Mere *et al.*, 2025; Ubajekwe *et al.*, 2025). The liver plays an important role in humans as it has diverse functions which includes digestion, immunity, bile production, blood sugar level regulation, detoxification and metabolism. However, the liver may develop functional problems as a result of complications. This may be caused by invasion of some parasites such as the tapeworm cysts which likely results to hydatid disease (Alisa *et al.*, 2020; Anukam *et al.*, 2020a; 2020b).

Allium sativum has been used as both herbal medicine and flavouring agent. Accumulating evidence has shown that garlic has many beneficial effects to the health which includes antioxidants, anti-inflammatory, anti-cancer, lipid regulation, improving blood glucose levels and blood pressure regulation as well as an effective antiparasitic remedy best in treating tapeworm infections and plasmodium infections like-wise (Banerjee *et al.*, 2001; Umeh *et al.*, 2020; 2021; Ele *et al.* 2025). Basically, the antioxidant value of garlic helps in detoxification of the liver and lower blood pressure of the liver. However, excess consumption of garlic could actually bring harm to the liver as garlic contains a compound, allicin which when taken in high quantities could lead to liver toxicity thus damaging the liver (Abdel-Naim *et al.*, 2002; Umennadi *et al.* 2026a; 2026b; Uba and Umennadi, 2026). The study is limited to the investigation of the biochemical and histological effects on the liver following oral administration of ethanolic extract of garlic on male wistar rats. Thus, the aim of this study is to investigate the biochemical and histological effects of graded doses of ethanolic extract *Allium sativum* in regards to liver health of male wistar rats.

MATERIALS AND METHODS

Location of Study

This research study was carried out in the school animal house of the Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University Uli Campus.

Materials

The materials used during the course of study include: 20 Adult male Wistar rats, Animal feed (Grower's mash), Distilled water, wire gauze cage, saw dust, Plates and cup, Hand gloves, Ethanolic extract of garlic, 5 mL syringe, electronic weighing balance (M-Metlr M3HL China), Chloroform, Oral cannula, Formalin, Dissecting Kits and dissecting board and Sensitive balance.

Animals

Twenty apparently healthy Wistar rats weighing about 90g to 180g, which was considered fit to withstand the experiment were used. The rats were purchased from Kennedy's Animal farm located at Nnamdi Azikiwe University and were transferred to the animal house of the Department of Anatomy, Faculty of Basic Medical Science, Anambra State University, Uli. They were fed with animal feed and water for a period of two weeks to enable them acclimatize with the new environment before the actual experiment began (Uba *et al.* 2020a; 2020b).

Animal Housing

The wire gauze cage were used to house the Wistar rats. The roofs of each cage were covered with wire gauze to allow for proper ventilation, the floor of the cage was lined with sawdust, this was done to prevent sores in the feet of the rats and also to provide bedding for them, the sawdust in the cages was changed thrice weekly to prevent infection of the animals (Uba *et al.* 2020a; 2020b).

Animals Feeding

They were all fed with vital feed growers animal feed produced by Grand cereals LTD, km 17, Zawan Roundabout Jos, Plateau State which was purchased from Nkwo market, Ihiala. The feed was provided in a stainless plate while the water was provided in stainless cup. Good ventilation system, favorable humidity and temperature condition of the room was ensured. They were fed with animal feed and water for a period of two weeks to enable them acclimatize with the new environment before the actual experiment began (Uba *et al.* 2020a; 2020b).

Preparation of the Garlic Extract

Fresh bulb of garlic was purchased from a commercial market at Onitsha, Anambra State and was identified by the Department of Biological Science, Nnamdi Azikiwe University (Iheukwumere *et al.*, 2012a; 2012b; Mundi *et al.*, 2013; 2014; Okoye *et al.* 2013; Okoye *et al.* 2014). The bulb was sliced into thin slice and was dried at room temperature. The next day the garlic bulb was dried with an oven at 50 °C and grounded into fine powder using a manual blender (Okoye *et al.* 2020a; 2020b; 2020c; Obiefoka *et al.*, 2023). Fine powder (1mL) was soaked in ethanol (70%; 5mL) and left in a conical flask at room

temperature for 3 days while employing rocking method. Then, the mixture was filtered through a fine muslin cloth and a filter paper. Evaporatory method is employed to make the extract concentrated using 52 °C water bath. Tween 80 was used to dissolve the extract in the concentrations of 100 mg/mL and 50 mg/mL respectively (Okoye et al., 2016a; 2016b; Anameze et al. 2023; Ezeamama et al. 2025a; 2025b; Umezulora et al., 2026a; 2026b).

Low dose= 30ml of tween 80 and 30ml of the garlic extract at the ratio of 1:1

Mid dose= 60ml of tween 80 and 30ml of garlic extract at the ratio of 2;1

High dose = 90ml of tween 80 and 30ml of garlic extract at the ratio of 3: 1.

Experimental Protocol

In this study as described by Uba et al. (2020b) and Afulukwe et al. (2026), 20 male Wistar rats were acclimatized for 2 weeks and their weights were measured prior to Garlic extract administration. They were divided into four groups (A, B, C, D) of 5 rats each. Base on their weights, group A (control) measures 90-100 g, group B measures (garlic under dosage) 110- 120 g, group C (normal dosage) measures 120-140 g, group D (over dosage) measures 140-180 g. Group A was not given extract. Group B received 0.1ml of Garlic extract, Group C received 0.15ml of Garlic extract, Group D received 0.2ml of Garlic extract (this is administered base on their body weight). The extract was administered for twenty-one (21) days through oral route.

Animal Grouping

Groups	Weight	Extract	Results
A (control)	90g-100g	Only food and water were given	Rats gained weight
B (low dose)	110g-120g	0.1ml of garlic extract low dose + food and water	Rats weight slightly increased
C (mid dose)	125g-140g	0.15ml of garlic extract mid dose + food and water	Rats weight slightly decreased
D (high dose)	150g-180g	0.2ml of garlic extract high dose + food and water	Rats weight decreased. Death occurred

Sacrifice And Harvesting of Organs/Collection of Blood Sample

The experiment was terminated on the 21st day and the weight of each rat in each group was measured with Camry Emperors (Made in China) weighing scale and was recorded according to group and labelling. Twenty-four (24) hours after the last administration, the animals were anaesthetized by chloroform in a closed and sacrificed. Blood samples were collected prior to harvesting the organ through ocular puncture and put into plain tubes and then sent to the lab for a liver function test. The liver was excised and fixed appropriately using 10% formalin. Histological studies were done using H&E Staining methods (Egurefa et al., 2020b; Uba et al., 2021).

Liver Function Assessment

Assays of Aspartate Amino Transferase (AST) Activity, Alanine Amino Transferase (ALT) Activity, Alkaline Phosphatase (ALP) Activity, Total and Direct bilirubin estimation were carried out according to the methods of Egurefa et al. (2020b) and Uba et al. (2021).

Histopathological Analysis

The methods of Egurefa et al. (2020b) and Uba et al. (2021) were adopted for the histopathological analysis. The fixed kidney tissues were dehydrated in alcohol, cut sagittally and embedded in paraffin wax mounted on slides. The paraffin-embedded sections were stained with haematoxylin and eosin (H and E stain) and slides were systematically examined by compound microscope (CX 23 Olympus, Japan) for histopathological effects at Ifes Specialist Diagnostic Laboratory and Clinic, Awka, Anambra State, Nigeria.

Data Analysis

The statistical analysis will be completed using Statview v5.0.1 (SAS instruments) for windows. Data collected from the open field will be analyzed using a repeated measures ANOVA (Uba et al., 2020a; 2020b; 2020c; 2020d; 2024; 2025; Chude et al., 2021).

RESULTS

Effects of Ginger Extract on the Weight of the Wistar Rats

Table 1 below depicts the result of paired sample t-test of the mean weight difference of wistar rats before and after administration with garlic extract. It was revealed that Group B showed no statistically significant difference in the initial and final weight of the subjects when administered with low dose of garlic extract at ($p>0.05$). On the contrary, a statistically significant mean difference in weight of the rats was observed in Group A, C and D before and after administration with garlic extract at ($p<0.05$).

Table 1: Weight of Wistar Rats before and after Administration of Garlic Extract (n=5)

Weight	Group A (Control)	Group B (Low Dose)	Group C (Mid Dose)	Group D (High Dose)
Initial	84.78 ± 3.21	99.06 ± 5.86	120.55 ± 2.63	160.56 ± 21.30
Final	89.09 ± 4.17	103.10 ± 10.95	116.83 ± 3.38	143.54 ± 17.96
t-statistic	-3.799	-1.474	5.526	3.171
Sig.	0.032*	0.237	0.012*	0.050*

*Significant at 0.05 level (2-tailed). Data are mean and standard deviation.

Biochemical Effects of Garlic on The Liver Function Parameters

From Table 2 below, the Analysis of Variance (ANOVA) test showed no statistically significant difference in liver parameters (AST, ALT, ALP, CB and TB) among the groups (A, B, C and D) of the subjects at $p > 0.05$. This simply implies that the increase or decrease in dose of the garlic extract does not significantly affect the liver parameters of the wistar rats.

Table 2: Liver Parameters (n=5) of Wistar Rat across groups for Garlic Extract.

Dose	Mean ± SD	f-value	p-value
Control	69.28 ± 95.3795	0.062	0.979
Low Dose	64.96 ± 85.8097		
Mid Dose	50.58 ± 73.3865		
High Dose	52.70 ± 71.0741		
Total	59.3800 ± 75.68867		

* = significant at 0.05 level (2-tailed). Data are mean and standard deviation.

Multiple Comparison of Wistar Rats Liver Parameters (n=5) across groups for Garlic Extract

Table 3 shows the ANOVA test performed to determine if there is a any significant mean difference in the effect of at least two of the groups feed with varying doses of garlic extract on the subjects and it revealed no significant difference at $p > 0.05$.

Table 3: Multiple Comparison of Wistar Rats Liver Parameters (n=5) across groups for Garlic Extract.

	Dose	Mean ± SD	p-value
Control	Low Dose	64.96 ± 85.8097	1.000
	Mid Dose	50.58 ± 73.3865	0.983
	High Dose	52.70 ± 71.0741	0.988
Low Dose	Control	69.28 ± 95.3795	1.000
	Mid Dose	50.58 ± 73.3865	0.992
	High Dose	52.70 ± 71.0741	0.995
Mid Dose	Control	69.28 ± 95.3795	0.983
	Low Dose	64.96 ± 85.8097	0.992
	High Dose	52.70 ± 71.0741	1.000
High Dose	Control	69.28 ± 95.3795	0.988
	Low Dose	64.96 ± 85.8097	0.995
	Mid Dose	50.58 ± 73.3865	1.000

* = significant at 0.05 level (2-tailed). Data are mean and standard deviation.

Histological Profile

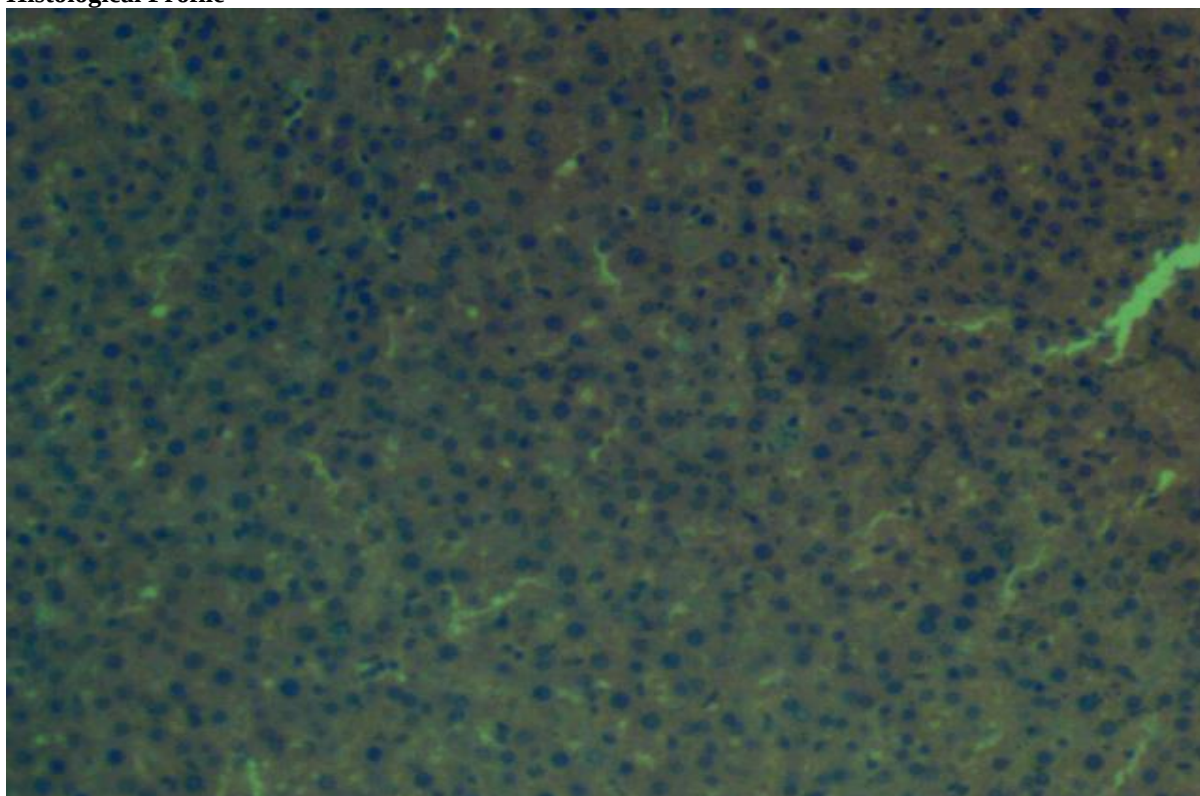


Figure 1: Liver (control): Photomicrograph of liver tissue shows morphology consistent with normal liver histology. The hepatocytes (arrowhead) are normal with no obvious sign of injury (H&E X 400).

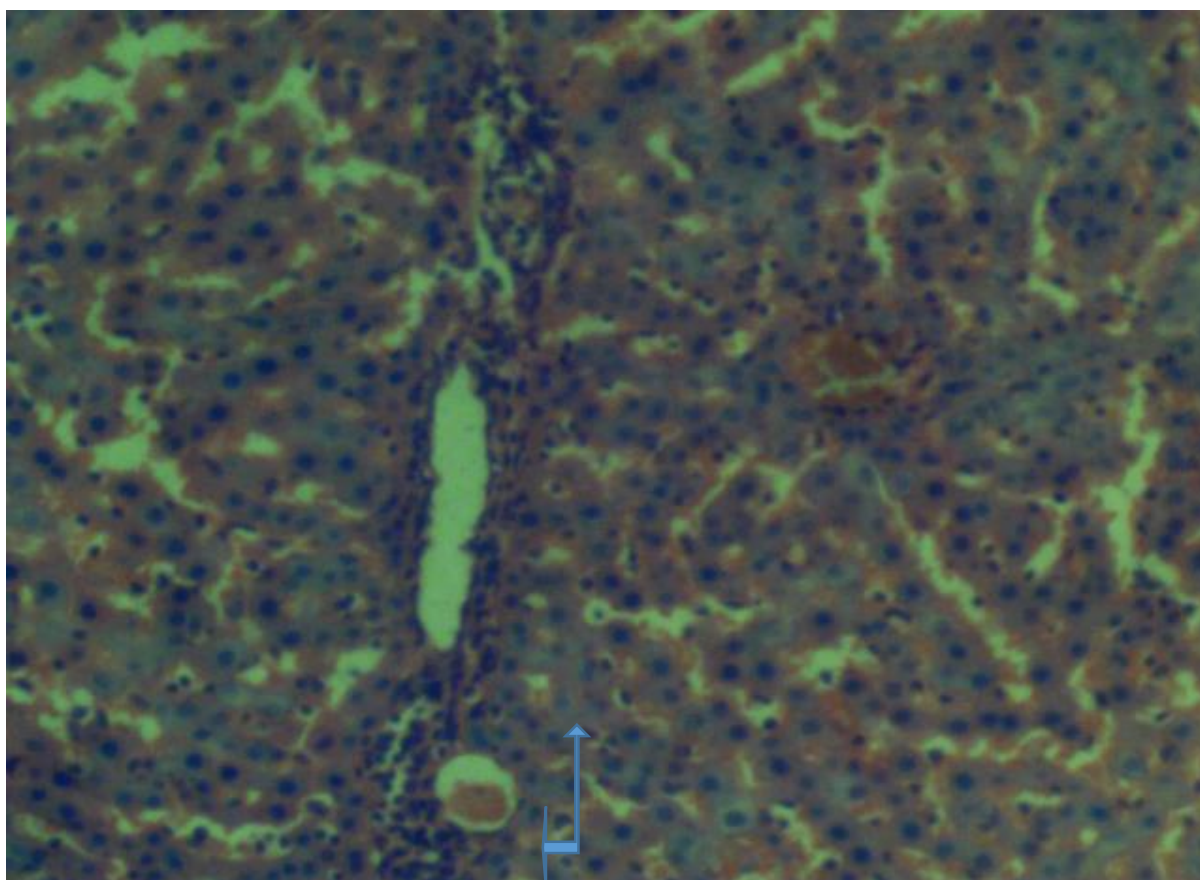


Figure 2: Liver (Low Dose): Photomicrograph of liver tissue shows focal areas of inflammatory background with mild vascular congestion (arrowhead) (H&E X 400).

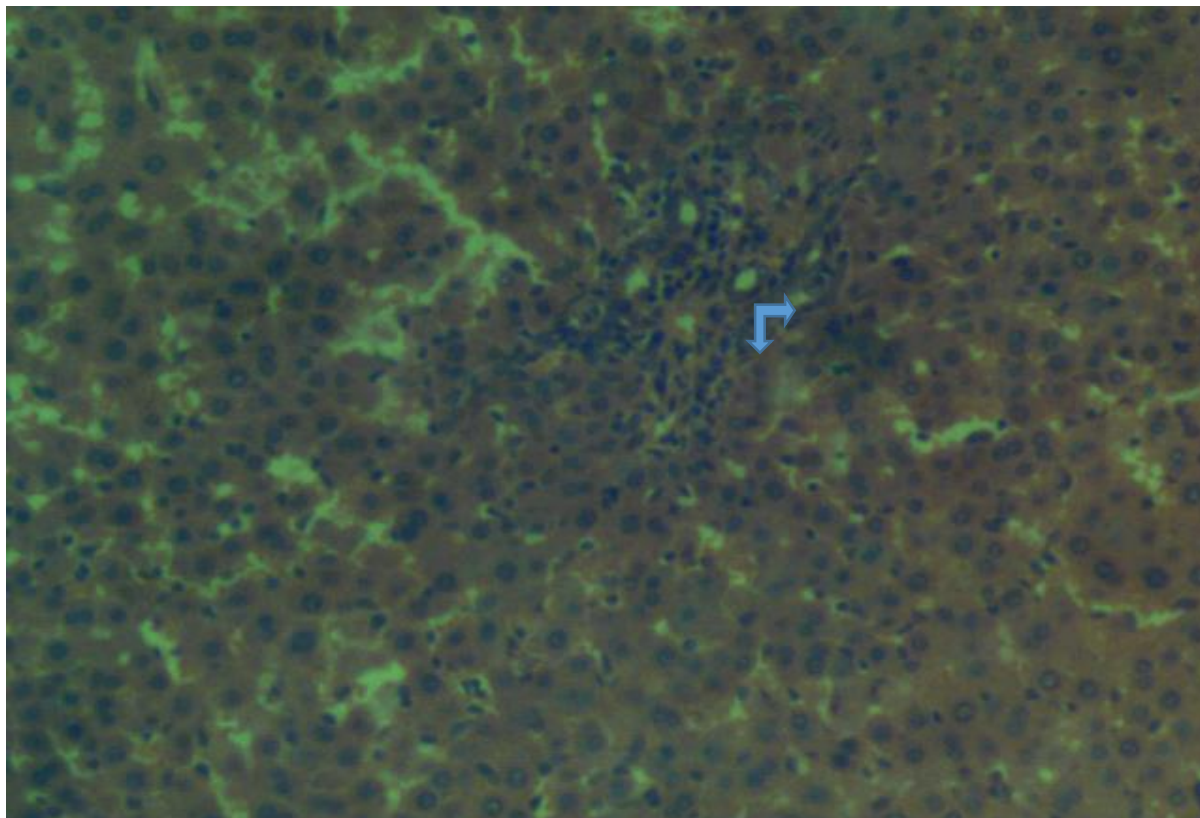


Figure 3: Liver (Medium Dose): Photomicrograph of liver tissue shows focal areas of inflammatory background (arrowhead) (H&E X 400).

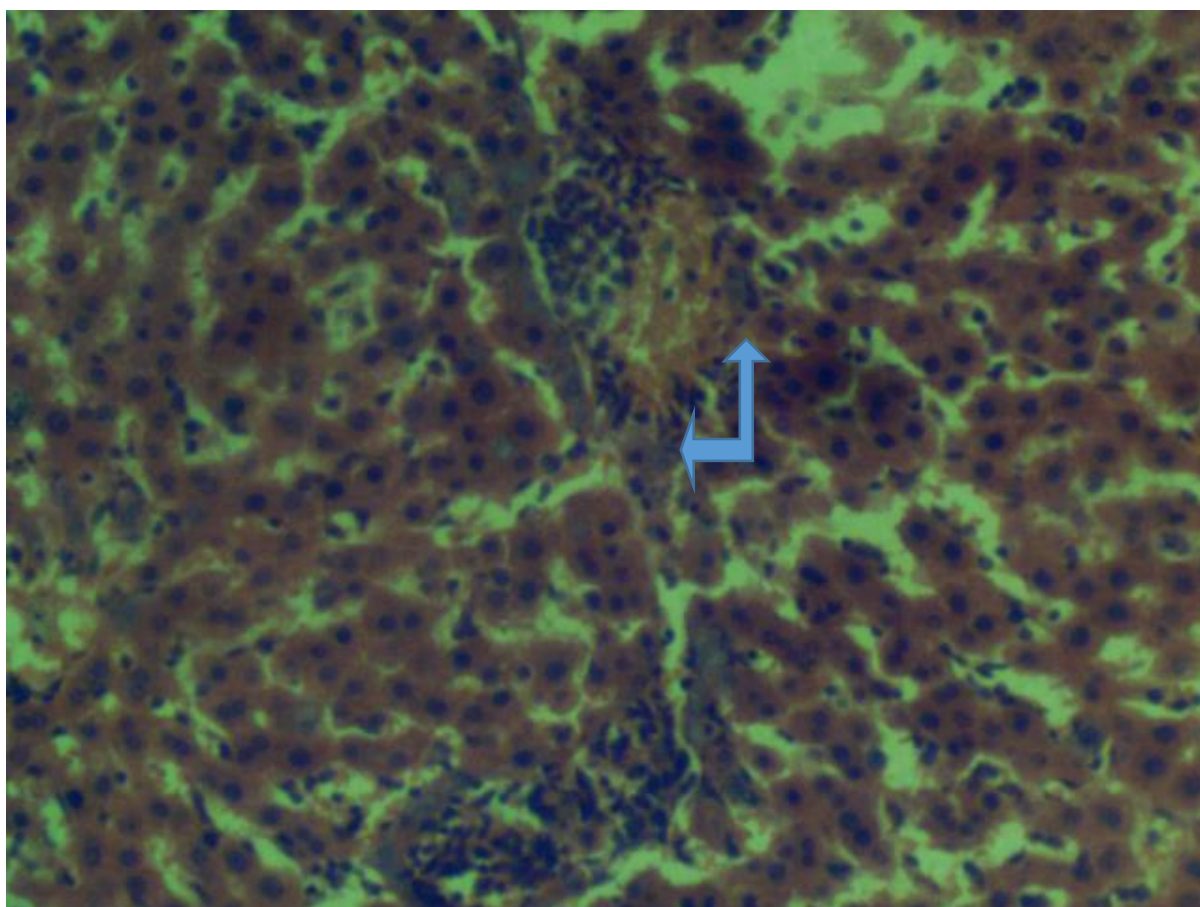


Figure 4: Liver (High Dose): Photomicrograph of liver tissue shows focal areas of inflammatory background with mild vascular congestion (arrowhead) (H&E X 400).

DISCUSSION

Liver is one of the vital organs of the animal body and plays a central role in transforming and clearing the chemicals, but it is susceptible to the toxicity from these agents (Krishna *et al.*, 2018). Garlic has particularly attracted special attention for its widespread potential medicinal benefits (Asman *et al.*, 1977; Hammami *et al.*, 2013). Nonetheless, there has been little scientific support for its therapeutic and pharmacological properties as well as its potential toxic effects. The increasing usage of medicinal plants in alternative medicine, justifies the need for toxicological assessment (Fowotade *et al.*, 2017). The aim of this study was to investigate the biochemical and histological effects of ethanolic extract of *Allium sativum* on the liver of adult male wistar rats.

The findings of this study showed that there was an increase in the body weight of the treated animals which is dose-dependent. Again, the findings of this study also revealed that Group B showed no statistically significant difference in the initial and final weight of the subjects when administered with low dose of garlic extract at ($p > 0.05$). On the contrary, a statistically significant mean difference in weight of the rats was observed in Group A, C and D before and after administration with garlic extract at ($p < 0.05$). The above findings are in conformity with study by Fowotade *et al.* (2017) who reported increase in the weights of the treated rats with progressively increasing doses of *Allium sativum*. This finding is also in accordance with the observations of Behnaz (2014), which showed a reversal of radiation-induced weight loss in experimental rats, thus showing that garlic was able to restore the weight loss caused by radiation.

Liver enzymes such as AST, ALT and ALP are marker enzymes for liver function and integrity (Rao, 2006). These enzymes are usually elevated in acute hepatotoxicity or mild hepatocellular injury, but tend to decrease with prolonged intoxication due to liver damage (Arti *et al.*, 2010). When the liver cell membrane is damaged, varieties of enzymes normally located in the cytosol are released into the blood stream (Rao, 2006). The findings of this study also demonstrated no statistically significant difference in liver parameters (AST, ALT, ALP, CB and TB) among the groups (A, B, C and D) of the subjects at $p > 0.05$. This simply implies that the increase or decrease in dose of the garlic extract does not significantly affect the liver biochemical parameters of the wistar rats. This is consistent with the findings of Bright *et al.* (2022) who equally reported significant reduction ($P < 0.05$) in the liver enzymes of the rats fed with garlic powder. They also noted that the reduction in the activities of ALT (8.54 ± 0.04 I/U) and AST (22.00 ± 0.58 I/U) in the liver was due to interference with protein metabolism in the cells (Rao, 2006). Reduced ALT and AST activities in garlic supplementation studies using humans showed protection of the liver against damage and improved hepatic features (Rao, 2006). Bright *et al.* (2022) also asserted that the concentration-and time-dependent changes in the AST and ALT enzymatic activities in the serum of the rats fed with garlic powder. The present findings are consistent with the observations by Fowotade *et al.* (2017) who revealed a significant decrease in the levels of serum AST, ALT and ALP) when compared with the control after the administration of garlic extract at different concentrations for 38 days. It has been reported that fresh garlic protects the liver against induced toxicity (Sabah *et al.*, 2009; Ezeala *et al.*, 2009).

The photomicrographs of liver tissue treated with *allium sativum* extract shows focal areas of inflammatory background with mild vascular congestion when compared with the control group which showed normal morphology consistent with normal liver histology with no obvious sign of injury. The histopathological findings of this study are in consonance with the cytoarchitectural of findings of Bright *et al.* (2022), Afulukwe *et al.* (2025); (2026) and Afulukwe (2026) who reported distortion of the liver tissue, necrosis, congestion of the central vein, and vacuolations were due to the increased concentrations of the garlic powder. The present study supports the findings of Fowotade *et al.* (2017) who observed cellular hypertrophy of the liver tissue treated with garlic extract. This was also 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 (Sangouni *et al.*, 2020; Al-Salahy and Mahnond, 2003). Interestingly, the observed histological changes in the liver following garlic administration at different doses for each group further support the biochemical findings shown in Tables 2 and 3.

CONCLUSION

The present study has clearly revealed that oral administration of ginger extract *Allium sativum* ethanolic extract to healthy adult rats showed no effect on the liver functions. The administration of *Allium sativum* ethanolic extract to healthy rats had no effect on the body weights of the treated animals. It was also concluded in this study that the observed distortion of the liver observed in the photomicrographs of the treated animals does not imply serious damage of the liver cells as this can be reversible upon withdrawal of the administration of garlic extract. It is recommended in this study that garlic consumption should be with caution in order to prevent the possible dose dependent toxic effects of garlic on the liver as observed in this study. Further studies are needed to investigate the exact mechanism(s) of action of the garlic extract which is not well understood presently.

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