

Phytochemical composition, acute toxicity, hypoglycemic and hypolipidemic effects of ethanol extract of *Allium sativum* (garlic) bulb in male Wistar rats

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Abstract

Allium sativum (garlic) has been used in traditional medicine in Nigeria in the treatment of various ailments such as cold, cough, and microbial infections; and there have been various claims about its pharmacological activities including anti-inflammatory, antimicrobial, hypoglycemic, hypolipidemic, antioxidant, antidiabetic, and anticancer properties. However, there are insufficient data to back up these claims. It is against this backdrop, this study evaluated the phytochemical composition, acute toxicity, hypoglycaemic and hypolipidemic potentials of ethanol extract of *A. sativum* bulb in male Wistar rats in order to validate its therapeutic use as hypoglycaemic and hypolipidemic agent. Twenty adult male Wistar rats were randomly divided into four groups of five rats each where group 1 (control) received normal saline while group 2-4 received the extract at the doses of 200, 400, and 1000mg/kg b.w respectively for fourteen days. T-test and one-way ANOVA were used to test for significant ($p < 0.05$) differences among the groups. Qualitative and quantitative phytochemical screening of the extract indicated the presence of flavonoids (17.59 ± 0.01), saponins (13.55 ± 0.01), alkaloids (2.06 ± 0.06), tannins (0.04 ± 0.00), phenols (0.01 ± 0.00), phlobatanin, phytosterols and terpenoids whereas steroids, anthraquinones and glycosides were not detected. The administration of the extract produced a significant ($P < 0.05$) decrease in the blood glucose concentration in the treatment groups compared to the control group, whereas a significant ($P < 0.05$) decrease was observed in the total cholesterol concentration of group 4 only relative to the control. Furthermore, there was a significant ($P < 0.05$) increase in the HDL concentrations and a significant ($P < 0.05$) decrease in the triglycerides and LDL concentrations respectively in all treatment groups compared to the control group. From these findings therefore, we conclude that *A. sativum* offers both hypoglycemic and hypolipidemic effects in male Wistar rats.

Keywords: *Allium sativum*; Phytochemical; Acute toxicity; Blood glucose; Serum lipids; Hypoglycemia; Hypolipidemia

1. Introduction

Natural plant products have historically been used to cure human maladies and are becoming increasingly popular within the past few years for combating numerous illnesses, and they are now an invaluable resource for developing novel drugs [1]. Due to the growing use of chemically made culinary flavorings in both industrialized and emerging nations, there are a growing number of humans plagued by certain degenerative diseases. These seasonings are becoming more expensive, and do not improve the nutritional quality of foods. Additionally, they frequently contain some suspicious ingredients that may be detrimental to human health when consumed constantly. Furthermore, herbal remedies have long been an important facet of traditional medical practices; unlike chemical or manmade medications, natural remedies are also readily obtainable and affordable.

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Allium sativum, a bulbous perennial flowering plant also known as garlic, belongs to the genus *Allium*. Its close relatives include the Chinese onion (*Allium chinense*), leek (*Allium ampeloprasum*), welsh onion (*Allium fistulosum*), onion (*Allium cepa*), shallot (*Allium ascalonicum*), and chive (*Allium schoenoprasum*) [2]. The bulb of *A. sativum* plant is the most common part of the plant that is most frequently utilized. Typically, the bulb is separated by several fleshy portions known as cloves. The cloves can be used either as food (raw or cooked) or as medicine. They have a distinctive pungent, spicy sensation of taste that significantly softens and sweetens when cooked. The bulb is odorous and has the cloves housed by an inner shaft that is surrounded by exterior layers of thin shearing leaves. The bulb commonly has 10 to 20 symmetrical cloves, with the exception of those closest to the center. *A. sativum*, like other plants, has a superb defense mechanism made up of as many unique components that boost the human immune system. Due to its preventive and therapeutic properties, it is one of the world's oldest medicines as well as a flavoring agent [3]. Some phytochemicals found in this plant material are known to have pharmacological effects. Phytochemicals are plant-derived compounds [4] that are produced by plants through primary or secondary metabolism [5]. As medicinal compounds and nutrients, they promote human health. Recently, it has been proven that they can even play important protective roles in human health when their dietary intake is reasonable [6, 7]. Mother Nature's green belt is the richest source of bioactive phytochemicals and natural dietary supplements [8]. The naturally occurring phytochemicals with therapeutic effects contribute to the demonstrated medicinal importance of herbal remedies [9].

Phytochemicals are naturally occurring non-nutritive plant compounds that play protective or preventive roles in plants against disease. Each class of these active compounds is made up of a variety of chemicals with varying potencies, and some of these phytochemicals have been discovered to be multifunctional [10]. These naturally occurring phytochemicals (secondary metabolites), include alkaloids, phenols, flavonoids, terpenoids, and others, possess an extensive array of therapeutic advantages against a variety of ailments [11]. Alkaloids, terpenoids, flavonoids, steroids, phenols, Anthraquinones, saponins, tannins, and glycosides were detected in *A. sativum* aqueous and ethanol extracts [12]. Phytochemical analysis of garlic bulb extracts revealed the presence of glycosides, steroids, phlobatanin, flavonoids, alkaloids, terpenoids, and carbohydrates, whereas saponins, phenolics, or tannins were not detected [13].

The presence of flavonoids, alkaloids, saponins, tannins, and cardiac glycosides was demonstrated in the phytochemical screening of garlic [14], whereas proteins, phlobatanin, ketones, phenolic compounds, cardiac glycosides, flavonoids, alkaloids, and tannins were detected in another phytochemical screening of garlic [15]. *A. sativum* extracts and isolated chemicals have been tested for a variety of biological activities including anti-inflammatory, antibacterial, antiprotozoal, antioxidant, antiviral, anticancer, and antifungal properties [16]. Sulfur compounds present in garlic have been demonstrated to offer protection against damage to the organs caused by the toxic effects of heavy metals at high doses. Research has shown that garlic has anti-stress and anti-aging activities, enhances memory and learning; also, aged garlic extract provides protective effects on the brain and prevents amnesia, neuronal death, Alzheimer's, and other neurodegenerative diseases [17]. Garlic has been shown to be linked to enhanced overall cardiovascular wellness; reduced blood pressure, plasma lipids, an anti-platelet impact, and other cardiovascular markers [18]. Garlic and its secondary metabolites have been shown in clinical investigations to lower the risk of developing human diseases such as blood pressure, cardiovascular and metabolic disorders, cancer, and diabetes. Specific features of garlic, such as anti-inflammatory and antioxidant properties, and the capacity to lower lipids percentage allow for this medicinal application of garlic in medicine [19].



Figure 1 *A. sativum* (garlic) bulb

However, the popular use of herbal remedies is riddled with many problems such as lack of sufficient studies on therapeutic properties and toxicity in order to provide satisfactory basis for their use. Based on this, this study aimed

at evaluating the phytochemical composition, acute toxicity, hypoglycaemic and hypolipidemic potentials of ethanol extract of *A. sativum* bulb in male Wistar rats.

2. Materials and methods

2.1. Plant Material

2.1.1. Collection and Preparation

Fresh *A. sativum* bulbs were purchased in the month of June 2022 from Relief market, Onitsha, Anambra state. The plant material was identified to specie level at Department of Botany, Faculty of Biological Sciences, Nnamdi Azikiwe University Awka by Mr. Finian Iroka with the Herbarium number: NAUH- 015 (BULB) and deposited in the herbarium. It was freshly peeled, air dried, blended and weighed.

2.1.2. Plant Extraction

The ethanol extract was obtained by soaking 468.43g of blended *A. sativum* in 1000ml of ethanol and shaken intermittently for 72hours. The mixture was first sieved with cheese cloth and then with Whatmann filter paper number: 42 (125mm). The solution was concentrated using rotary evaporator and the crude extract was obtained and weighed.

$$\text{Percentage (\%)} \text{ yield} = (A1/A0) \times 100$$

Where:

A1= weight of extract

A0= weight of sample

2.2. Phytochemical Screening

2.2.1. Qualitative Analysis

The screening of *A. sativum* for various phytochemical constituents like terpenoids, alkaloids, flavonoids, steroids, glycosides, saponins, anthraquinones, phlobatanin, phytosterols, phenols, and tannins was conducted using standard methods. The analysis was carried out as described by Trease and Evans [20].

2.2.2. Quantitative Analysis

Alkaloids

Five (5) g of the extract was weighed into a 250ml beaker and to the beaker; 200ml of 20% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated to one-quarter of the original volume using water bath. Drop by drop, concentrated ammonium hydroxide was added to the extract until the preparation was completed and the entire solution was allowed to settle, then filtered and the precipitate was collected and weighed [21].

$$\text{Percentage (\%)} \text{ alkaloids} = (\text{weight of alkaloid}/\text{weight of sample}) \times 100$$

Tannins

A known quantity, 0.2g of sample was measured into a 50ml beaker, and 20ml of 50% methanol was added, covered with paraffin, placed in a water bath at 77-80°C for 1 hour and stirred with a glass rod to prevent bumping. Using a double layer of Whatman No 1 filter paper the extract was filtered into a 50ml volumetric flask. Then 20ml distilled water, 2.5ml Folin-Denis reagent and 10ml of 17% of sodium trioxocarbonate IV (Na_2CO_3) were added and left to stand for 20 minutes during which when a bluish-green colouration developed. Standard tannic acid solutions of range 0-10 ppm were treated similarly as 1ml of sample above. The absorbances of the tannic acid standard solutions as well as samples were read after color development at 760nm [22].

Flavonoids

The flavonoids content was quantified using the method of Harborne [21], by weighing out 1.0 g of the sample which was macerated with 20 ml of ethyl acetate for 5 minutes and then filtered. Five (5) ml of dilute ammonia solution was added to 5 ml of the filtrate, the entire mixture shaken for 5 minutes. The upper layer formed was collected, and the absorbance read at 490 nm.

Saponins

Saponins content was determined by the method of Harborne [21]. One (1.0) g of the sample was macerated twice in a beaker with 10 ml of petroleum ether. The filtrate was combined and evaporated to dryness. The recovered residue was dissolved with 6 ml of ethanol. Two (2) ml of chromate solution was also added to 2 ml of the dissolved residue in a test tube, was allowed to stand for 30 minutes and the absorbance read against an ethanol blank at 550 nm.

Phenols

The extract (0.5 ml) was transferred into a test tube containing 0.2 ml of 1/10 dilution Folin reagent, 0.2 ml of 2 % Na₂CO₃ was added to it and allowed to stand for 30 minutes at room temperature. After addition of 2.1 ml of distilled water, the absorbance was read at 640 nm against the blank [21].

2.3. Animal model

Twenty (20) adult male Wistar rats of about 12 weeks of age weighing between 120-150g and thirteen (13) male mice weighing 30-44g were used for this study. The animals were housed in clean rat cages in the animal house of Department of Biochemistry, Faculty of Natural and Applied Science, Legacy University Okija, Anambra State Nigeria and were allowed to acclimatize for seven (7) days under standard photoperiodic condition and fed with Chikun broiler pellet. All animals were allowed access to food and water *ad libitum*.

2.4. Acute Toxicity Test

Acute toxicity test was carried out according to the method of Lorke [23]. The study was carried out in two phases as follows:

2.4.1. Phase 1

Nine (9) male mice were randomly divided into three groups of three mice each. Each group of animals was administered different doses (10, 100 and 1000mg/kg body weight) of ethanol extract of *A. sativum*. The animals were observed for 24 hours for abnormal behavior and mortality.

2.4.2. Phase 2

Four (4) male mice were randomly divided into four groups of one animal each. The animals were administered higher doses (1600, 2900, 3600 and 5000mg/kg body weight respectively) of ethanol extract of *A. sativum* and then observed for 72 hours for abnormal behavior as well as mortality.

2.5. Experimental Design

Twenty (20) male Wistar rats were randomly divided into four groups of five (5) rats each. The experimental animals were given ethanol extract of *A. sativum* once daily by gavage for fourteen (14) days as follows:

- Group 1: Normal rats treated with normal saline.
- Group 2: Normal rats treated with 200mg/kg body weight of ethanol extract of *A. sativum*.
- Group 3: Normal rats treated with 400mg/kg body weight of ethanol extract of *A. sativum*.
- Group 4: Normal rats treated with 1000mg/kg body weight of ethanol extract of *A. sativum*.

A day after the final exposure, blood samples were collected through the tail for the determination of blood glucose concentration using Accucheck glucometer and through ocular puncture for the analysis of serum lipid profile.

2.6. Biochemical Analysis

2.6.1. Determination of Blood Glucose Concentration

A drop of blood was collected from the tail of each animal and the baseline blood glucose concentration was measured using Accu-check glucometer prior to the commencement of treatment. After 14 days of extract administration, blood sample was also collected through the tail and their blood glucose concentrations were also measured.

2.6.2. Serum Lipid Profile

After fourteen (14) days, the animals were sacrificed and by ocular puncture blood was taken into a plain sample tube (containing no anticoagulant), allowed to clot, centrifuged and then the serum lipid profile such as triglycerides (TG), total cholesterol (TC), and high-density lipoprotein (HDL) were determined using commercially available kits. Serum low density lipoprotein (LDL) was also determined mathematically using the Friedewald formulae [24].

Serum Total Cholesterol

Serum total cholesterol was determined according to the method of Allain *et al* [25] using commercially available kit. Twenty two (22) test tubes were set up in a test tube rack and labelled blank (BL), standard (ST) and the rest were labelled sample (SA) respectively. Ten (10) μl of distilled H_2O , 10 μl of standard solution and 10 μl of serum were added to the test tube labelled blank, standard and samples respectively. 1000 μl of the cholesterol reagent was added to each of these test tubes, and was thoroughly mixed and incubated for 10 minutes at room temperature (20-25°C). The absorbance of the sample (A_{sample}) against the blank was measured at 500nm within 60 minutes. The total serum cholesterol concentration in the sample was calculated using the general formula:

$$\text{Cholesterol concentration in mg/dl} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

High Density Lipoprotein (HDL)

Serum HDL-Cholesterol was determined according to the method as described by Assmann [26] using commercially available kit. Low density lipoproteins (LDL and VLDL-) and chylomicron fractions are precipitated from serum by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction which remains in the supernatant is determined. Two hundred (200) μl of the sample and 200 μl of the cholesterol standard were pipetted into the centrifuge tubes labelled samples and standard respectively and 500 μl of the precipitant was added to each of the tubes. The solution was mixed and allowed to stand at room temperature for 10 minutes, then centrifuged at 400rpm for 10minutes. The supernatant was separated within two hours and the cholesterol content was determined by the CHOD-PAP method.

Cholesterol CHOD-PAP assay

One hundred (100) μl of distilled water, 100 μl of standard supernatant and 100 μl of sample supernatant were pipetted into test tubes labelled blank, standard and samples respectively and 1000 μl of cholesterol reagent was added to each of the test tubes. The solutions were mixed and incubated at 20-25°C for 10 minutes and the absorbance of the sample (A_{sample}) and standard (A_{standard}) against the reagent blank at 546 nm were measured within 60 minutes. The HDL cholesterol concentration in the supernatant was calculated using the following general formula:

$$\text{HDL concentration in mg/dl} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Triglycerides (TG)

Serum Triglycerides was determined using the method of Fossati and Prencipe [27] using commercially available kits. Twenty two (22) test tubes were set up in a test tube rack and labelled blank (BL), standard (ST) and the rest were labelled sample (SA). Ten (10 μl) micro-litre of the sample and 10 μl of the standard solution were pipetted into the test tubes labelled sample and blank respectively and 1000 μl of the cholesterol reagent was added to each of the test tubes. The solutions were incubated at 37 °C for 5 minutes and the absorbance of the sample (A_{sample}) and standard (A_{standard}) against the reagent blank were measured at 546 nm within 60 minutes. The concentration of triacylglycerol in serum was calculated as follows:

$$\text{TG concentration in mg/dl} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Low Density Lipoprotein (LDL)-Cholesterol

Normally, triglycerides, HDL-cholesterol and total cholesterol are measured while LDL-cholesterol is calculated. The LDL-cholesterol concentration in the sample was calculated using the Friedewald *et al.* formula [24].

$$\text{LDL cholesterol} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL cholesterol (mg/dl)}$$

2.7. Statistical Analysis

All values were expressed as mean \pm SD (standard deviation). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by t-test using IBM SPSS software version 23. $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Percentage yield

The percentage yield of *A. sativum* was calculated as follows:

$$\text{Percentage (\%)} \text{ yield} = \frac{A1}{A0} \times 100$$

Where, A1 = Weight of extract (29.449g)

A2 = Weight of sample (468.43g)

$$\therefore \% \text{ yield} = \frac{29.449}{468.43} \times 100$$

$$\% \text{ yield} = 0.629 \times 100$$

$$\% \text{ yield} = 6.29\%$$

3.2. Phytochemical screening

The result of the qualitative phytochemical screening of ethanol extract of *A. sativum* showed the presence of saponins, tannins, flavonoids, phenols, alkaloids, terpenoids, phlobatanin and phytosterols as shown in table 1 below.

Table 1 Qualitative phytochemical screening of *A. sativum* ethanol extract

s/n	Phytochemicals	Status
1	Saponins	+
2	Tannins	+
3	Flavonoids	+
4	Phenols	+
5	Steroids	-
6	Alkaloids	+
7	Terpenoids	+
8	Phlobatanin	+
9	Phytosterols	+
10	Glycosides	-
11	Anthraquinones	-

Key: + = Present; - = Not detected

Table 2 Quantitative phytochemical screening of *A. sativum* ethanol extract

s/n	Phytochemical	Percentage composition (g/100g)
1	Alkaloids	2.06±0.06
2	Flavonoids	17.59±0.01
3	Saponins	13.55±0.01
4	Phenols	0.01±0.00
5	Tannins	0.04±0.00

n=3; mean ± standard deviation.

3.3. Acute toxicity

In phase 1 of the acute toxicity study, no death was observed at all the administered doses of the ethanol extract of *A. sativum*. The animals were apparently healthy with no sign of toxicity up to the dose of 1000mg/kg body weight within and after 24 hours of administration.

In phase 2 of the acute toxicity study, no death was observed at all the administered doses of the ethanol extract of *A. sativum*. The animals were apparently healthy with no sign of toxicity up to the dose of 5000mg/kg body weight within and after 72 hours of administration. Thus, the LD₅₀ of the garlic extract was more than 5000mg/kg body weight.

Table 3 Acute toxicity study

Phase I		
Dose (mg/kg body weight)	Number of animals	Number of deaths
10	3	0
100	3	0
1000	3	0
Phase II		
Dose (mg/kg body weight)	Number of animals	Number of deaths
1600	1	0
2900	1	0
3500	1	0
5000	1	0

3.4. Effect of Ethanol Extract of *A. sativum* on Glucose Concentration of Male Wistar Rats

Figure 1 above represents the effect of ethanol extract of *A. sativum* on glucose concentration of the experimental rats. The result revealed that there was no significant ($p>0.05$) difference in the concentrations of glucose down the groups prior to the administration of the extract. However, after the administration of the extract for 14 days, blood glucose concentrations of groups 2, 3 and 4 were found to be significantly ($p<0.05$) lower than the normal (group 1) group.

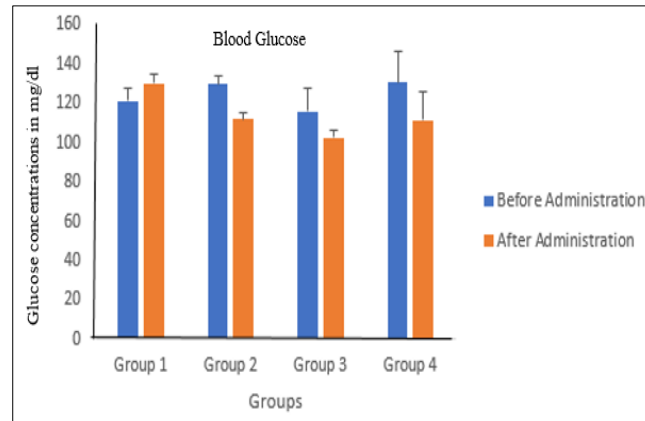


Figure 1 Effect of ethanol extract of *A. sativum* on blood glucose concentrations of male Wistar rats

Legends: Group 1: Normal control received normal saline; Group 2: Received 200mg/kg body weight of ethanol extract of *A. sativum*; Group 3: Received 400mg/kg body weight of ethanol extract of *A. sativum*; Group 4: Received 1000mg/kg body weight of ethanol extract of *A. sativum*

3.5. Effect of Ethanol Extract of *A. sativum* on Total Cholesterol concentration of Male Wistar Rats

After the 14 days administration of ethanol extract of *A. sativum*, group 4 was found to be significantly ($p < 0.05$) lower than groups 1 and 2 in total cholesterol concentration while there was no significant ($p > 0.05$) difference between groups 1, 2 and 3 as shown in figure 2 below.

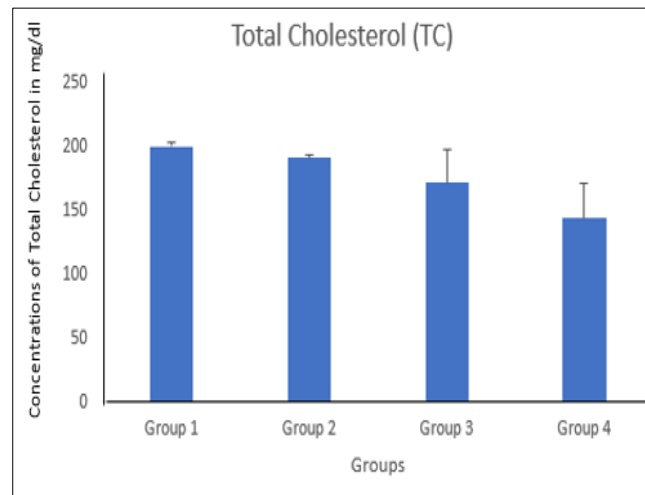


Figure 2 Effect of ethanol extract of *A. sativum* on total cholesterol concentration of male Wistar rats.

Legends: Group 1: Normal control received normal saline; Group 2: Received 200mg/kg body weight of ethanol extract of *A. sativum*; Group 3: Received 400mg/kg body weight of ethanol extract of *A. sativum*; Group 4: Received 1000mg/kg body weight of ethanol extract of *A. sativum*

3.6. Effect of Ethanol Extract of *A. sativum* on HDL Concentration of Male Wistar Rats

Figure 3 below shows that the administration of the extract led to a significant ($p < 0.05$) increase in the concentration of high density lipoprotein (HDL) of the treatment groups (2, 3, 4) compared to the normal control (group 1).

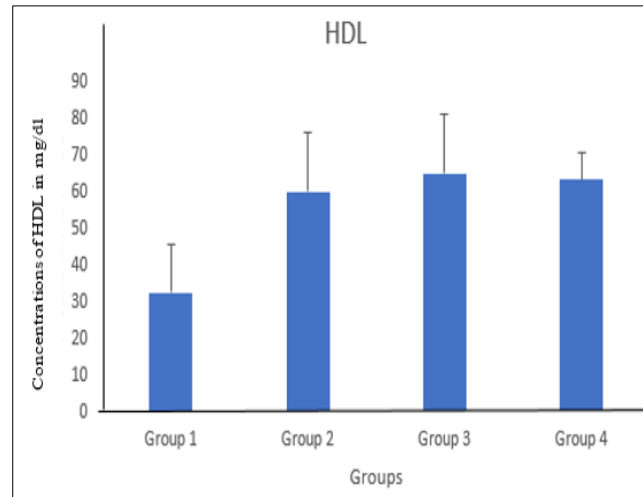


Figure 3 Effect of ethanol extract of *A. sativum* on HDL concentration of normal Wistar rats.

Legends: Group 1: Normal control received normal saline; Group 2: Received 200mg/kg body weight of ethanol extract of *A. sativum*; Group 3: Received 400mg/kg body weight of ethanol extract of *A. sativum*; Group 4: Received 1000mg/kg body weight of ethanol extract of *A. sativum*

3.7. Effect of Ethanol Extract of *A. sativum* on Triglycerides Concentration of Male Wistar Rats

From figure 4 below, the administration of the extract led to a significant ($p < 0.05$) reduction in the concentration of triglyceride of groups 2, 3 and 4 compared to group 1 (normal control).

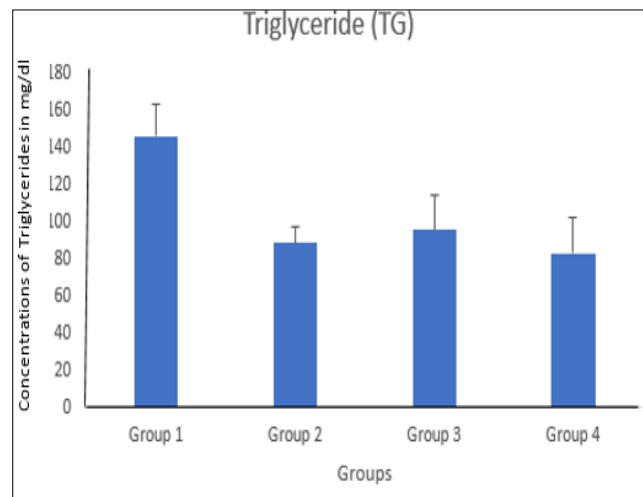


Figure 4 Effect of ethanol extract of *A. sativum* on triglyceride concentration of male Wistar rats.

Legends: Group 1: Normal control received normal saline; Group 2: Received 200mg/kg body weight of ethanol extract of *A. sativum*; Group 3: Received 400mg/kg body weight of ethanol extract of *A. sativum*; Group 4: Received 1000mg/kg body weight of ethanol extract of *A. sativum*

3.8. Effect of Ethanol Extract of *A. sativum* on Low Density Lipoprotein Concentration of Male Wistar Rats

The concentration of low density lipoprotein (LDL) of the treatment groups (2, 3 and 4) were found to be significantly ($p < 0.05$) lower in a dose-dependent manner compared to normal control (group 1) as shown in figure 5 below.

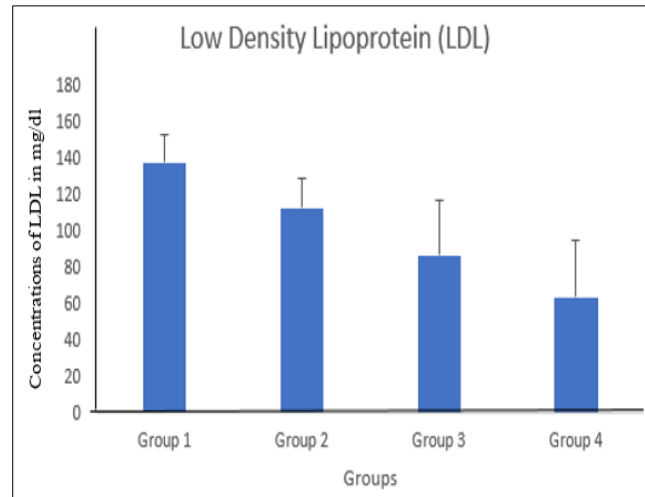


Figure 5 Effect of ethanol extract of *A. sativum* on LDL concentration of male Wistar rats

Legends: Group 1: Normal control received normal saline; Group 2: Received 200mg/kg body weight of ethanol extract of *A. sativum*; Group 3: Received 400mg/kg body weight of ethanol extract of *A. sativum*; Group 4: Received 1000mg/kg body weight of ethanol extract of *A. sativum*

4. Discussion

In this study, the qualitative phytochemical screening of *A. sativum* indicated the presence of saponins, tannins, flavonoids, phenols, terpenoids and alkaloids which is in accordance with the findings of Ali and Ibrahim [12] and in contrast to the findings of Fadiji [13], whose work indicated the absence of saponins, phenolics and tannins: whereas the findings of this research also indicated the absence of glycosides and anthraquinones as opposed to the works of Ali and Ibrahim [12] as well as Fadiji [13], respectively whose works indicated the presence of the two.

The result of this study which showed that fourteen days administration of ethanol extract of *A. sativum* significantly lowered the blood glucose concentrations of the treated groups (groups 2, 3 and 4) compared to normal control (group 1) is in conformity with the findings of Hamid *et al.* [28], which stated that garlic exerted hypoglycemic effect in the livers of type-1 diabetic rats; and that of Thomson *et al.* [29] which also stated that treatment with aged garlic extract positively reversed the indicators of diabetes in streptozotocin (STZ)-induced diabetic rats as well as that of Otunola and Afolayan [30], which equally stated that aqueous extract of a combination of garlic, ginger and cayenne pepper has a strong hypoglycemic potential in alloxan-induced diabetic rats. The hypoglycemic effect exerted by *A. sativum* could be as a result of the presence of flavonoids, alkaloids, terpenoids and saponins in the ethanol extract of *A. sativum* because these phytochemicals are said to exert hypoglycemic effect by acting mainly on the inhibition of intestinal glucose absorption and repair of insulin responses and these effects on the beta pancreas result in stimulation of insulin secretion from the cells [31, 32].

Furthermore, the result of this study showed that after the 14 days administration of the ethanol extract of *A. sativum*, a significant decrease in total cholesterol (TC), low density lipoprotein (LDL) levels and significant elevation of high density lipoprotein (HDL) level of the treated groups (groups 2, 3 and 4) compared to the control group were observed which is in accordance with the findings of Siddiqui *et al.* [33], in a cross sectional study which stated that the intake of garlic (300mg/day, 8 weeks) was shown to reduce the levels of cholesterol and low density lipoprotein and elevate the levels of high density lipoprotein in patients with diabetic dyslipidemia. In addition, the result of this study also demonstrated a significant decrease in the triglycerides of treated groups (groups 2, 3 and 4) relative to the control group (group 1) which contravenes the findings of Siddiqui *et al.*[33], that *A. sativum* has no effect on the level of triglycerides in patients with diabetic dyslipidemia. The observed hypolipidemic effects of ethanol extract of *A. sativum* could be due to the presence of phytosterols which are shown to exert hypolipidemic effect by suppression of cholesterol absorption [34, 35], because phytosterols are known to possess structural similarity with cholesterol and therefore compete with cholesterol for absorption in the gastrointestinal tract [36]. Due to their higher affinity for micelle formation than cholesterol, phytosterols possess a higher level of absorption by the intestine than dietary cholesterol by mass action and consequently, cholesterol will be precipitated out into the lumen, thereby lowering the intestinal solubility of cholesterol and hydrolysis of cholesterol esters [37]. The hypolipidemic effects of ethanol extract of *A. sativum* could also be as a result of the high content of sulfur compounds in garlic which confers on it its antioxidant

properties [38], and this antioxidant effect of garlic offers protection against lipid peroxidation [39] and cholesterol fraction (LDL) oxidation, a precursor for the development of atherosclerotic disease [40].

Conclusion

The ethanol extract of *A. sativum* was composed of saponins, tannins, flavonoids, phenols, phlobatanin, alkaloids, terpenoids, and phytosterols but not anthraquinones, glycosides and steroids. Also it can be concluded from the experimental findings that the ethanol extract of *A. sativum* is safe and possesses a hypoglycemic effect on the blood glucose concentration of normal male Wistar rats and can be used in the control of diabetes mellitus. Furthermore, the hypolipidemic effect observed in this study indicates that consumption of *A. sativum* can aid in the prevention of cardiovascular diseases as well as coronary heart disease.

Compliance with ethical standards

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Disclosure of Conflict of interest

Authors declare no competing interests.

Statement of ethical approval

This research was approved by The Research and Ethical Review Committee of Legacy University Okija, Anambra State Nigeria, with the approval number: LUO/FNAS/EC/0001.

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