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# Effect of garlic juice administration on the lipid profile of male albino rats fed with high cholesterol diet

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**Abstract**
Hypercholesterolemia is a lipoprotein metabolic disorder with raisedserum concentration of low-density lipoprotein (LDL) and total serum cholesterol as its characteristic disorders. These disorders are well known risk factors in the pathogenesis of atherosclerosis. Recently, herbal therapies, including garlic extracts has been proposed as effective regimen for a number of metabolic disorders. Garlic extract has been proposed as therapy for lowering serum cholesterol level thereby contributingto improved cardiovascular health. It is proposed to be effective in the control of hypercholesterolemia due to its rich bioactive ingredients. In this study, the effect of garlic on the lipid profile of male albino rats fed with high cholesterol diet using standard methods were examined. Twenty-four male albino rats were divided into four groups with six rats each. Group 1 was fed with normal diet only, group 2 was fed with normal diet and administered 1ml of garlic juice daily for 21 days, group 3 was fed with high cholesterol diet (HCD) enriched with 30% soybean and 2% cholesterol only for 21 days and group 4 was fed with the same high cholesterol diet but administered 1ml garlic juice daily throughout the 21 days period of the experiment The administration of the garlic juice was done orally with the use of gavage tube. The rats were sacrificed under anesthesia and blood samples were collected and assayed for plasma lipid profile parameters using the spectrophotometric method. The results showed that there was significant ($p$<0.05) decrease in plasma concentration of total cholesterol and triglyceride and a significant ($p$<0.05) increase in High Density Lipoprotein (HDL) cholesterol for the garlic treated groups respectively when compared with the untreated groups. There was no significant difference ($p$>0.05) in the concentration of the Low-Density Lipoprotein (LDL) cholesterol although lower in the treated groups. Results from this work suggests that the administration of garlic juice exerted antilipidemic effect in the albino rats and useful in preventing cardiovascular diseases that may occur as a result of increase in plasma lipid.

**Keywords:** garlic juice, lipid profile, high cholesterol diet, wistar albino rats

## Introduction
Cardiovascular disease is reported as one of the primary causes of death worldwide and accounts for a significant fraction of untimely death [1]. It includes coronary heart diseases (CHD) such as myocardial infarction and anginia. Intricate and multifactorial etiologies that are strongly attributable to cardiovascular diseases includes but not limited to hypercholesterolemia, hypertension, elevated platelet aggregation, increased blood clotting time and reduction in fibrinolytic activities of the blood [2]. Increased serum cholesterol (hypercholesterolemia) have been implicated in Atherosclerosis [3], which is a lipoprotein metabolic disorder that has raised serum concentration of low-density lipoprotein (LDL) and total serum cholesterol as its characteristic disorder [4].Hypercholesterolemia is a malady that poses a significant threat to various nations and also to the health professionals at large because of the massive mortality rate recorded from ischemic heart disease (IHD) [5].Elevated total serum cholesterol and low-density lipoprotein (LDL) level are well known risk factors in the pathogenesis of atherosclerosis [6].

Over the years, clinical findings have been made on extractions from most of the traditional medicinal plants and many were found to possess potent antilipidemic effects hence they were recognized as antilipidemic agents [7].Amongst these plants is Garlic (*Allium sativum Linn*). Garlic (*Allium sativum*) is a bulbous perennial plant with a powerful aroma and pungent taste and for over 5,000 years it has been employed as a flavoring agent, condiment with medicinal purposes [8] available commercially in the form of garlic extract, oil macerate, garlic essential oil and garlic powder. It is mostly explored as therapy for lowering blood pressure, serum cholesterol level and improving fibrinolytic activities of the blood thereby contributing immensely to improved cardiovascular health [9]. Several organosulfur compounds, trace elements like selenium, calcium, magnesium, potassium, zinc and iron and compounds of phenolic and steroidal origin, with carbohydrates, proteins and fiber are the commonly known contents of fresh garlic [10, 11]. It is also a good source of some vitamins like vitamin C, vitamin

A, vitamin $B_6$ and $B_{12}$ [12]. More than 70 fatty acids have been discovered in Garlic, with linoleic (46-53%), palmitic (20-23%), Oleic (4-13%), and α-linolenic (3-7%) acids being the most abundant, giving rise to 80% of the total lipids [13]. The active content of Garlic *(Allium sativum)* is divided into two main groups on the basis of the solubility of their contents. The first group is the lipid-soluble allyl sulfur compounds such as diallyl disulfide (DADS) and diallyl trisulfide (DATS) and the second group is the water-soluble compounds g-glutamyl S-allylcysteine (SAC) group such as SAC and S-allylmercaptocysteine (SAMC) [14]. The metabolism of allicin the major allylcysteine sulfoxide to allicin is catalyzed by allicinase when the bulb is chopped, cut or squeezed. A wide variety of therapeutic agents in modern medicine are available for the treatment of hyperlipidemia. However, most hypolipidemic drugs cause potentially harmful side effects including digestive disturbances, nausea and vomiting [15].The World Health Organization endorsed the assessment of plant's efficacy in some complications where there is dearth of safe and modern drugs [16]. This conduces to a high demand of investigations on antilipidemic plants of naturally origin which produces little or no side effects to cardiovascular disease. This study is intended to evaluate the effect of oral administration of garlic juice on the lipid profile of male albino rats fed with high cholesterol diet.

## 2. Materials and Methods

### 2.1 Preparation of Garlic Juice

Fresh Garlic (*Allium sativum* L.) bulbs were purchased from Mile 3 market, Diobu, Port Harcourt, Rivers State, Nigeria. The bulbs were peeled, washed with several changes of water, and chopped into small pieces. One hundred grams of the chopped garlic were blended with a blending machine with the addition of 250ml of distilled water. The slurry produced was then compressed and sieved through a fine clothe (sieve bag) according to El-Gamal & El-Gazzarmode of preparation [5] and the filtrate was immediately refrigerated at 4 ºC until used as a garlic juice.

### 2.2 Preparation of the High Cholesterol Diet

The design of the test meal was a modification of the Matos *et al*. [17] dietary models for inducing hypercholesterolemia in rats. The diet is composed of 68% grower (chicken) marsh, 30% soybean oil and 2% cholesterol. It was compounded by dissolving 20g of cholesterol in 300g of soybean oil, stirring thoroughly and mixing it evenly with 680g of grower chicken marsh to make up 1000 grams of the high cholesterol diet. The crude fat content of both the normal and high cholesterol diets fed to the rats were determined using Soxhlet Extraction method.

### 2.3 Animals

A total of twenty-four healthy male albino rats weighing approximately 120g were purchased from the Department of Animal and Environmental Biology, Faculty of Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria. The rats were caged in an iron cage with plastic tray at the base for waste retrieval and with provisions to hold feeds and water which were changed daily and the cages cleaned on a daily basis. These cages were well-ventilated with uniform temperature and the rats were acclimatized for two weeks. The animals had free access to food (grower chicken marsh) which was gotten from Top Feed Company, Eastern Premier Feed Mill Ltd, Aba, Abia State, Nigeria and water. They were all handled in accordance with international guidelines for Care and Use of Laboratory Animals according to the conditions and standards outlined by the National Academy of Science [18, 19].

### 2.4 Experimental Design and Treatment of Animals

The rats were divided into four groups (n-6 each) and fed with different dietary regimens. The first group, which is the negative control group had rats that were fed with grower marsh and water only. The second group had rats fed with grower marsh and water with administration of 1ml of garlic juice by orogastric gavage tube daily. The third group were the positive control group having rats fed with the constituted high cholesterol diet (HCD) and water only, while the final group were fed with high cholesterol diet, water and then administered 1ml of garlic juice by orogastric gavage tube daily, throughout the 21 days of the experiment.

### 2.5 Sacrifice of Animals

Twenty-four (24) hours of fasting after the last administration of garlic juice, the rats were anaesthetized with chloroform and sacrificed. 5ml of blood sample was collected from the jugular vein into lithium heparin bottles with needle and syringe. The blood samples were spun and the plasma collected into plain tubes for estimation of the lipid profile parameters.

### 2.6 Methods

#### 2.6.1 Biochemical Determination

Total cholesterol was estimated by an enzymatic reaction according to Allain *et al.* [20]. HDL cholesterol was estimated as for total cholesterol after precipitation of the other lipoproteins. Triglyceride was also estimated by enzymatic reaction of Buccola& David [21] and the LDL-cholesterol was calculated using the formula of Friedwald *et al.* [22]. LDL-cholesterol (mg/dl) =Total cholesterol (mg/dl)-(HDLC (mg/dl) +TG/5) (mg/dl)

### 2.7 Quality Control Measures

External quality control sera were assayed alongside the test samples during the analyses. Standard operating procedures and good laboratory practices were duly adhered to while carrying out the analysis.

### 2.8 Ethical Approval

All experiments have been examined and approved by the Rivers State University research/ethics committee following the guideline and principles of laboratory animal care and usage for experimental purposes.

### 2.9 Statistical Analysis

All data obtained from the result of the experiment were expressed as mean ± standard deviation (SD). Analysis of Variance (ANOVA) and Tukey's Multiple Comparison Test were used to compare the significant differences between mean values in each group using the Graph Pad Prism, version 5.03 statistical package. All values of results were considered significant at $p<0.05$

## 3. Results

The results for total cholesterol (TC), triglyceride (TG), High Density Lipoprotein Cholesterol (HDL-C) and Low-density Lipoprotein (LDL-C) are shown on table 1.

**Table 1:** Mean ± SD of Lipid Profile parameters of Rats in the experimental groups.

| Groups | TC (mmol/l) | | TG (mmol/l) | | HDL-C (mmol/l) | | LDL-C (mmol/l) |
|---|---|---|---|---|---|---|---|
| Group 1 (ND) | 2.20 | ± 0.21$^{a}$ | 0.84 | ± 0.22$^{a}$ | 0.85 | ± 0.15$^{a}$ | 0.86 ± 0.29 |
| Group 2 (NDG) | 1.85 | ± 0.14$^{ab}$ | 0.75 | ± 0.18$^{b}$ | 0.90 | ± 0.29$^{ab}$ | 0.78 ± 0.21 |
| Group 3 (HCD) | 2.28 | ± 0.08$^{a}$ | 1.46 | ± 0.35$^{abc}$ | 0.73 | ± 0.28$^{bc}$ | 0.90 ± 0.17 |
| Group 4 (HCDG) | 2.10 | ± 0.19$^{ab}$ | 1.04 | ± 0.19$^{c}$ | 0.94 | ± 0.26$^{ac}$ | 0.72 ± 0.19 |
| F-value | 5.043 | | 10.08 | | 2.6329 | | 0.2392 |
| p-value | 0.0097 | | 0.0003 | | 0.0028 | | 0.8679 |
| Remark | S | | S | | S | | NS |

**Key:** Mean with same superscripts are significantly different ($p$<0.05) from each other in the same column. S – Significant, NS – not significant, ND – normal diet, NDG – normal diet + 1ml garlic juice, HCD – high cholesterol diet, HCDG – high cholesterol diet + 1ml garlic juice, n= 6

## 4. Discussions

Results obtained from this study show significant reduction in the values of total cholesterol, triglyceride and increased High-Density Lipoprotein Cholesterol (HDL-C) levels for the various groups while there was no significant difference in the mean value of the Low-Density Lipoprotein Cholesterol (LDL-C) level. The total cholesterol levels in the treated rats (group 2 and group 4) show significant decrease as seen in Table 1 when compared with group I (ND) which is the negative control, fed with normal diet and water, The observations in the test animals implies that garlic had an effect of reducing the cholesterol level in the treated group that was administered garlic and this is in corroboration with a similar study by Mohammadi and Oshaghi [23-24]. This may be explained in the efficacy of allicin in garlic which acts by inhibiting the rate limiting enzyme (3-Hydroxylmethylglutaryl-CoA reductase (HMG-CoA), in the biosynthetic pathway of cholesterol as reported by [5], thereby halting cholesterol biosynthesis. Also, there was a significant increase (P<0.05) in the total cholesterol level in group 3 compared to group 1 as reflected in the results. This agrees with the work done by Matos *et al.* [17] which states that cholesterol rich diet increases the total cholesterol concentration in the plasma. Triglyceride levels in treated rats across the various groups showed there was obvious significant decrease. This may be adduced to the stimulation of lipoprotein lipase on triglycerides [25] which helps in the regulation of triglyceride level by its hydrolytic actions on the core triglycerides in the triglyceride-rich lipoproteins which includes the chylomicrons and the very-low-density lipoproteins (VLDLs) thereby producing glycerol and free fatty acids (FFAs) which the tissues utilizes. Also Lipoprotein lipase fastens the uptake of lipoprotein particles into tissues by attaching them to the vessel wall serving as a ligand for the lipoprotein receptors [26] This is also in line with a study carried out by Thomson *et al.* [27] and a review by Chan *et al.* [28] with similar results showing reduced values of triglycerides in the treated groups with garlic. On the other hand, triglyceride value in group 3 (HCD) was significantly increased when compared with the triglyceride value in group 1 (ND) in agreement with a similar higher level as been reported by Getz and Reardon [29] which reported that cholesterol enriched diet triggers an increase of triglyceride in the blood. The results for the High-Density Lipoprotein Cholesterol (HDL) shows that there was no significant difference ($P$>0.05) in the concentration of group 3 rats fed with high cholesterol diet compared with the group 1 rats fed with normal diet. This is in agreement with the study [5] with similar result though HDL concentration in group 4 was significantly higher than that in the group 3 fed with a high cholesterol diet as seen in this study. This reduction in HDL level was also reported in a study by [7]. It is important to note that increases in HDL-C always occur with cholesterol intake even when LDL-C is not increased [30] but that was not seen in this study where the HDL concentration was lower in the rat fed with high cholesterol diet. There was also a remarkable increase in the concentration of the HDL cholesterol in the garlic treated rats compared to the concentration in the untreated rats. This corroborates the work carried out by Ali *et al.* [31] and Santhosha *et al.* [32] who both observed a higher concentration of serum High Density Lipoprotein (HDL) incholesterol fed rats treated with garlic. This may imply that garlic juice could directly alleviate atherosclerosis by its ability to suppress intracellular lipid accumulation, inhibit lipogenic activities and suppressarterial cell lipid contents [33, 34] hence boosting HDL cholesterol concentration show ever this work is in disagreement with a review by Sanjay & Subir [35] who reported that garlic had no effect on the HDL cholesterol level of rats fed with hypercholesteremic diet though it reduce the level of total cholesterol, triglyceride and low density lipoprotein. Low density lipoprotein values were not statistically significant in this study among the various group however the values in the treated groups (group 2 and 4) were reduced compared to the control (group 1). This observation may be adduce to the ability of garlic to retard arteriosclerosis progression and the suppression of LDL oxidation [36]. Allicin in garlic is also seen to act on the uptake of LDL as described by Gonen [37].

## 5. Conclusion

Albino male rats fed on high cholesterol diet and treated with garlic juice showed significantly better improved lipid profile status than that of the normal control ones as evident in lower serum triglycerides, cholesterol and low-density lipoprotein (LDL), with a higher serum high density lipoprotein (HDL) level suggesting that garlic juice has hypolipidaemic effects and its consumption is beneficial to improving cardiovascular health. Hence, recommending garlic supplementation into our routine diet.

## 6. Competing Interests

Authors have declared that no competing interests exist.

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