



The effect of garlic on plasma lipids and lipoproteins in rats fed on high cholesterol enriched diet

Maria Onomhaguan EBESUNUN^{1*}, Omolara Oluwatosin POPOOLA, Emmanuel Oluyemi AGBEDANA², Japheth Madu OLISEKODIAKA³, Jude Analachi ONUEGBU³, Augustine Anayo ONYEAGALA¹

¹*Chemical Pathology Department, University College Hospital, Ibadan, Nigeria*

²*Department of Chemical Pathology College of Medicine, University of Ibadan, Ibadan, Nigeria*

³*Department of Biomedical Science, Ladoke Akintola University, Oshogbo, Nigeria*

Received 4 March 2007

MS/No BKM/2007/015, © 2007 Nigerian Society for Experimental Biology. All rights reserved.

Abstract

Garlic has been reported in some populations to possess hypolipdemic effect in particular on low density lipoprotein cholesterol, thus preventing cardiovascular disease risk. Sixteen male albino rats of seven weeks old were purchased for this study. These were divided into four groups of four per group. Group A was fed on a high cholesterol diet and garlic, group B was fed on high cholesterol diet only, group C was fed on normal diet (controls) and group D was fed on normal diet and garlic. The plasma lipids, lipoproteins and tissues histological appearances were determined using standard procedures. Results showed significant decreased in the mean wet tissue weight of the kidney ($p < 0.001$) in the group fed on high Cholesterol+garlic diet. The plasma LDLC was markedly reduced in the groups fed on high cholesterol diet + garlic as well as normal diet+garlic. The mean plasma HDLC although not statistically significant was higher in these groups. Histological findings showed pronounced atheromatous changes in the coronary artery of rats fed on high cholesterol diet. The consumption of raw garlic has beneficial effect on plasma total cholesterol, and LDLC in rats fed on high cholesterol diet.

.Keywords: Garlic, cholesterol, LDLC, HDLC

*To whom correspondence may be addressed,

E-mail onoebe@yahoo.com, **Tel.** +234- 8055307626, **Fax** (+234)-2-2413545

INTRODUCTION

Previous studies have demonstrated that lowering plasma total cholesterol (TC), low density lipoprotein cholesterol (LDLC) and increasing high density lipoprotein cholesterol (HDL) are beneficial in preventing risk of cardiovascular disease (CVD)^{1,2}. There are divergent views on the effect of garlic on plasma cholesterol concentration and coronary heart disease. Garlic (*Allium sativum*)^{3,4}, is a bulb which belongs to the family liliaceae⁴. The well-known members of this family all belong to the genus "Allium"⁴. Available report has shown that it could be used for any ailments such as parasitic infections, respiratory problems, poor digestion and low energy⁵. Recent studies have reported the ability of garlic to reduce plasma cholesterol in humans⁵⁻⁷. Evidence on the role of garlic in lowering plasma cholesterol in developed populations abounds^{6&8}. Several studies have also reported the antihypertensive effects of garlic^{8,9}.

In this community however, where the consumption of garlic is uncommon, there is little or no information on the role of garlic on plasma lipids and lipoproteins. This study was therefore designed to assess the beneficial effects of consuming raw garlic on plasma lipids and lipoproteins in albino rats fed on high cholesterol diet for four weeks.

MATERIALS AND METHODS

Animals

Sixteen albino rats age seven weeks old were purchased from the University of Ibadan Animal House. These were divided into four groups (ABCD), kept in cages in a well ventilated room and fed *ad libitum* for 4 weeks with free access to clean water. A locally prepared commercial diet composed mainly of carbohydrate, protein, mineral salts and minimally of fat, antioxidants and antibiotics was purchased from Ladokun Feeds, Ibadan, Nigeria,. To this diet was added 1.75g raw garlic/kg body weight (Table 1) and the mixture was homogenized in a mortar and formed into pellet with clean water. The pellets were sun dried immediately for several days.

Table 1: Feed composition

Ingredients(g)	Groups			
	A	B	C	D
Pellet	900	900	900	900
Cholesterol	8.75	8.75	-	-
Raw garlic	1.75	-	-	1.75

A =High cholesterol diet and garlic; B =High cholesterol diet only Group; C =Normal diet only; D = Normal diet and garlic

Blood sample and tissue collection

Animals were sacrificed under light chloroform anesthesia after 4 weeks of feeding and overnight fasting of 10-14 hours. Blood samples were collected by cardiac puncture into EDTA-bottles for lipid and lipoprotein profiles. The plasma samples were separated from the cells by centrifugation. The plasma samples stored frozen until analyzed. The heart, kidney, liver and spleen organs of each animal were also excised, blotted on a filter paper to remove blood and the wet weights were measured. These organs were fixed in 10% formalin for histological examinations

Biochemical determinations

Total cholesterol was estimated by an enzymatic reaction¹⁰. HDL cholesterol was estimated as for total cholesterol after precipitation of the other lipoproteins. Triglyceride was also estimated by enzymatic reaction¹¹ and the LDL-cholesterol was calculated using the formula of Friedwald et al,¹²

$$LDL\text{-cholesterol (mg/dl)} = Total\ cholesterol\ (mg/dl) - (HDL\ C\ (mg/dl) + TG/5)(mg/dl)$$

Histological examination

Blood was blotted from each organ with a filter paper and weighed using weighing balance. The organs were then fixed in 10% formalin for a minimum of 48 hours. These were then processed on the automatic tissue processor through various grades of alcohol and xylene for a specified period. The tissues were impregnated and embedded in molten wax. These were allowed to solidify in wax at room temperature and immersed in water to harden. The tissues were then cut on Rotary Microtome to 5 microns thickness

using disposable microtome knives. Tissues were floated out at 56°C in floating out water bath onto a slide on which 20% bovine albumin solution had been previously smeared. The glass slides were allowed to dry on hot plate and stained for nuclei and cytoplasmic structures using haematoxylin and eosin stains and thereafter examined for atheromatous changes

Statistical analysis

All results were subjected to statistical analysis using Computer Software Statistical Package of

Social Sciences (SPSS). The values were expressed as Mean ± Standard error of mean (X ± S.E). Differences within groups were assessed using analysis of variance. Student “t” test was used to assess the differences between two groups and these were regarded as significant at p<0.05.

RESULTS

Tissue weight

Table 2 shows tissue weight (mean±S.E) in all dietary groups and controls at sacrifice.

Table 2: Tissue weight (mean+SE) in all dietary groups at sacrifice

VARIABLE	GROUPS				F-VALUE	P-VALUE
	A	B	C	D		
HEART(g)	1.25±0.15	1.65±0.17	1.05±0.17	1.03±0.03	4.19	<0.05
SPLEEN(g)	2.80±0.36	3.80±0.50	3.00±0.50	2.03±0.03	4.76	<0.01
LIVER (g)	9.03±0.90	8.43±0.45	4.93±0.45	10.03±0.14	1.33	ns
KIDNEY(g)	0.63±0.05	1.45±0.93	0.73±0.12	0.53±0.05	9.73	<0.001

ns=Not significant; A=High cholesterol diet and garlic; B=High cholesterol diet only Group; C=Normal diet only; D = Normal diet and garlic

Table 3: Plasma lipids and lipoproteins (mean ± S.E) in all the groups

VARIABLE	A	B	C	D	F	P
T.C (mg/dl)	72 ± 7.56	110±16.89	91 ± 3.48	74 ± 3.3	3.50	<0.01
TG(mg/dl)	80±13.35	144 ± 2.69	109± 22.50	112±28.1	1.82	ns
HDLC(mg/dl)	52 ± 5.22	41 ± 4.31	44±.1.32	50 ± 4.87	1.50	ns
LDLC(mg/dl)	16 ± 4.51	40 ± 7.86	19 ± 3.90	16±3.48	2.58	ns

A=High cholesterol diet and garlic; B=High cholesterol diet only; C=Normal diet only; D=Normal diet and garlic; ns = not significant

Table 4: Statistical comparison of the different groups

	A Vs B	A Vs C	A Vs D	B Vs C	B Vs D	C Vs D
TC	<0.05	ns	ns	ns	<0.01	<0.01
TG	<0.05	ns	ns	ns	ns	ns
HDL-C	ns	ns	ns	ns	ns	ns
LDL-C	ns	ns	ns	ns	<0.01	ns

A=High cholesterol diet and garlic; B=High cholesterol diet only; C=Normal diet only; D=Normal diet and garlic; ns = non significant

Within group analysis showed significant variations in the mean wet heart ($p < 0.05$), spleen ($p < 0.01$) and the kidney ($p < 0.001$) tissue weights in the experimental animals with the group fed on high cholesterol diet showing the highest mean wet weights in the spleen heart and kidney tissues.

Plasma lipids and lipoproteins

The mean plasma total cholesterol (TC) was significantly different in all the groups ($p < 0.01$) while the plasma HDLC, LDLC, as well as triglyceride showed no significant changes within the groups (Table 3). Table 4 shows the statistical comparison of the different groups. There was significant increase in the plasma TC of group B when compared with the corresponding values in groups A ($p < 0.05$) and D ($p < 0.01$) respectively. The plasma T.G level in group A was significantly reduced ($p < 0.05$) when compared with the corresponding value in group B. The plasma LDLC was markedly elevated in group B ($p < 0.01$) when compared with the corresponding value in group D. No significant changes were observed in the plasma HDLC in all the groups.

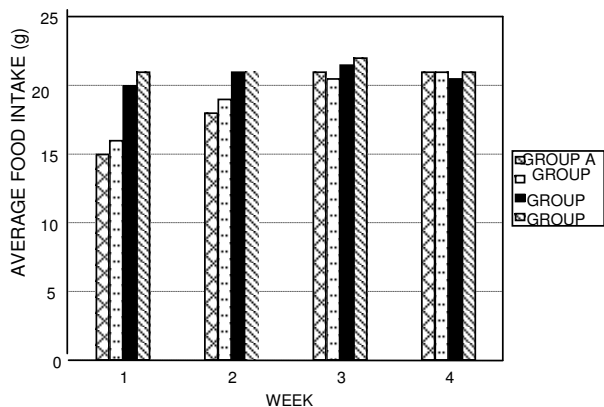


Figure 1: Average food intake in the different dietary groups.

- A= High cholesterol diet and garlic
- B= High cholesterol diet only
- C= Normal diet only
- D= Normal diet and garlic

As shown in Figure 1, the animals on high cholesterol diet showed a reduced food intake, probably due to the fact that the diet was unfamiliar. Thereafter there were consistent increases in food intake from the 2nd week in all

the different groups. The body weights gained in the first week in all the four groups were similar (Figure 2). However, from the 2nd week, there was exponential increased in the body weight in the group fed on high cholesterol diet only.

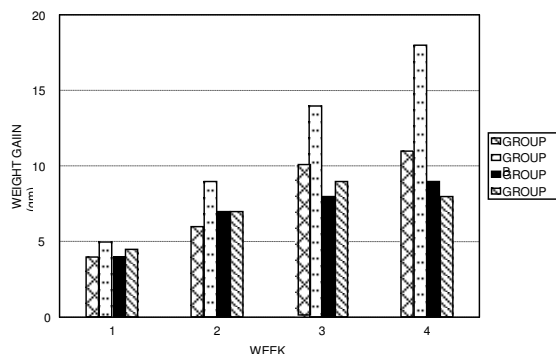


Figure 2: Body weight gain (gm/rat) in rats fed with different diets.

- A = High cholesterol diet and garlic
- B = High cholesterol diet only
- C = Normal diet only
- D = Normal diet and garlic

Histological investigations

The haematoxylin and eosin stained coronary artery sections from the rats fed on high cholesterol diet only and high cholesterol diet + raw garlic showed varied degree of atheromatous changes in the two groups. The group fed on high cholesterol diet only showed a higher degree of atheromatous changes and this was more pronounced (figures not shown).

DISCUSSION

The diet used in this study differs from that of other studies which used a 2% high cholesterol diet¹³. The high cholesterol diet was constituted based on 2.5g/kg body weight of animal suggested to be sufficient to induce hypercholesterolemia as reported in an earlier study¹³.

The two groups on normal diet tended to consume more food than the groups on high cholesterol diet because they were more familiar with the commercial diet. The experimental rats on high cholesterol diet, however, adjusted to the new diet with time and the total food intake

in each dietary group was adequate as evidenced from the growth rate of the different groups of animals. But the animals on high cholesterol diet showed exponential increase in body weight gain, thus suggesting that consumption of high cholesterol diet may be a contributory factor to body weight changes. The reduction in body weight in the group fed on normal diet + garlic may be suggesting that consumption of raw garlic could reduce body weight.

At sacrifice, there were statistical significant increases in the mean wet tissue weights of the heart, kidney and spleen in the animals fed on high cholesterol diet only when compared with the groups on normal diet, normal diet + garlic and high cholesterol diet + garlic. The reason for this observation is largely unknown since no available reports have suggested any significant specific relationship between organ weight and the type of dietary fats consumed. It could be speculated that the increase in the wet tissue weight in these organs, was probably due to the absence of garlic in the diet coupled with the high cholesterol content. However, study on rats fed on palm kernel oil for twelve weeks showed a similar finding¹⁴.

In the present study, within group analysis showed a significant increase in the plasma total cholesterol level in the groups fed on high cholesterol diet. There were however no significant changes on plasma TG, HDLC and LDLC when compared with the animals on normal diet. Reduced plasma TC, and LDLC and increased mean plasma HDLC were obtained in rats fed on high cholesterol diet + garlic and normal diet + garlic, these reductions and increases were not statistically significant, this could be attributed to the short duration of feeding. An earlier study^{7,8} showed that garlic when administered raw caused significant alterations in total cholesterol in subjects with raised plasma cholesterol in their subjects after a long period of feeding.

Although no significant difference was observed in the HDL cholesterol level between studied groups, the groups that had garlic incorporated into the diet had the highest mean plasma HDLC level, this increase was however not significant. The most probable reason for the lack of significance in the plasma HDLC could be attributed to the fact that garlic does not interfere with HDLC synthesis but its

mode of action is by inhibiting cholesterol biosynthesis. This is through the inhibition of HMG-CoA reductase, the rate-limiting enzyme that mediates the first step in cholesterol biosynthesis.

It could also be speculated that the short period of feeding may not have allowed for a notable significant changes in the mean plasma HDLC in these groups of animal fed on diet containing garlic.

On the other hand the plasma LDLC was significantly decreased in the groups of animals whose diet had garlic incorporated. This perhaps supports the ability of garlic to reduce LDL cholesterol concentration. Evidence from available studies showed that garlic can affect vasculature by improving aortic elasticity as well as retardation of atherosclerosis progression^{6-9,15,16,18} perhaps through increase excretion of LDL-cholesterol.

As evident from this study, garlic also decreases plasma triglyceride level in the rats fed on garlic containing diets, most likely through the stimulation of lipase. Available reports from a similar study indicated that garlic is a potential stimulant of lipase^{7,15,17}. The results of this study suggest that garlic has hypolipidemic effect. Available report shows that garlic consumption is beneficial in the prevention of cardiovascular disease^{2,5,7,15,17}.

Histological examinations of the different groups revealed pronounced atheromatous changes in the coronary arteries of all the rats fed on high cholesterol diet. Although the rats fed on high cholesterol diet and garlic also showed atheromatous changes but the degree of changes was not as pronounced as the ones observed in the group on high cholesterol diet only. This in part suggests that garlic when consumed raw can cause remarkable delay/regression in atherosclerosis development.

The rats used in the present study were matured animals and are therefore more

likely to be prone to the development of atherosclerosis.

The results of this study suggest that garlic when consumed raw could reduce the plasma level of circulating LDL cholesterol and therefore reduces the risk of developing hypercholesterolemia in rats. This could also be applicable in human individual and thus preventing premature atherosclerotic vascular disease. Further study on longer period of feeding is highly warranted before firm conclusion can be made.

REFERENCE

1. **Bordia, A. (1981)** Effect of garlic on blood lipids in patients with coronary heart patients. *Am. J. Clin Nutr.* **34**: 2100-2103.
2. **Agbedana, E. O. and Akanji, A. O. (1988)** Lipid profiles and vascular disease in type 2 (non-insulin dependent) Nigerian Diabetic Patients. *Trop. Geogr. Med.* **40**:88-92.
3. **Chutani, S.K. and Bordia, A.G. (1981)** The effect of fried versus raw garlic on fibrolytic activity in man. *Atherosclerosis* **38**:417-21
4. **Kenderler B.S. (1985)** Garlic (*Allium sativum*) and onion (*Allium cepa*): A review of their relationship to cardiovascular disease *Prev Med.* **16**:670-685
5. **Ernst, E. (1987)** Cardiovascular effects of garlic: A review. *Pharmatherapeutica* **5**:85-89.
6. **Jain A.K., Vegas R, Gotzkaowska S M. Mahon FG (1993)** Can garlic reduce levels of serum lipids? A controlled clinical study. *Am J. Med.* **94**:632-635
7. **Watkins R.W. (2002)** Herbal Therapeutics, the Top 12 remedies. *Annals Internal Med.* **133**: 420-429
8. **Rotzch, W. J. and Azneim F. (1992)** Postprandial lipemia under treatment with *Allium sativum* controlled double blind study in healthy volunteers with reduced HDL2-cholesterols. *Arzneim Forsch* **42**: 1223-1227.
9. **Sainani, G.S. Desai D. B., Gorthan, N. H., Sainani, P. G., Natu. S. M. and Pise, D. V. (1979)** Effect of dietary garlic and onion on serum lipid profile in the Jain community. *Ind. J. Med. Res.* **69**: 776-780.
10. **Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W. and Fu, P.C. (1974)** Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20**: 470-3
11. **Buccolo, G. and David, H. (1973)** Quantitative determination of serum triglycerides by the use of Enzymes. *Clin. Chem.* **19**:476-482.
12. **Friedwald, T.W, Fredrickson, D.S. and Levy, R.J. (1972)** LDL cholesterol estimation. *Clin. Chem.* **18**:499-501.
13. **Sanjay, K. B. and Subir, K. Maulik (2002)** Effect of garlic on cardiovascular disorders: a review. *Nutr J.* **1**: 4.
14. **Ebesunun, M.O. Agbedana, E. O. and Taylor, G.O. (2003)** Selective cholesterol deposition in the kidney tissue of rats fed palm kernel oil diet. *Afr. J. Med. Med. Sci.* **32**:41-47.
15. **Warshafsky, S. Russel, S. K. and Steven, L. S. (1993)** Effect of garlic on total serum cholesterol. *Annals Intern. Med.* **119**:599-605.
16. **Mader, S.H (1990)** Treatment of hyperlipidemia with garlic powder tablets. *Arneim Forsch* **40**:1111-1116.
17. **Lau, B.H., Adetumbi S., M. A and Sanchez A. (1983)** *Allium sativum* (Garlic) and atherosclerosis. A review. *Nutr. Res.* **3**:119-28
18. **Stevinson, C., Gridley, D. S. and Fittler, E. (2000)** Garlic for treating hypercholesterolemia; a mental analysis of randomized clinical trial. *Annals Intern Med.* **133**: 420-429