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Effect of dry garlic powder on plasma lipid profile and enzyme activities in some tissues of hypercholesterolemic rats

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Abstract: The effect of garlic powder at 5% and 10% level on plasma lipid profile was investigated in hypercholesterolemic rats. Male albino rats were fed a diet containing 20% fat and 1% cholesterol for two weeks to provoke hypercholesterolemia. The hypercholesterolemic rats were divided into three groups B, C and D. Group A rats were fed with normal diet, Group B were maintained on the hyper diet, Group C were fed hyper diet +5% garlic powder, Group D were fed hyper diet with +10% garlic powder. They were maintained on this diet for four weeks. Results showed that the plasma total cholesterol and LDL-C was significantly reduced in the treated groups (C and D) compared to the hypercholesterolemic control (Group B), while the HDL-C was significantly increased ($P<0.05$). Also plasma enzyme activities of Aspartate and Alanine Aminotransferases (AST. ALT) and Alkaline Phosphatase (ALP) showed a slight decrease in the treated rats. It is concluded that consumption of dry garlic powder at the level used in this study could be beneficial on the plasma lipid profile in hypercholesterolemia.

Keywords: Hypercholesterolemia, Aminotransferases, Lipid Profile

1. Introduction

Hyperlipidemia is defined as the condition in which the level of plasma lipids primarily cholesterol and triacylglycerol are higher than normal range. Hyperlipidemia is an important risk factor in atherosclerosis which can lead to cardiovascular disease (CVD).

In most African countries, the mortality rate due to CVD is high, likely because of the fact that the population consumes traditional diet rich in carbohydrates and animal fats especially red meat, and relatively low in plant foods.

A large number of modern drugs are available for the treatment of hyperlipidemia with potentially dangerous side effects. Inrecent years, the use of herbs like ginger, garlic,onions and cinnaminns in the management of hypercholesterolemia has been beneficial to health.

Garlic (*Allivum sativa*) has been used over the years, for it's the rapeutic effects such as in the treatment of some cancers and antihypertensive properties(1). Non-pharmacological treatment with garlic preparation wasfound to reduce blood pressure in hypertensive individual (2,3).

In this study we explore the effect of garlic powder at two different levels (5% and 10%) on the plasma lipid profiles and enzyme activities in some organs of hypercholesterolemic rats.

2. Materials and Methods

Dry fruit of garlic (*Allivum sativa*) was bought from the Oja-Oba market in Ado-Ekiti, cleaned, sundried, powdered and stored until required for diet composition. All chemicals used for the study were of analytical grade (ANALAR).

3. Animal Groupings

Twenty four (24) male white albino rats (*Rattus novergicus*) with average weight of about 120g were used for the study. They were obtained from the Department of

Biochemistry, University of Ilorin, Kwara –State, Nigeria. The rats were kept in good conditions and were given normal rat feed and water *ad libitum*. They were randomly divided into four experimental groups (A, B, C and D). Group A served as the normal control fed with standard commercial diet, Group B were fed modified diet containing 20% fat and 1% cholesterol (Table 1) and after establishing hypercholesterolemia, this group was subdivided into Group C and D. Group B was still maintained on the hypercholesterolemic diet. In addition, Group C and D were supplemented with 5% and 10% dry garlic powder (*Allivum sativa*) respectively while the experimental period lasted for four weeks.

4. Preparation of Serum and Tissue Homogenate

The rats were sacrificed at the end of the experimental period and blood was collected from the heart into lithium-heparin bottles. It was centrifuged and the plasma was put into labeled bottles and used fresh.

The liver and kidney were removed quickly, drained of blood and weighed. It was then homogenized in sucrose buffer (0.25M) solution according to (4) and the homogenate kept frozen until required for analysis.

5. Enzyme and Protein Measurements

Alkaline phosphatase (ALP) (E.C.3.1.3.1) activity was assayed using the method of(5) where p-nitrophenyl phosphate was hydrolysed and the absorbance read at 400nm.

Table 1. *Diet composition (g/kg)*

	A	B	C	D
Soyameal	510	510	510	510
Vegetable oil	50	200	200	200
Cholesterol	_	10	10	10
Sucrose	100	100	100	100
Vitamin mineral mix	50	50	50	50
Cellulose	30	30	30	30
Corn starch	260	100	50	-
Garlic powder	_	_	50	100

A – Control group C – Test group 1 (5% Garlic powder (*Allivum sativa*)
B – Hyper control groupD – Test group 2 (10% Garlic powder (*Allivum sativa)*.

Aspartate aminotransferase (AST) (E.C. 2.6.1.1) and alanine aminotransferase (ALT)(E.C 2.6.1.2) activities were determined using appropriate buffer systems by measuring the pyruvate resulting from transamination reactions at 546nm (6).

Protein concentration was measured by the Biuret method (7). All measurements were done using Spectronic 20.

5.1. Assay of Lipid Profile

Total Cholesterol (TC), HDL Cholesterol (HDL-C), LDL-Cholesterol, Triacylglycerol (TG) were estimated from the plasma. Plasmatotal cholesterol was estimated using Randox laboratory kit based on the enzymatic end point method. The HDL-Cholesterol was determined by the method of (8). LDL-Cholesterol was calculated with the Friedweld formula(9).

5.2. Statistical Analysis

The results are expressed as Mean ± standard deviation. Analysis of variance was used to test for differences in the groups. All the values were expressed as mean± standard deviation (SD). Differences were considered to be statistically significant at $P<0.05$.

Table 2. *Plasma lipid profile after treatment with garlic powder.*

	A	B	C	D
TC(mg/dl)	2.9 ± 0.6^a	4.0 ± 0.9^b	2.8 ± 0.1^a	3.1 ± 0.5^a
TG(mg/dl)	0.9 ± 0.1^a	1.3 ± 0.5^b	1.6 ± 0.5^b	1.3 ± 0.5^b
HDL(mg/dl)	1.1 ± 0.05^a	1.4 ± 0.02^b	1.6 ± 0.02^c	1.7 ± 0.01^c
LDL(mg/dl)	1.30 ± 0.2^a	2.0 ± 0.5^b	0.9 ± 0.1^c	0.9 ± 0.1^c
LDL/HDL	0.85	1.43	0.56	0.52
CRI	2.64	2.84	1.75	1.82

Results are mean± SD ($P<0.05$) from 4 observations. Values with the same superscript letter(s) along the same row are not statistically different. LDL - Low density lipoprotein; HDL – High density lipoprotein; TG – Triglycerides; TC – Total cholesterol.

Table 3. *Plasma enzyme activities after treatment(U/L)*

	A	B	C	D
AST	116.3 ± 25.7^a	153.7 ± 10.7^b	99.4 ± 7.0^a	101.6 ± 3.0^a
ALT	21.9 ± 0.8^a	22.3 ± 1.5^a	32.0 ± 6.5^b	30.2 ± 5.0^b
ALP	370.9 ± 78.2^a	367.3 ± 62.1^a	334.7 ± 6.6^b	380.7 ± 54.9^c
AST/ALT	5.31	6.89	3.10	3.36

Results are mean± SD ($P<0.05$) from 4 observations. Values with the same superscript letter(s) along the same row are not significantly different. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

Table 4. *Kidney enzyme activities(U/mgprotein)*

	A	B	C	D
AST	158.1 ± 26.9^a	171.8 ± 22.5^b	165.8 ± 39.8^c	154.2 ± 34.0^c
ALT	37.0 ± 9.5^a	44.8 ± 8.8^b	38.4 ± 13.1^a	40.0 ± 16.1^a
ALP	476.2 ± 17.4^a	521.0 ± 65.4^b	481.4 ± 7.0^a	530.0 ± 8.1^c

Results are mean± SD (P<0.05) from 4 observations. Values with the same superscript letter(s) along the same row are not significantly different. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

Table 5. *Liver enzyme activities(U/mgprotein)*

	A	B	C	D
AST	108.0±8.7[a]	330.1±19.2[b]	303.2±1.2[c]	437.2±15.5[d]
ALT	30.0±7.0[a]	176.0±26.7[b]	105.0±5.3[c]	144.1±4.7[c]
ALP	293.3±10.6[a]	580.0±45.3[b]	446.1±24.9[c]	599.2±22.0[d]

Results are mean± SD (P<0.05) from 4 observations. Values with the same superscript letter(s) along the same row are not significantly different. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

6. Results and Discussion

Table 2.0 shows the plasma lipid profile six weeks post treatment of hypercholesterolemic rats with garlic powder at 5% and 10% level. There was a significant increase (P<0.05) in the total cholesterol (TC) and low density lipoprotein (LDL), of the hypercholesterolemic rats compared with the control, while a significant decrease (P<0.05) was observed in the treated rats compared to the hypercholesterolemic rats. However, a significant increase was observed in the high density lipoprotein (HDL) fraction of the treated rats compared to the hypercholesterolemic rats and significant reduction in the LDL, LDL/HDL, and coronary index (CRI) of the treated rats. The observed increase in plasma TC, and LDL cholesterol in the hypercholesterolemic rats may be as a result of the increased lipid content of the food by 20% and incorporation of 1% cholesterol in the diet.

Elevation of blood cholesterol has been associated with diet, genetic factors and the presence of other diseases such as diabetes and an underactive thyroid (10). The observation made in this study is consistent with previous reports (11,12), High lipid intake is a risk factor for hyperlipidemia, i.e. an excess of fatty substances called lipids largely cholesterol in the blood. It has been reported that high serum abnormal levels of TC and LDL are associated with an increased risk for atherosclerosis (13). Also (14) reported that feeding 2% cholesterol for a period of one month increased serum and tissue lipid levels of rats. However, the rats treated with dry garlic powder at 5% and 10% level had reduced TC and LDL-cholesterol but increased HDL-levels. This agrees with previous findings (15,16)

It had also been reported that garlic constituents can prevent the biosynthesis of cholesterol in the liver by inhibiting HMG CoA reductase which is the rate limiting enzyme in cholesterol biosynthesis and other lipogenic enzymes (17). Also garlic powder preparation has been reported to reduce lipoprotein oxidation susceptibility in vitro and in vivo (18,19). This may be responsible for the observations made in this study.

High HDL-C levels in human blood protects against heart attack since the lipoprotein has been reported to carry cholesterol away from the arteries back to the liver where it is excreted in the bile as free cholesterol or as bile salts following conversion to bile acids (20). The observed increase in HDL is one of the most important criteria of an anti- hypercholesterolemic agent. Several studies have demonstrated that high levels of HDL are associated with a lower incidence of cardiovascular disease (CVD) (21,22). It therefore, means that HDL may play a protective role through reversing cholesterol transport by inhibiting the oxidation of LDL and thus neutralizing the atherogenesis effect of oxidized LDL.

The observation on garlic treated rats (group C & D) made in this study may likely be due to garlic derived substances (which have sulphur containing compounds) which may effectively decrease cholesterol synthesis. Garlic contains, S-allyl-cysteine, vitamin C and selenium which are strong antioxidants (23,24). Several reports have also indicated that the LDL decreasing effect of garlic maybe as a result of its antioxidant compounds including vitamin C and germanium. It plays its role by scavenging free radicals, inhibition of the HMG CoA reductase and hence, biosynthesis of cholesterol.

Garlic extract had been reported to suppress blood lipid level by suppressing lipogenic and cholesterolemic activities (25,26)

The LDL/ HDL ratio is a more predictive atherogenic index the lower the ratio the less atherogenic the lipoprotein profile is thought to be. In this study the hyper control rats had significantly higher ratio compared to the control while the garlic treated (5% & 10%) rats had a significantly lower LDL / HDL ratio compared to the hyper control, this further lends support to the hypocholesterolemic effect of garlic.

Table 3.0 shows the plasma enzyme activities six weeks after treatment of hypercholesterolemic rats with garlic powder at 5% and 10% level. A significant increase (P<0.05) was observed in the activities of AST while there was no significant (P>0.05) difference in the ALT and ALP activities of the hypercholesterolemic rats compared to the control. High AST and ALT are usually present within hepatocytes and plasma levels increase as hepatocytes membrane integrity is distorted during hepatocellular cell injury (27,28) . Rise in the level of AST is of ten accompanied by significant elevation in the levels of ALT which is not observed in the present work. It could possibly mean that the hepatotoxic effect of the hypercholesterolemic rats is mild. Since ALT is more specific for the liver because it is mainly present in the cytosol of the liver and in low concentration elsewhere both enzymes play important roles in the conversion of amino acids to keto acid sand they are major markers of liver damage caused by exposure to toxic substances (29). However on treatment with 5% and 10% garlic significant

($P<0.05$) reduction was observed. This implies that garlic powder at the two levels used in this study helps to alleviate the injury caused by the hypercholesterolemic diet. The observed increase in liver and kidney AST and ALP activities in hypercholesterolemic rats could be due to *denovo* synthesis, however a significant reduction in activities of AST and ALP were observed in both organs in the group fed 5% garlic powder but an increase observed in the liver of rats fed 10% garlic powder only. This report agrees with previous reports and it shows that garlic contains some constituents that could counteract the atherogenicity of high fat diet, but the fact that the activities of AST and ALP activities were significantly increased at 10% level of garlic in the diet suggests that the consumption of garlic should be with caution. From the foregoing, it can be concluded that garlic powder at 5% and 10% levels in the diet reduced atherogenicity while its effects on organ enzymes at 10% level suggests mild injury.

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