EFFECTS OF ETHANOLIC EXTRACTS OF *Allium sativum Linn. Liliaceae* (GARLIC) ON SERUM CHOLESTEROL AND BLOOD SUGAR LEVELS OF ALBINO RABBITS

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ABSTRACT

Administration of the ethanolic extracts of Allium sativum Linn (Liliaceae) lowered the cholesterol and blood sugar levels in the albino rabbits. The animals treated with higher concentration of the extract recorded more reduction in cholesterol (115 ± 19.2 mg/dl), and blood sugar levels (31.40 ± 31.1 mg/dl), with significant reduction in their body weights (P < O. 05)

INTRODUCTION

Several factors influence the human plasma or serum lipid and sugar levels. Some of these factors could be genetic, resulting in the hypo- or hyper-responsiveness to increased dietary levels of these substances (Clarkson *et al*, 1985). Other factors which influence the serum lipid and sugar levels include drugs, nutritional state, as well as the nature of the dietary fatty acids (Davidson, 1979).

Incorporation of triglyceride fatty acids of high density lipoproteins into adipose tissues of an animal has been affected by the nutritional state of the animal (Gutman *et al*, 1962). Again diets deficient in protein have been observed to result in sub-normal rates of lipid synthesis (Cantarow and Schepartz, 1967). However, polyunsaturated and monounsaturated fatty acids were found to have lowered cholesterol levels (Dubey *et al*, 1987).

The reason for this cholesterol lowering effects of polyunsaturated fatty acids is still not clear. Several reasons have been advanced to explain the effect in the stimulation of cholesterol excretion into the intestine and its oxidation to bile acids. This effect is largely due to a shift in distribution of cholesterol from the plasma into the tissues. Also, it is because of increased catabolic rate of low density lipoprotein due to regulation of low density lipoprotein receptors, by polyunsaturated and mono unsaturated fatty acids (Lehninger, 1990).

Cholesterol is a member of a large group of substances called steroids, of which the steroid hormones and adreno-corticosteroids are included. It is the most naturally occurring steroid (White, 1959) .Cholesterol is synthesized in the intestines, nervous tissues, adrenal glands and gonads. It is absorbed in the intestine, and incorporated into the chylomicrons, formed in the mucosa, and subsequently transported to the liver. Some of these are excreted in the bile in free form, as bile acids, and the rest are incorporated into very low density lipoproteins (Lehninger, 1990).

It was estimated that an adult man synthesizes approximately 80mg of cholesterol, while diet also provides substantial amounts (Armstrong, 1983). In pathological conditions, substantial quantities of cholesterol have been found to be esterified with long chain fatty acids (Myrant, 1981). Of interest is the role of cholesterol in the aetiology, of the pathological condition known as atherosclerosis. The formation of an atheroma was found to be initiated by a thickening of the arterial intima due to smooth muscle cell proliferation. Under this condition cholesterol was deposited on the inner walls of blood vessels, together with other lipids. This leads to the occlusion of blood vessels in the heart and brain resulting in heart attacks and strokes (Cantarow and Schepartz, 1967).

The process of aging which predisposes one to myocardial infarction, cerebral thrombosis and other serious illness is characterized by the infilteration of cholesterol into certain tissues of the arterial walls, distorting the vessels, and making them rigid (Lehninger, 1990). Hyper-cholesterolemia has been associated with pathological conditions like diabetes mellitus (Daniel *et al*, 1983), nephritis, myxoedema and xanthomatosis. People with high level of cholesterol in their blood are more likely to build up fatty deposits in their blood vessels, and are prone to heart diseases. (McGill and Mott, 1976).

Diabetes mellitus is a syndrome characterized by a raised glucose concentration in the blood, due to deficiency or

decreased effectiveness of insulin (Davidson, 1979). The disease produces an increased risk of athereosclerosis and other health problems (Stryer, 1981). Diabetes affects the metabolism of fat and protein. Glucose usually spills into the urine, and this is associated with polyuria and other diseases. There is always the hazard of acute complications of ketoacidosis, and other ocular and vascular complications. Most of these complications, though treatable are dangerous medical emergencies (Glew and Peters, 1990).

Dietary fiber consumption has been shown to alter the digestion and absorption of carbohydrates and lipids in the small intestine. It promotes the lowering of blood lipid and sugar levels (Cummings, 1978). Similar lowering effects, especially in serum cholesterol and glucose levels have been achieved by the consumption of certain plants or their extracts (Dubey *et al*, 1987). Consumption of raw garlic and or its oil has been observed to exert reduction in serum cholesterol of atherosclerotic, as well as sugar levels in diabetic rats (Adoga, 1975; Heinemann, 1995).

Garlic has been found to be antimicrobial (Zwergal, 1952; Barone and Tansey, 1977; Yamaskiet *et al*, 1991), antitumor (Weisberger and Pensky, 1958) and antimutagenic (Yamaskiet *et al*, 1991). In folk medicine, it has been found to be useful in the treatment of high blood pressure, rheumatic and muscular pains, giddiness and sore eyes, for stimulation of rapid contraction of the uterine smooth muscles and return of menstrual cycle, as diuretic, in cases of myocardial infarction, diabetes, dyspepsia, and as natural antiseptic for the body (Houghton, 1995).

Most of these diseases are now on the increase, and could be treated satisfactorily with garlic. The objective of this study, therefore, is to determine the effects of various concentrations of the extracts of garlic on serum cholesterol and blood sugar levels in the albino rabbits. The study is also meant to show the degree of esterification of serum cholesterol as a result of the administration of the extract. Garlic (*Allium sativum*) is a member of the plant family Liliceae (Dutta, 1981). Garlic and Onion (*Allium cepa*) belong to the same genus Allium, but the degree of pungence and effectiveness, among other factors may be used to differentiate between the two.

MATERIALS AND METHODS

Plant and Animal Materials: Bulbs of *Allium sativum (Garlic)* used for this work were purchased from Eke – Okigwe Market, Imo State, Nigeria. It was identified by Dr. C.U. Okeke of the Department of Botany, Abia State University, Uturu.

The albino rabbits used were purchased from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

Preparation of Plant Extracts: The garlic cloves were separated from the bulb. They were peeled and air dried for about one week. 50g of the dried garlic cloves were ground to powder with pestle and mortar. The ground garlic sample was soaked in 750ml of 50% ethanol and left to stand for 24 hours. Thereafter, it was sieved with a cheese cloth. Ethanol was removed from the filterate using rotary evaporator. The extracted garlic oil weighing about 5g was used for the work.

Administration of the Extracts: 15 albino rabbits were shared into 3 groups of 5 animals in each. The extracts were administered with syringes of 1.0ml size. Group A (Control) was given 1.0ml of water per kg body weight of animal twice daily. Group B was given 0.5ml (0.25g) of garlic extract per kg body weight twice daily while group C was given 1.0ml (0.5g) of garlic extract per kg of body weight, also twice daily. The treatments were carried out for 21 days. The body weights of the animals were measured daily. The animals were maintained on normal rabbit feed bought from a feed manufacturer.

Blood Sample Collection and Serum Preparation: At the end of every week 1ml of blood was collected from each of the animal through the ear vein using a hypodermic needle and syringe. The sera were separated from the blood through ultracentrifugation for 20 min at 1000 rpm. The sera were used for the assay, to determine total cholesterol, free cholesterol and blood sugar levels.

Quantitative Determination of Total Serum Cholesterol: Total Serum cholesterol was determined using standard procedures (Searcy and Bargquist 1960, Durrington *et al*, 1975). To each test tube containing 0.1ml of serum, 0.9ml of acetone-ethanol solution was added, stoppered, mixed well and centrifuged at 3000 rpm. for 15 minutes. 0.4ml of the clear supernatant from each test tube was than pipetted out and placed into separate test

tubes and then diluted with 3.0ml of $FeSO_4$-acetic acid reagent. Concentrated sulphuric acid (1.0ml) was added into each of the test tubes. The contents were mixed thoroughly by shaking. The test tubes were then allowed to stand for 10 minutes. The absorbance of each test tube content were read at 490nm in a spectrophotometer against a reagent blank (acetone-ethanol reagent).The concentration of cholesterol was determined by extrapolation from the cholesterol standard curve.

Quantitative Determination of Free Serum Cholesterol: Free Serum Cholesterol was determined by using standard methods (Searcy and Bergquist 1960). To another set of test tubes containing 0.4ml of the serum, 1ml of digitonin solution was added and allowed to stand for one hour.
The mixture was centrifuged at 3000g for 15 minutes. The supernatant was carefully decanted and the precipitate re-suspended again at 3000g for 15 minutes. The supernatant was carefully decanted and the precipitate re-dissolved in 3.0ml of $FeSO_4$-acetic acid reagent, 1.0ml of concentrated sulphuric acid and reaction mixture were allowed to stand for 10 minutes. The content of each test tube was read at 490mm against a reagent blank. The concentrations of free cholesterol in the sera were determined by extrapolation from the cholesterol standard curve.

Quantitative Determination of Blood Sugar: Standard method of determining Blood sugar was adopted (Asator and king 1954, Baker *et al,* 1998). 0.1ml each of the serum was placed in 3.8ml of sodium-sulphate/copper sulphate solution and mixed well, 0.1ml of 10% sodium tungstate was added to each test tube and mixed well before centrifuging at 3000 r.p.m for 15 minutes. To 1ml each of the supernatant working standards and isotonic sodium sulphate-copper sulphate (blank), 1ml of alkaline tartarate solution was added, plugged lightly with cotton wool and heated in a boiling water bath for 10 minutes. The test tubes and their contents were cooled and 3ml of phosphomolybdic acid solution and 3ml of distilled water were added to each test-tube. The contents were mixed well and their absorbance read after 5 minutes at 680mm. The concentration of blood sugar was extrapolated from the blood sugar standard curve.

RESULTS

The results of the study are shown in Tables 1- 4. Table 1 showed that the total cholesterol level increased from 210 ± 8.7 mg/dl in week 1 to 250 ± 14.3 mg/dl in week 3. Treatment with the garlic extract reduced the total serum cholesterol. The reduction in the total serum cholesterol was of the of the order of 190 ± 18.3 mg/dl in week 1, 150 ± 4.8 mg/dl in week 3, and 135 ± 13.5 mg/dl for group B, while treatment C reduced from 185 ±21.2 mg/dl in week 1 to 145 ± 1.9 mg/dl in week 2 and 114 ± 19.2 mg/dl in week 3. Comparatively, the animals in treatment C showed remarkable reduction in the total serum Cholesterol. These decreases were statistically significant.

Table 1: Effect of garlic extract on total serum cholesterol level

	TREATMENTS		
WEEKS	A Cholesterol Level (mg/dl)	B Cholesterol Level (mg/dl)	C Cholesterol Level (mg/dl)
WEEK 1	210 ±8.7	190 ± 18.3	185 ± 21.2
WEEK 2	215 ± 5.8	150 ± 4.8	145± 1.9
WEEK 3	250 ± 14.3	135 ±13.5	115 ± 19.2

Table 2 showed that serum free cholesterol was lowest in week 3 in all the animals in treatment A. In all the three treatment, the values recorded in treatment B were the lowest.

Table 2: Effect of extract on free serum cholesterol levels

	TREATMENT		
WEEKS	A Cholesterol Level (mg/dl)	B Cholesterol Level (mg/dl)	C Cholesterol Level (mg/dl)
WEEK 1	75 ± 10.6	35 ± 3.9	35 ±7.7
WEEK 2	55 ± 1.0	40. ±1.0	45 ±14.4
WEEK 3	40 ± 9.6	50 ±4.8	65 ±1.9

Table 3 showed that there were reduced serum esterified Cholesterol in treatment B and C than in treatment A. The reduction was more in treatment C than in treatment B.

Table 3: Effects of the extract on the esterified serum cholesterol levels

	TREATMENTS		
WEEKS	A (Control) Cholesterol Level (mg/dl)	B Cholesterol Level (mg/dl	C Cholesterol Level (mg/dl
WEEK 1	1.35 ± 19.1	155 ± 22.1	150 ± 31.8
WEEK 2	160 ± 4.81	110 ± 3.90	100 ± 2.90
WEEK 3	210 ± 24.1	85 ± 18.3	35 ± 3.46

Table 4 showed that the blood sugar level in the animals in treatment A was increasing as the weeks and highest in week 3. Treatment of the animals with the garlic extract reduced the blood sugar levels as shown in treatment B and C. The highest reduction was achieved in treatment c with the higher concentration of the garlic extract.

Table 4: Effects of the extract on blood sugar levels

	TREATMENT		
WEEKS	A Blood Sugar Level (mg/dl)	B Blood Sugar Level (mg/dl)	C Blood Sugar Level (mg/dl)
WEEK 1	94.4 ±1.0	111.7 ± 18.3	24.2 ±22.5
WEEK 2	95 ± 1.0	95 .6±9.0	100 ± 8.6
WEEK 3	99.8 ±2.1	32.6±27.3	31.4±31.1

Table 5 showed that the body weight of the animals increased as the weeks in the animals in treatment A. The treatment B and C animals decreased on treatment with the garlic extracts. Greater decreases in body weights were achieved in the treatment C animals that were treated with higher concentration of the extract.

Table 5: Effect of the extract on the average body weights of the animals (g)

	TREATMENT		
WEEKS	A	B	C
WEEK 1	973.20 ±10.85	975.6 ±20.60	978.10 ±15.10
WEEK 2	996.67 ±24.40	893.33 ±8.35	865.70 ±16.03
WEEK 3	1200 ±11.55	870 ±8.60	760 ±38.50

DISCUSSION

The results of the study showed that the administration of the ethanolic extracts of garlic lowered the cholesterol levels in the albino rabbits. During the third week of the study, the total serum cholesterol level reduced to 135 ± 13.5 (mg/dl) in treatment B. This reduction was significant (Table 1). The reason for the greater reduction in treatment C animals was because they were treated with higher concentration of the test sample (1.0 ml/kg body weight). The extract was able to reduce the concentration of the esterified cholesterol level, thereby increasing the free cholesterol level (Table 2 and 3).

This result was in agreement with the results obtained on similar works in rats. The report of that study observed that hyper-cholesterolemic rats fed on high sucrose, high alcohol, high fat and high cholesterol diets, and in the condition of streptozotocin induced diabetes, indicated a reduction of the serum cholesterol levels. This was as a result of the oral administration of garlic oil (Adoga, 1995). The result also showed that the total serum cholesterol was the sum of the free serum cholesterol and the esterified serum Cholesterol (Table I and 2). As the free serum Cholesterol increased, the esterified serum cholesterol decreased, and vice versa.

The treatment of the albino rabbits with the garlic extract, as shown in treatment B and C, reduced the blood sugar levels in the animals (Table 4). There was greater reduction in group C than in treatment B. The reason for this was that the concentration of the test extract administered in treatment C was higher (1.0 ml/kg body weight) than that given in treatment B (0.5 ml/kg body weight).

This reduction in the blood sugar concentration showed that the ethanolic extract of garlic had anti-hyperglycemic effect on the albino rabbits. This result agreed with earlier reports on the effects of the oral administration of raw garlic on rats. (Adoga, 1995).

It was shown that garlic was able to lower the blood sugar levels in diabetic rats, and even revived them when they appeared fatigued (Heinemann, 1995). Again, allyl propyl disulphide and diallyl sulplude oxide extracted from *Allium cepa* (Onion), a relative of garlic, had successfully reduced the blood sugar levels and glucosuria in the alloxan diabetic rabbits.

There was significant loss in the body weights of the animals during the third week in treatment B (870 ± 8.60g) and C (760 ± 38.50g). The reason for this was that the animals were treated with the ethanolic extract of garlic. Greater lowering effect was observed in the treatment C animals, where they were treated with higher concentration of the extract. The higher the concentration of the extract administered, the lower the body weight of the animals (Table 5). Reduction in the body weight of the albino rabbits treated with the garlic extract could be because of the lowering in the total serum cholesterol and total blood sugar. It could be recalled that the individuals with excess cholesterol in their blood are more likely to have more fatty deposits in their tissues (McGill and Mott, 1976). The beneficial effect of low body weight in control of diabetes cannot be over emphasized (Heinemann, 1995).

The interference with the carbohydrate metabolism has been shown to have reflected on the lipid metabolism as well. This is

probably because lipid and carbohydrate metabolisms are somehow related (Davidson, 1979, Glew and Peters,1990). The lipid lowering diets have been regarded as necessary for the control of atherosclerosis and myocardial infarction (Davidson,1979, Myrant, 1981).However because garlic has lowering effects on the total serum cholesterol and blood sugar concentrations in albino rabbits and other animals, it is therefore recommended for the management of diabetes mellitus and hypercholesterolemia in the humans. In conclusion we substantiate that garlic produces significant reducing effect on both cholesterol and blood sugar levels in rabbits.

ACKNOWLEDGEMENT

The authors wish to acknowledge the efforts of Ukonu Nwadiuto during the course of this study.

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