Sokoto Journal of Veterinary Sciences
(ISSN 1595-093X)
Aka *et al.* /*Sokoto Journal of Veterinary Sciences* (2010). **8**(1):26-30.

The effects of dietary supplementation of *Allium sativum* on some vital biochemical parameters in male Albino rats.

LO Aka[1&2]*, NN Pilau[3], & RI Obidike[2]

[1]*Department of Veterinary Physiology & Biochemistry, Usmanu Danfodiyo University, Sokoto.*
[2]*Dept. of Veterinary Physiology &Pharmacology, University of Nigeria, Nsukka,*
[3]*Dept. of Veterinary Medicine, Usmanu Danfodio University, Sokoto*

*Correspondence: Tel.: 08134576173 e-mail Lawrence_aka@yahoo.com

Abstract

This study investigated the effects of Garlic (*Allum sativum*) on plasma lipid levels, serum proteins and electrolytes in albino rats. Graded dietary supplementations of garlic viz: 0, 5, 10, 15, 20, and 25 % administered to sixty (60) apparently healthy male albino rats of the Wistar strain in five groups of ten (10) rats each. The *Allium* was supplemented for 30 days in a complete randomized manner. A sixth group of ten rats served as the unsupplemented control. The result showed a significantly ($p<0.05$) decreased plasma lipid (total cholesterol) level in a dose dependent manner. The serum albumin was significantly ($p<0.05$) decreased at 20 and 25 % supplementation levels. Though there was an increase in serum globulin at higher supplementation levels, the increase was not significant. There was also significant ($p<0.05$) reduction in serum sodium and calcium levels as the garlic supplementation levels increased. There was however no significant ($p>0.05$) effect on serum potassium levels in the supplemented groups. This study reveals that garlic has an effect on the dynamics of plasma lipids and serum proteins, and key electrolytes concentrations. These investigations infer that garlic intake has some desirable effects, but caution should be exercised in its consumption to minimize some possible adverse effects which may be associated with an overdose or excess intake.

Keywords: Electrolytes, garlic, lipids, proteins, rats.

Introduction

Allium sativum commonly known as garlic is a specie in the onion family *Alliaceae*. (Eric, 2010). Garlic has been used throughout recorded history for both culinary and medicinal purposes. It has a characteristic pungent spicy flavour that mellows and sweetens considerably with cooking (Gernot, 2005). *Allium sativum* grows in the wild. It is easy to grow and can be grown year-round in mild climates. Garlic is widely used around the world and is readily available (Charlson, and McFerren, 2007).

Garlic has been used as both food and medicine in many cultures for thousands of years, dating at least as far back as the time that the Giza pyramids were built. In vitro studies shows garlic has antibacterial, antiviral and antifungal activity (Dura key al., 2002). It is also claimed to help prevent heart diseases these includes atheriosclerosis, high cholesterol, and high blood pressure and cancer (Durak *et al.*, 2002). Animal studies and some early investigational studies in humans, have suggested possible cardiovascular benefits of garlic (Sovova & Sova, 2004). It has been demonstrated that garlic supplementation reduce accumulation of cholesterol on the vascular walls of animals (Sonova & Sova, 2004). Another study had similar results, with garlic supplementation significantly reducing aortic plaque deposits of cholesterol-fed rabbits (Durak *et al.*, 2002). Studies again showed that supplementation with garlic extract inhibit vascular calcification in human patients with high blood cholesterol (Durak *et al.*, 2004). Although these studies showed protective vascular changes in garlic fed subjects, a randomized clinical trial found that the consumption of garlic in any form did not reduce blood cholesterol levels in patients with moderately high baseline cholesterol levels (Chan *et al.*, 2007). Also no effects of raw garlic or garlic supplements on low density lipoprotein (LDL), high density lipoprotein (HDL) or triacylglycerols has been reported (Gardner *et al* 2007). Likewise, information on the hematological effects of *Allium Sativum* have been widely documented such as reduced platelet aggregation (Borreli *et al.*, 2007; Rahman, 2007) and hyperlipidemia (Steiner & Lin 1998; Kojuri *et al.*, 2007), blood sugar levels (Chang & Johnson, 1980). The hitherto conflicting finding on the effect of garlic on plasma lipid, low density lipoproteins and on body fluid electrolytes as well the paucity of information relating level of supplementation of *Allium sativum* to its effects suggest that more work is required to elucidate the ambiguity therein. The aim of this study was to further assess the effect of different levels of dietary supplementation of raw garlic on some vital biochemical parameters using albino rats.

Materials and Methods

Study location

The study was carried out at the Physiology and Biochemistry laboratory, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Experimental animals

Sixty (60) apparently albino rats of the Wistar strains were used for this study. On arrival they were divided into six groups of ten rats each and weighed. After an acclimatization period of 21 days during which they were fed with poultry growers mash (Vital Feeds®, Jos-Nigeria) only, they were weighed and observed for disease symptoms, agility, and appetite. Those that showed unthriftness were removed and replaced with other apparently healthy ones from the stock.

Plant collection, identification and preparation

Dried cloves of garlic obtained commercially from the Sokoto central market and identified by a botanist using a sample specimen were pulverized, using a local mortar, into fine granules of powder. After pulverization, the powder was further dries under the sun for proper desiccation. A total of 3 kilograms of dries garlic powder was obtained and stored in well cocked plastic container, at room temperature until use.

Experimental design and experimentation

The five groups of ten rats each were designated groups A, B, C, D and E. A sixth group of ten rats served as the control. The average daily ration of each group, as obtained during the acclimatization period were supplemented with garlic powder at 5, 10, 15, 20 and 25 % supplementations for groups A, B, C, D and E respectively and offered daily for 30 days after which samples were collected for analysis.

Collection of blood sample and analysis

On day 30, seven rats per group were anaesthetized using chloroform and blood was collected from each through the medial canthus of the eyes using a capillary tube. Blood was collected into two Bijou bottles, one containing an anticoagulant and the other without anticoagulant. Serum was obtained from coagulated blood by centrifugation (1500 x G for 15 minutes) and analyzed for serum proteins as described by Obidike *et al*., (2009). Serum was also used for the determination of sodium, calcium and potassium concentrations by colorimetric method. Uncoagulated blood samples were centrifuged at 5000 G for 20 minutes and the plasma samples obtained for analysis. Plasma total cholesterol was measured by the procedure described by Allain *et al*., (1974) and high density lipoprotein (HDLP) cholesterol according to Warrick *et al*., (1982). Low-density lipoprotein (LDLP) cholesterol was deduced from the values of total and HDL- cholesterol according to the modified procedure described by Friedewald *et al*., (1972), while triacylglycerol determination was by the method of Foster & Dunn (1973).

Statistical analysis

Treatment effect was analyzed using the Multiple Range Test and differences between means were established using the Least Significant method of mean comparison (Steel & Torrie, 1980).

Results

The results of the study are presented in tables 1 and 2. It was observed that total cholesterol was significantly ($p<0.05$) decreased at 20 % (59.87±4.66 mg/dl) and 25% (54.02±3.81 mg/dl) supplementation from a control value of 76.68±3.99 mg/dl. Other treatments also recorded decreases which were not significantly different from each other as well as the control. Similarly, the high density lipoprotein (HDLP) was significantly ($p<0.05$) reduced at 20 and 25 % garlic supplementation levels with values at 44.34±5.10 mg/dl and 41.37±4.27 mg/dl respectively.

The low density lipoprotein (LDLP) was in like manner reduced significantly to 10.53±3.18 and 12.65±5.15 mg/dl at 20 and 25% supplementation levels compared to a control value of 21.74±2.88. The triacylglycerol was also significantly reduced at 15% (56.31±2.18 mg/dl), 20% (50.11±3.61 mg/dl) and 25 % (44.27±0.83 mg/dl) supplementations compared to the control value of 65.96±0.14 mg/dl (table 1).

As shown in table 2, there was a significant ($p<0.05$) decrease in total protein concentration at 20 and 25 % garlic supplementation with a plasma concentrations of 2.9±2.8 and 1.4±3.1 mg/dl respectively in comparison with a concentration of 4.8±0.1 mg/dl. At 5% supplementation there was significant ($p<0.05$) increase, 6.3±4.6 mg/dl of total protein compared to the control value of 4.8±0.1 mg/dl was recorded. There was also significant ($p<0.05$) decrease in serum albumin concentration at 15, 20 and 25 % supplementation levels (Table 2). There was a concomitant increase in fibrinogen, compared to the control (0.3±0.1mg/dl), at 15, 20 and 25 % garlic supplementation with values recorded as 1.8±0.4, 1.3±0.2 and 1.1±0.2 mg/dl respectively with its peak occurring at 15 % supplementation. Globulin significantly increased from a control value of 2.3±0.7 to 4.3±0.6 and 5.1±1.1 mg/dl at 20 and 25 % supplementations respectively. Sodium on the contrary, significantly ($p<0.05$) decreased with increasing level of garlic supplementation commencing particularly from 15 % supplementation and above. Serum calcium level was significantly ($p<0.05$) reduced as the supplementation levels increased. From a control value of 110.8±2.2 ME/L to 88.3±4.1, 70.3±3.2 and 68.4±4.7 ME/L at 15, 20 and 25 % supplementation levels respectively. Garlic supplementation had no significant treatment effect on serum potassium concentration.

Table 1: Plasma lipids levels in rats fed graded supplemental levels of *A. Sativum*

Treatment	TC	HDL	LDL	Triacylglycerol
A(5%)	72.31 ± 2.66^{a}	53.12 ± 2.99^{a}	$19.19\pm3.77^{a,c}$	62.72 ± 5.11^{a}
B (10%)	$70\text{-}02\pm3.17^{a}$	50.84 ± 4.13^{a}	$19.18\pm4.11^{a,c}$	9.56 ± 3.99^{a}
C (15%)	68.64 ± 3.63^{a}	50.93 ± 2.83^{a}	17.71 ± 3.59^{a}	6.31 ± 2.18^{b}
D (20%)	59.87 ± 4.66^{b}	44.34 ± 5.10^{b}	10.53 ± 3.18^{b}	$0.11\pm3.61^{b,c}$
E (25%)	54.02 ± 3.81^{b}	41.37 ± 4.27^{b}	12.65 ± 5.15^{b}	44.27 ± 0.83^{c}
Control	$76.68\pm0.78^{a,c}$	54.94 ± 3.99^{a}	21.74 ± 2.88^{c}	65.96 ± 0.14^{a}

a, b,c,d= Means within column with same superscript letters are not significantly different at p<0.05

TC: Total cholesterol, HDL: High density lipoprotein, LDL: Low density lipoprotein

Table 2: Serum protein and some electrolyte levels in rats fed graded supplemental levels of *Garlic Sativum*

Trt	TP	Albumin	Fibrin	Glob	Na	Ca	K
A(5%)	6.3 ± 4.6^{c}	2.8 ± 4.9^{a}	0.2 ± 0.2^{a}	2.3±1.2	122.6±4.2	100.2± 3.2	2.1±0.0
B (10%)	5.6 ± 3.8^{a}	3.6 ± 5.2^{b}	0.4 ± 0.1^{a}	2.6±1.4	101.5±3.9	95.8± 6.1	2.6±0.1
C (15%)	4.5 ± 3.1^{a}	0.9 ± 4.7^{c}	1.8 ± 0.4^{b}	2.8±0.9	88.3 ± 4.1^{a}	69.3 ± 4.2^{a}	2.4±0.1
D (20%)	2.9 ± 2.8^{d}	0.6 ± 3.9^{c}	1.3 ± 0.2^{b}	4.3 ± 0.6^{a}	70.3 ± 3.2^{a}	60.2 ± 3.6^{a}	2.7±0.3
E (25%)	1.4 ± 3.1^{d}	0.4 ± 2.3^{c}	1.1 ± 0.2^{b}	5.1 ± 1.1^{a}	68.4 ± 4.7^{a}	55.3 ± 3.9^{a}	2.3±0.0
Control	4.8 ± 0.1^{a}	1.7 ± 4.1^{a}	0.3 ± 0.1^{a}	2.3±0.7	128.3±3.1	110.8±2.2	2.6± 0.1

a,b,c= means within column with same superscript are not significantly different at p<0.05
[*]measured in ME/L. Albumin, fibrinogen and globulin are measure in mg/dl
Trt=Treatment, TP= Total Proteins, Fibrin=Fibrinogen, Glob=Globulins, Na=Sodium, Ca= Calcium, K=Potassium

Discussion

Dietary supplementation of garlic, from this study has clearly demonstrated that garlic is capable of reducing the plasma lipid levels particularly total cholesterol and triacylglycerol. The reductive effect on the total cholesterol was a reflection of marked reduction on both the high density and low density lipoproteins. This cholesterol reducing effect (hypolipidemic effect) has been reported earlier (Chi *et al*., 1982). This is rather a beneficial effect as it could exert its anticholesterol effect in the control of hyperlipidemia, a predisposing factor to hypertension. Chang & Johnson (1980) had earlier reported that the addition of garlic to a sucrose and cholesterol diet in rats effectively prevented the rise in serum and liver cholesterol, triglycerides and free fatty acids. The result of this experiment is however consistent with these earlier observations. Chi *et al*., (1982) ascribed the hypolipidemic action of garlic to its sulphur compounds. This hypolipidemic effect of garlic probably explains why garlic has been traditional considered as an excellent medicinal plant particularly as an anti-hypertensive agent. The cholesterol lowering effect of garlic as observed in this study agrees with the report of Rahman, (2002) and Siligaly & Neil, (1994) this may probably account for its use in the treatment of atheriosclerosis. Protective effect of garlic on atherosclerosis has been attributed to its capacity to reduce lipid content in arterial wall (Orekhov & Grunwald, 1997). Garlic causes direct antiatherogenic (preventive) and antiatherosclerotic (causing regression) effects at the level of artery wall (Orekhov & Grunwald, 1997). Garlic depressed the hepatic activities of lipogenic and cholesterogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6 phosphate dehydrogenase and 3-hydroxy-3-methyl-glutaryl-CoA reductase (Yu-yan, 2001). Garlic also increased the excretion of cholesterol, as manifested by enhanced excretion of acidic and neutral steroids after garlic diet (Chi *et al*., 1982).

The chronic effects of garlic on lipid metabolism in rats have been previously reported (Chi *et al*., 1982). In their study, garlic (1–4% in diet) and garlic protein administration in hypercholesterolemic rats induced by a high-cholesterol diet, significantly reduced serum cholesterol, triglyceride and LDL cholesterol [Orekhov & Grunwald, 1997; Rajasree *et al*., 1999) but there was less reductive effect on serum HDL. This observation agrees with the findings in this present study. Total lipid content and cholesterol levels in liver were also decreased in rat after chronic garlic consumption (Yu-Yan, 2001).

Table 2 shows the effect of garlic on serum proteins and some electrolytes. Garlic supplementation increased the total serum proteins only at 10 and 15 % supplementation. This was characterized by relative increase in albumin level. This increase in total proteins with relative increase in albumin levels alludes to the hepatoprotective effect of garlic as earlier reported (Ajayi *et al*., 2009). In their studies, post-lead treatment with *A. sativum* and vitamin C significantly reduced the activities of amino alanine transferase (ALT), amino aspartate transaminase (AST) and alkaline phosphate

(AP). They showed that hepatoprotective activity of *A. sativum* lies in its ability to decrease the production of ALT, AST and ALP thus, giving credence of no sipage of these biomarkers into the blood.

The relative increase in serum albumin at 10 and 15 % indicated that garlic is probably beneficial in the maintenance of the colloidal osmotic or oncotic pressure of plasma at these supplementation levels. In so doing, it is physiologically competent in regulating capillary reabsorption at the venous end, thus preventing accumulation of fluid in the interstitial spaces, hence preventing edema. Plasma proteins are known to maintain colloidal osmotic pressure, contribute to viscosity of blood, influence the suspension stability of erythrocytes and help to regulate acid-base balance, transport substances (vitamins, hormones, nutrients etc) and affect solubility of carbohydrates, lipids and other substances held in solution in the plasma.

Hanaa *et al*., (2009) showed that supplementation of sodium nitrite ($NaNO_2$) intoxicated rats with garlic oil ameliorated the nitrite adverse effects as evidenced by a significant increase in serum total protein content. According to Sodimu *et al*., (1984) garlic oil had significant (p>0.05) effect on body weight, haematological parameters (PCV, Hb and WBC), and serum total protein. However, the spices mixture exerted significant (p<0.05) hypoglycemic and hypolipidaemic effects at 2% w/w of the supplement beyond which there was significant increase in both mean plasma glucose and serum total cholesterol. The report concluded that while spice mixture containing raw garlic powder is beneficial at culinary doses, its benefits and safety at higher doses remain unclear. This observation tends to buttress the results of this study since supplementation levels higher than 15 % caused a marked reduction in serum total proteins and albumin, indicating possible adverse effect on the liver. This could compromise protein synthetic ability under the prevailing circumstance.

The study also revealed a significant reduction in serum sodium and calcium levels as garlic supplementation increased up to 15 % and beyond. A reduction in serum levels of sodium and calcium probably resulted from a decrease in renal reabsorption of sodium and demineralization of bone with consequent excretion of sodium and calcium in urine. Increased excretion of sodium is usually associated with excess water loss as uraemia. Therefore, this observation suggests that excess intake of garlic could predispose an animal or human individuals to hyponatranemia and consequently water loss. Physiologically, this is usually compensated for by excess secretion of aldosterone with resultant increase in sodium reabsorption particularly at the distal convoluted tubule and the collecting duct leading to water retention to compensate the effects of excess water loss following loss of sodium. However, this compensatory mechanism could be overwhelmed by excess intake of garlic. This work suggests that high intake of garlic probably would disrupt the water balance by impairing its homeostatic mechanism.

On the other hand the reduction in serum calcium concentration is suggestive of the potentials of high levels of raw garlic in diets to cause hypocalcaemia. Since the absorbed dietary calcium is usually filtered in the kidney (98-99 %) and reabsorbed from renal tubules into blood, it does appear that its decrease in serum at high level (15 % and above) of supplementation probably resulted from either poor intestinal absorption or decreased renal reabsorption. Another possible mechanism for this observation could be a humoral effect of raw garlic, at high supplemental levels, on the calcium metabolic hormones such as parathormone, 1, 25-dihyroxycholecalceferol and calcitonin. These remain a subject for further investigation.

In conclusion, dietary supplementation of raw garlic show some lowering effects on total lipids, cholesterol and increasing effect on serum proteins particularly at dietary supplementation not exceeding 10 – 15 % levels. There are contrasting views, in literature, on the effects of raw garlic. From this study however, the generally held view that raw garlic reduces plasma cholesterol, increases serum proteins particularly albumin at levels not beyond 10 % supplementation has been validated. Additionally, beyond this supplemental level some key serum electrolytes are more likely to decrease. A situation capable of predisposing animals to some metabolic disorders. It is therefore recommended, that garlic intake should not exceed 10 % of daily ration.

References

Ajayi GO, Adeniyi TT & Babayemi DO (2009). Hepatoprotective and some haematological effects of *Allium sativum* and vitamin C in lead-exposed Wistar rats. *International. Journal of Medicine and Medical Science* **1**(3)64-67.

Allain CC, Poon LS, Chan CS, Richmond W & Fu PC (1974). Enzymatic determination of total cholesterol. *Clinical Chemistry.* **20**(1):470-475.

Borreli F, Capasso R & Izzo AA (2007). Garlic *(Allium sativum)*: Adverse effects and drug interaction in humans. *Molecular Nutritional Food Research.* **51**(11):1386-1397.

Chan KC, Yin MC & Chao WJ (2007). Effects of diallyl trisulfide-rich garlic on blood coagulation and plasma activity of anticoagulation factors in rats. *Food Chemistry and. Toxicology.* **45**(3):502-507.

Chang MLW & Johnson MN (1980). Effect of garlic on carbohydrate metabolism and lipid synthesis in rats. *Journal of Nutrition.***110**:931-936.

Chi MS, Kohi ET & Stewart TJ (1982). Effect of garlic on lipid metabolism in rats fed cholesterol or lard. *Journal of Nutrition.* **112**:241-248.

Durak I, Kavutcu M & Aytac B (2004). Effects of garlic extract consumption on blood lipid and oxidant/antioxidant parameters in humans with high blood cholesterol. *Journal of Nutrition and. Biochemistry*. **15**(16):373-377.

Durak I, Ozturk HS, Olcay E & Guven C (2002). Effects of garlic supplementation on blood lipid and antioxidant parameters and atherosclerotic plaque formation process in cholesterol-fed rabbits. *J. Herb. Pharmacothem* **2**(2): 19-23.

Eric B (2010). "Garlic and Other Alliums: The Lore and the Science" *Cambridge, Royal Society of Chemistry,* **34**:152 166.

Foster BL & Dunn RT (1973). Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. *Clinical. Chemistry.* **19**:338-340.

Friedewald WT, Leve RI & Fredrickson DS (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative centrifuge. *Clinical Chemistry.* **18**(6)499-502.

Gardner CD, Lawson LO. Block E, Chatterjee LM, Kiazand A, Blaise R.Q & Kraemer, H.C. (2007). The effect of raw garlic supplement on plasma lipids concentration in with moderate hypercholesterolemia: A Clinical Trial *"Archives of Internal Medicine"* **167**:346-354.

Gernot K (2005). "Spices Pages": Garlic (*Allium sativum*, garlic)" *http:llwww.unigraz.at/-katzer/engl/Alli_sat.html. Retrieved 2007-08-28.*

Hanaa AH, Sherif ME, Rajiv LG, Augusta F, Madhiva HGR & Allal O (2009). In vivo evidence of hepato and reno-protective effect of garlic oil against sodium nitrite-induced oxidative stress. *International Journal of Biological Science.* **5**(3):249-255.

Kojuri J, Vosoughi AR & Akrami M (2007). Effects of *Anethum graveoleus* and garlic on lipid profile in hyperlipidemic patients. *Lipids Health Dis*., 6:5.

Obidike RI, Aka LO & Okafor CI (2009). Time-dependent peri-partum haematological, biochemical and rectal temperature changes in West African Dwarf ewes. *Small Ruminant Research **72**:*234-238.

Orekhov AN & Grunwald J (1997). Effects of garlic on atherosclerosis. *Nutrition* **13**:656-663

Rahman K (2007). Effects of garlic on platelet biochemistry and physiology. *Molecular Nutritional. Food Research*. **51**(11):1335-1344.

Rahman K & Billington D (2000). Dietary supplementation with aged garlic extract inhibits ADP-induced platelet aggregation in humans. *Journal of Nutrition.* **130**:2662-2665.

Rajasree CR, Rajmohan T & Austi KT (1999). Biochemical effects of garlic on lipid metabolism in alcohol fed rats. Indian *Journal of Experimental. Biology.* **37**:243-247.

Silagy C & Niel A (1994). Garlic as a lipid lowering agent-a meta-analysis. *Journal of Royal College of Physician*. **28**:39-45.

Sodimu O, Joseph PK & Augusti KO (1984). Certain Biochemical effects of garlic oil on rats maintained on high fat-high cholesterol diet. Experientia. **40**:78-81.

Sovova M & Sova P (2004). Pharmaceutical importance of *Allium sativum* L. 5. Hypolipidemic effects in vitro and in vivo. *Ceska. Slov. Farm* **53**(3):117-123.

Steel RGD & Torrier JD (1980). Principles and procedures of Statistic: A biometric approach. 2nd edition, McGraw-Hill, New York.

Steiner M & Lin RS (1998). Changes in platelet function and susceptibility of lipoproteins to oxidation associated with administration of aged garlic extract. *J. Cardiovasc Pharmacol.* **31**(6):904-8

Warrick GR, Berdersond J & Alberts JJ (1982). Dextran sulphate-mg precipitation procedures for quantitation of high-density lipoprotein cholesterol. *Clinical Chemistry.* **28**:1379-1388.

Yu-Yan Y, & Liu L (2001). Cholesterol lowering effect of garlic extracts and organosulfur compounds: Human and animal studies. *Journal of Nutrition.* **131**:9895-9935.