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

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**Abstract**

This study investigated the effects of Garlic (*Allium 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 as least as far back as the time that the Giza pyramids were built. In vitro studies show garlic has antibacterial, antiviral and antifungal activity (Dura key al., 2002). It is also claimed to help prevent heart diseases these includes atherosclerosis, 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 triglycerides 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 lipoprotein, and 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 dried 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 pH, 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 HDLp 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 line 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. Globulins 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TC</th>
<th>HDL</th>
<th>LDL</th>
<th>Triacylglycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(5%)</td>
<td>72.3±2.66</td>
<td>53.12±2.99</td>
<td>19.19±3.77</td>
<td>62.72±5.11</td>
</tr>
<tr>
<td>B (10%)</td>
<td>70.0±2.17</td>
<td>50.84±4.13</td>
<td>19.18±4.11</td>
<td>9.6±3.99</td>
</tr>
<tr>
<td>C (15%)</td>
<td>68.64±3.63</td>
<td>50.93±2.83</td>
<td>17.71±3.59</td>
<td>6.31±2.18</td>
</tr>
<tr>
<td>D (20%)</td>
<td>59.87±4.66</td>
<td>44.34±5.10</td>
<td>10.53±3.18</td>
<td>0.11±3.61</td>
</tr>
<tr>
<td>E (25%)</td>
<td>54.02±3.81</td>
<td>41.37±4.27</td>
<td>12.65±5.15</td>
<td>44.27±0.83</td>
</tr>
<tr>
<td>Control</td>
<td>76.68±0.78</td>
<td>54.94±3.99</td>
<td>21.74±2.88</td>
<td>65.96±0.14</td>
</tr>
</tbody>
</table>

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

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

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

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 traditionally considered as an excellent medicinal plant particularly as an antihypertensive 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 atherosclerosis. Protective effect of garlic on atherosclerosis has been attributed to its capacity to reduce lipid content in arterial wall (Orekhov & Gronwald, 1997). Garlic causes direct antitherogenic (preventive) and antiatherosclerotic (causing regression) effects at the level of artery wall (Orekhov & Gronwald, 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 & Gronwald, 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 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.


