Effects of Raw and Boiled Garlic Extract on Body Weight, Fasting Blood Glucose and Lipid Profile of Alloxan Induced Adult Male Wistar Rats

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ABSTRACT
Background: The burden of diabetes is high and rising in every country, fueled by the global rise in the prevalence of obesity and unhealthy lifestyles.
Objective: This study assessed the effects of raw and boiled garlic extract on body weight, fasting blood glucose, and lipid profile of alloxan-induced adult male Wistar rats.
Materials and methods: Fresh garlic bulbs were peeled, washed, and drained after which they were divided into 2 equal portions. The rats were grouped into seven based on body weight with a difference not more than ±5g per group. Each group was labeled A, B, C, D, E, F and G with 4 rats in each group. Rats in groups A, B, and C were administered 10mg, 20mg and 30mg of raw garlic extract per kilogram body weight, respectively while rats in groups D, E, and F were administered 10mg, 20mg, and 30mg of cooked garlic extract per kilogram body weight respectively then group G rats (control; induced but not treated) were administered with 10mg of plain water per kilogram body weight. All statistical analysis was carried out using IBM SPSS statistical software, version 21. All values obtained were expressed as mean and standard deviation. Paired sample t-test was used to compare baseline and end values. Differences in mean were considered significant at P <0.05.
Results: There was a significant (P <0.05) decrease in the fasting blood glucose level of the rats administered the experimental diets. A significant (P <0.05) decrease in body weight was observed in rats fed the experimental diets while the weight of the control group increased significantly. The total cholesterol and triglyceride levels of all the groups increased, however, the control had the highest (38.56% & 40.45%) percentage increase (P <0.05).
Conclusion: Garlic extract has a significant effect in reducing body weight and blood glucose levels.

Keywords: Garlic, diabetes, lipid profile, body weight

INTRODUCTION
Dietary factors play a key role in health and longevity, among dietary factors include the number of calories consumed has been unequivocally and causally associated with the risk of many prominent age-related diseases (1). Diabetes mellitus comprises a group of chronic diseases characterized by hyperglycemia or diminished insulin secretion, or both (2). In the year 2004, according to the World Health Organization reports, more than 150 million people throughout the world suffered from diabetes (3) while mankind has been unable to solve this problem. The prevalence of diabetes mellitus in Nigeria has increased from 2.2% in 1997 to 5.0% by 2013 (4). Type 2 diabetes is increasing in adolescents and more than half of the deaths due to diabetes occur in people less than 60 years old. Diabetes is accompanied by different complications and in a recent study the prevalence of hypertension and peripheral neuropathy in Nigeria for patients who are diabetic were more than 50% while the prevalence of retinopathy was 35%, cataract 25%, cardiovascular disease 5%, foot ulcer 16% and nephropathy 3% (5).

Although current diabetes treatment relies mainly on a medication arsenal established since the advent of insulin (6), diabetes treatment prior to 2000 was based on dietary changes, including the use of traditional anti-hyperglycemic herbs (7). Traditional plant remedies for diabetes, according to the World Health Organization, should be investigated further (8). For the treatment of diabetes, many herbal medications have been suggested (9, 10). Plant medications are often thought to be less harmful and have fewer negative effects than manufactured drugs (11). Garlic's anti-hyperglycemic (12-13) and anti-microbial (14-18) properties have been confirmed in some investigations.

Garlic has several beneficial compounds, including selenium and germanium, which are sulphur antioxidants property as well as vitamins A, C, and E, which aid in the scavenging of damaging free
radicals and the elimination of low-density lipoprotein from the bloodstream, thereby increasing high-density lipoprotein levels (14). According to Sukander et al. (19), garlic has been proposed to have direct preventive and anti-atherosclerotic (causing regression) effects at the artery wall. Garlic also increases the body’s metabolic rate by stimulating the adrenal gland to release adrenaline which increases the rate of fat metabolism in the body and in turn helps burn more calories to decrease weight (20). Some researchers have proved that the regular addition of garlic to the diet can help reduce blood cholesterol and glucose levels (21-23).

In the current literature, there is a paucity of information regarding the efficacy of garlic extract. Thus the purpose of the present study was to compare the efficacy of raw and boiled garlic extract on body weight, fasting blood glucose, and lipid profile of alloxan-induced adult male Wistar rats.

**MATERIALS AND METHODS**

**Study design**
An experimental study design was employed.

**Collection and identification of samples**
Garlic was procured from Ogige market in Nsukka and taken to the Department of Plant Science and Biotechnology Department, Faculty of Biological Sciences, University of Nigeria, Nsukka where it was identified as *Allium sativum*.

**Preparation of sample**
The garlic was peeled and thoroughly washed with clean water and put in a colander to drain water for 15 minutes. After the water had been completely drained, the fresh garlic was blended using a binatone blender of BLG 450 model. The garlic was filtered using a porous sieve of 0.45-micron pores in order to extract the juice (extract). Fresh juice extract was prepared every day during the feeding trial. 10mg, 20mg, and 30mg of raw garlic extract per kilogram body weight of the rats were administered in three different rat groups whereas 10mg, 20mg, and 30mg of cooked garlic extract per kilogram body weight of the rats were administered in another three different rat groups, therefore a total of 6 groups of rats were fed the garlic extract.

**Rat study**

**Animal and housing**
Twenty-eight adult male Wistar rats of about 24 weeks old weighing between 120-140g was purchased from the Faculty of Veterinary Medicine, Department of Veterinary Pathology, University of Nigeria, Nsukka, Nigeria. The rats were grouped into seven (24) based on body weight with a difference not more than ±5g per group. Each group was labeled A, B, C, D, E, F, and G with 4 rats in each group.

**Induction of diabetes in rats and blood glucose determination**
The 28 rats fasted overnight prior to injection of alloxan dissolved in iced cold normal saline at a dose of 150mg/kg body weight and the routes of administration were intraperitoneal injection (25). After 1 hour of alloxan administration, the rats were fed 5% dextrose solution in a feeding bottle for a day to overcome the early hypoglycaemic phase. The rats were kept under observation and after two days the blood glucose was confirmed using an Accu Chek glucometer. The diabetic rats with blood glucose levels 200mg/dl and above were separated into 8 different groups with each containing 4 rats and used for the experimental studies (25).

**Feeding the rats**
The rats were fed with commercial grower vital feed (rat chow) and water *ad libitum* for 5 days to acclimatize them to laboratory hygienic conditions and diet. The individual weights of the rats were taken on the 1st and final day to determine the weight gain. All the rats were induced with alloxan so they can become diabetic. After 48 hours of induction, the blood glucose levels of the rats were determined using an accurate glucometer. The rats are said to be diabetic if their fasting blood glucose level is more than 11.1mmol/l or 200mg/dl. All the rats received rat chow and water *ad libitum* throughout the study.

**Biochemical analysis**

**Fasting blood glucose determination**
After 8-12 hours of fast, the blood glucose of the rats was measured 48 hours after diabetes induction and on the final day (26). An Accu-chek active glucometer whose capacity is 600ml (33mmol/L) was used to check the blood glucose level. An Accu-chek active glucose test strip was inserted into the glucometer. This automatically switches the glucometer on. Cotton wool was used to apply methylated spirit on the rats’ tails to sterilize the area. An Accu-cheksoftellix lancet was inserted into the Accu-cheksoftellix which was used to prick the rats’ tails. When a dropping sign is seen on the display of the glucometer, a small drop of the rats’ blood was applied in the middle of the orange-coloured, squared application area of the test strip. The glucometer then measured and displayed the level of glucose in the blood. Reading was taken in mg/dl (26).
Blood lipid profile determination
Blood samples were collected by ocular punctures into plain bottles. The supernatant (serum) collected was used for the lipid analysis. Triglycerides were determined by the method of Richmond (27). Total cholesterol was determined after enzymatic hydrolysis and oxidation of the sample as described by Roeschla (28). HDL-Cholesterol and LDL-Cholesterol was estimated according to the method described by Peter (29).

Statistical Analysis
All statistical analysis was carried out using IBM SPSS statistical software version 21. All values obtained were expressed as mean and standard deviation. Paired sample T-test was used to compare baseline values and end values. The Differences in mean were considered significant at p< 0.05.

RESULTS
Table 1 shows the mean total cholesterol levels of the rats before and after treatment. From the result of the experiment, those fed with 10mg of raw garlic extract (RGEss) showed no significant difference while those fed with 10mg of CGE showed a significant difference. Those fed with 20mg of RGE and 20mg of cooked garlic extract (CGE) were not significantly different (P<0.05). Those fed with 30mg of RGE and 30mg of CGE were significantly different (P<0.05). The control made a significant increase when compared to those fed with 10mg, 20mg, and 30mg of raw and cooked garlic extract respectively.

Table 1: Mean total cholesterol level (mg/dl) of rats before and after treatments

<table>
<thead>
<tr>
<th>Groups &amp; Doses administered</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Percentage difference (Δ %)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mg RGE</td>
<td>80.25±8.18</td>
<td>90.50±3.41</td>
<td>12.77†</td>
<td>0.060</td>
</tr>
<tr>
<td>10mg CGE</td>
<td>94.25±6.55</td>
<td>112.50±3.42</td>
<td>19.36†*</td>
<td>0.003</td>
</tr>
<tr>
<td>20mg RGE</td>
<td>84.00±6.68</td>
<td>89.25±6.45</td>
<td>6.25†</td>
<td>0.301</td>
</tr>
<tr>
<td>20mg CGE</td>
<td>87.25±4.99</td>
<td>89.00±4.08</td>
<td>2.01†</td>
<td>0.607</td>
</tr>
<tr>
<td>30mg RGE</td>
<td>83.25±4.57</td>
<td>94.25±4.92</td>
<td>13.21†*</td>
<td>0.017</td>
</tr>
<tr>
<td>30mg CGE</td>
<td>82.75±2.75</td>
<td>91.75±5.06</td>
<td>10.88†*</td>
<td>0.020</td>
</tr>
<tr>
<td>Control</td>
<td>87.25±7.27</td>
<td>142.00±17.68</td>
<td>38.56†*</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Significant at P<0.05; RGE= Raw garlic extract; CGE= Cooked garlic extract; Control= No treatment

Table 2 shows the mean triglyceride levels of rats before and after treatments. The rats administered 10mg of RGE increased the triglyceride level while 10mg of CGE significantly increased it. Those administered 20mg of RGE and 20mg of CGE increased the triglyceride level. Those given 30mg of RGE significantly increased the triglyceride level while 30mg of CGE made no significant increase. The control made the highest increase as there was a significant difference (P<0.05).

Table 2: Mean triglyceride levels of rats before and after treatments

<table>
<thead>
<tr>
<th>Groups &amp; Doses administered</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Percentage difference (Δ %)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mg RGE</td>
<td>95.00±5.03</td>
<td>105.00±6.83</td>
<td>10.53†</td>
<td>0.057</td>
</tr>
<tr>
<td>10mg CGE</td>
<td>98.50±8.06</td>
<td>109.25±3.40</td>
<td>10.91†*</td>
<td>0.049</td>
</tr>
<tr>
<td>20mg RGE</td>
<td>100.75±8.14</td>
<td>103.50±3.11</td>
<td>2.73†</td>
<td>0.551</td>
</tr>
<tr>
<td>20mg CGE</td>
<td>104.50±7.55</td>
<td>114.75±9.07</td>
<td>9.81†</td>
<td>0.133</td>
</tr>
<tr>
<td>30mg RGE</td>
<td>100.25±5.91</td>
<td>115.25±2.22</td>
<td>14.96†*</td>
<td>0.003</td>
</tr>
<tr>
<td>30mg CGE</td>
<td>103.75±7.50</td>
<td>108.50±3.70</td>
<td>4.58†</td>
<td>0.314</td>
</tr>
<tr>
<td>Control</td>
<td>99.50±6.45</td>
<td>139.75±14.71</td>
<td>40.45†*</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Significant at P<0.05; RGE= Raw garlic extract; CGE= Cooked garlic extract; Control= No treatment
Table 3 shows the mean fasting blood glucose level of rats before and after treatments. The group fed with 10mg of RGE and 10mg of CGE decreased the fasting blood glucose level from 264.50 mg/dl to 98.50 mg/dl and 342.75 mg/dl to 115.00 mg/dl respectively. Those fed with 20mg of RGE and 20mg of CGE decreased the fasting blood glucose level from 351.25mg/dl to 93.50mg/dl and 300.00mg/dl to 99.50 mg/dl and those fed with 30mg of RGE and 30mg of CGE decreased the fasting blood glucose level from 269.50mg/dl to 113.25mg/dl and 391.25mg/dl to 96.25mg/dl respectively. The control showed no significant difference (P<0.05).

Table 3: Mean fasting blood glucose level (mg/dl) of rats before and after treatment

<table>
<thead>
<tr>
<th>Groups &amp; Doses</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Percentage difference (Δ%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mg RGE</td>
<td>264.50±47.25</td>
<td>98.50±6.19</td>
<td>62.75↓*</td>
<td>0.005</td>
</tr>
<tr>
<td>10mg CGE</td>
<td>342.75±103.25</td>
<td>115.00±6.83</td>
<td>66.44↓*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20mg RGE</td>
<td>351.25±44.91</td>
<td>93.50±6.19</td>
<td>73.38↓*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20mg CGE</td>
<td>300.00±61.71</td>
<td>99.50±7.19</td>
<td>66.83↓*</td>
<td>0.001</td>
</tr>
<tr>
<td>30mg RGE</td>
<td>269.50±92.87</td>
<td>113.25±25.26</td>
<td>57.98↓*</td>
<td>0.018</td>
</tr>
<tr>
<td>30mg CGE</td>
<td>391.25±58.37</td>
<td>96.25±5.56</td>
<td>75.40↓*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>432.25±91.05</td>
<td>362.50±164.60</td>
<td>16.14↓</td>
<td>0.486</td>
</tr>
</tbody>
</table>

*Significant at P<0.05; RGE= Raw garlic extract; CGE= Cooked garlic extract; Control= No treatment

Figure 4 shows the mean weight of rats before and after treatment. There was a significant decrease in the fasting blood glucose level and mean weight of the rats administered the experimental diets while the control group showed a significant (P < 0.05) increase in these parameters. The rats fed 20 and 30 mg of RGE had the highest percentage decrease in body weight while the control made a significant difference (P<0.05) as the mean weight of the rats increased.

Table 4: Mean weight of rats before and after treatment

<table>
<thead>
<tr>
<th>Groups &amp; Doses</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Percentage difference (Δ%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mg RGE</td>
<td>131.75±0.39</td>
<td>128.63±0.41</td>
<td>2.37↓*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10mg CGE</td>
<td>132.25±0.21</td>
<td>130.43±0.30</td>
<td>1.85↓*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20mg RGE</td>
<td>131.78±0.22</td>
<td>127.45±0.34</td>
<td>3.29↓*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20mg CGE</td>
<td>131.38±0.30</td>
<td>129.43±0.22</td>
<td>1.48↓*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30mg RGE</td>
<td>133.45±0.41</td>
<td>128.50±0.41</td>
<td>3.71↓*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30mg CGE</td>
<td>132.40±0.24</td>
<td>129.95±0.13</td>
<td>1.85↓*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>131.33±0.22</td>
<td>136.63±0.31</td>
<td>4.04↑*</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Significant at P<0.05; RGE= Raw garlic extract; CGE= Cooked garlic extract; Control= No treatment

DISCUSSION

Diabetes mellitus is a highly prevalent chronic illness and a major socio-economic burden with serious health consequences. A study conducted by the World Health Organization reported that the worldwide prevalence of diabetes in 2002 was 170 million, with the number predicted to grow to 366 million by 2030 (30).

The high concentration of total cholesterol and triglyceride observed in the diabetic rat in this study was inconsistent with the report of several studies demonstrating that a rise in glucose level on induction of diabetes, results in a corresponding increase in serum lipid (31). Hyperlipidaemia is a recognized complication of diabetes mellitus characterized by an elevated level of cholesterol and triglyceride (32) it has been reported that elevated serum lipids in diabetes are due to the increased mobilization of free fatty acids from peripheral fat depots as a result of inhibition of the hormones sensitive lipase (33). The excess fatty acid produced is converted into phospholipids and cholesterol, which together with excess triacylglycerols formed at the same time in the liver are discharged into the blood in the form of lipoproteins. Thus, the marked hyperlipidaemia observed in diabetic rats may be regarded as a consequence of the uninhibited action of lipolytic hormones in fat depots (34).

The observed significant reduction in the blood glucose level of the diabetic rats fed the raw and
cooked garlic extract is an indication that the extract contains bioactive phytochemicals with potent anti-diabetic properties. The result compared favorably with the fasting blood-lowering effect of *Barleriapriorities* (35). According to Iweala and Oludare (36), reduction in blood glucose by most bioactive compounds from plants might act by one of several mechanisms including the stimulation of insulin secretion, increased repair or proliferation of beta-cells, and enhancing the effect of insulin. The possible mechanism by which raw and cooked garlic extract reduced fasting blood glucose levels of diabetic rats in this study agrees with their findings.

The result of this study showed a decrease in the mean body weight of animals with significant percentage weight changes, indicating that the extracts might be toxic to the animal. The control rats showed an increase in body weight because they did not consume the extracts thereby, they had more body weight compared to the other groups. This observation disagreed with the reports of Mohamood (26) and Tanko (37) which recorded an increase in the mean body weight of rat-fed plant extract. This study, therefore, indicates that the level of the raw and cooked garlic extract had an adverse effect on the weight loss of the rats, which may be due to inadequate utilization of the extracts to maintain growth.

**CONCLUSION**

From this study there were significant changes in the lipid profile and body weight of the rats fed with the test materials (various groups) when compared with that of the control. Thus, garlic has effect on lipid profile and can also have such effects on cardiovascular diseases that are caused by excessive increases in cholesterol and low-density lipoprotein levels.

**Conflict of interest:** We declare that no conflict of interest exists.

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**Authors’ Contributions:** Peace N. Ani & Joy C. Obi conceived and designed of the study. Ogechukwu P. Umeakuka & Joy C. Obi procured and processed the samples. Peace N. Ani, Ogechukwu P. Umeakuka & Joy C. Obi performed data analysis, drafted the manuscript & interpreted the study. All authors reviewed and approved the final manuscript.

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