Comparative Evaluation of Hypoglycemic Properties of Raw And Boiled Allium cepa in Alloxan-Induced Diabetes Mellitus Rats

Ojieh, AE1*, Ugorji AE1, Ovuakporaye IS1, Ewhre OL2; Ossai NR2

1Department of Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, PMB1, Abraka Nigeria
2Department of Pharmacology and Biotechnology, Emma-maria Scientific Research Laboratories & Consultancy, P. O. BOX 85, Abraka, Nigeria

Abstract

Diabetes Mellitus is a chronic disorder of carbohydrate metabolism whose prevalence is rising globally. The hypoglycemic property of raw Allium cepa has been established by several authors, and this study, was carried out to investigate the effect of heat on the hypoglycemic property of Allium cepa. This is against the backdrop that Allium cepa is mostly consumed boiled in Africa and several parts of the World. 40 Wistar rats were used for the study and diabetes was induced in 35 of them using single intraperitoneal dose of 40mgkg\(^{-1}\) of alloxan monohydrate. There were eight groups (n= 5). Group one and two served as the control groups, group three is insulin and group four is metformin. Group five to eight were treatment groups. Result showed the following reduction at 400 mgkg\(^{-1}\) (raw -57.2%; boiled -41.3%). At 600 mgkg\(^{-1}\), (raw -30.9%; boiled-20.9%). It is therefore concluded based on these findings that boiling Allium cepa reduced its hypoglycemic potential and hence its effectiveness in ameliorating hyperglycemia associated with diabetes mellitus. In the same experiment, insulin at (40 µgkg\(^{-1}\)) and metformin at (50 mgkg\(^{-1}\)) show plasma glucose reduction of (52.7%) and (12.3%) respectively when compared with the control. Result shows that heat reduces hypoglycemic property of onions.

Keywords: Diabetes Mellitus, Herbal, Allium cepa, Alloxan, Hypoglycemia

1 Introduction

Diabetes Mellitus is a disease associated with severe derangement of carbohydrates, protein and lipids metabolism\(^1\). It continues to attract public concern, not just in developing countries but also in developed countries like United State\(^2\). It presents usually with hyperglycemia and other metabolic abnormalities, which are due to deficiency of insulin effect\(^3\)\(^-\)\(^5\). According to “The Centers for Disease Control and Prevention (CDC)\(^6\)”, estimated about 285 million people to be suffering form the disease as of 2010. An estimate by the International Diabetes Federation, also put the number of people suffering form the disease at 381 million by 2013 and forcast a geometrical increase to double the number by 2030. (IDF, 2014)\(^7\). Diabetes mellitus occurs globally, but more common (especially type 2) in the more developed countries\(^3\)\(^-\)\(^4\). The increase in incidence in developing countries follows the trend of urbanization and lifestyle changes, perhaps most importantly a “Western-style” diet. This has suggested a dietary etiological component to the occurrence of the diseases, especially the Type 2 diabetes. The prevention of type 2 diabetes is of great importance, especially when the disease is linked to obesity. Diabetes prevention is as basic as eating healthy, becoming more physically active and losing a few extra pounds\(^3\)\(^-\)\(^4\)\(^-\)\(^9\). The orthodox management for diabetes mellitus have several challenges notable side-effects and the high cost of the medication especially on longterm of treatment and these affect patient compliance; hence, alternative strategies to the pharmacotherapy of the disease are urgently needed.\(^10\) Well, over 400 medicinal plants globally have shown potentials as alternate or complimentary drugs for diabetes mellitus management, however they need to be scientifically authenticated to confirm their effectiveness as anti-diabetic
agents\textsuperscript{15}. Substances with hypoglycemic properties would be effective in the management of diabetes mellitus; and one of such substances has been reported to be an onion bulb (Allium cepa)\textsuperscript{22}.

1.2 Allium cepa Linn, (onion bulb)

Onion (Allium cepa L) is a vegetable and mostly used as condiment to food\textsuperscript{12}. There are several genus such as;

- *Allium fistulosum* - Japanese bunching onion
- *Allium proliferum* - Egyptian onion
- *Allium Canadense* - Canada onion\textsuperscript{14}

Apart from its anti-diabetic activities which have been reported by several researchers, onion (Allium cepa) has been reported to have various biological activities, such as anti-tumor, anti-bacterial and anti-viral activities\textsuperscript{15-19}.

Onions is found in almost all traditions and cultures and its consumption is increasing significantly, particularly in the United States and this is partly because of heavy promotion that links flavor and health\textsuperscript{20}. It is consumed a lot in Nigeria but mostly cooked and mixed in food. Scientific analysis shows two chemical groups that are thought benefits to human health; these are the flavonoids and the alk(en)yl cysteine sulphoxides (AcSOs)\textsuperscript{20,21}.

Two flavonoids sub-groups are found in onion (Allium cepa); Cleaving of AcSOs by the enzymes allinase, generate the characteristic odour and taste of onion\textsuperscript{20}. The downstream products are a complex mixture of compounds which includes thiosulphinates, thiosulphonates, mon-di- and tri-sulphides.\textsuperscript{20}

Composites from onion (Allium cepa) have been reported to have a range of health benefits which includes; Anticarcinogenic properties, Antiplatelet activity, Antithrombotic activity, Anti asthmatic effect, Antibiotic effect\textsuperscript{22,26}.

It is believed that methcysteine and flavonoids in onion (Allium cepa) helped to decrease elevated levels of serum blood glucose, lipids, oxidative radical and lipid peroxidation, while increasing the antioxidant enzymes activities and insulin secretion\textsuperscript{24}. Mohammed et al., (2007)\textsuperscript{13} has earlier reported the hypoglycemic activity of onion (Allium cepa). At the 97\textsuperscript{th} annual meeting and expos of the endocrine society of the United State, tagged, ‘ENDO 2015’, in San Diago, California, Anthony Ojieh, presented a research article on the hypoglycemic and hypolipidemic properties of raw extract of onion (Allium cepa) on diabetic rats. This article has been published, (Ojieh et al., 2015)\textsuperscript{19}.

1.3 Effect of Heat on some vegetables

When vegetables are exposed to higher temperatures as is the case during cooking, heat-sensitive compounds are destroyed reducing their nutritional value and antioxidant activity\textsuperscript{27}. Heating is accountable for the oxidation, thermal degradation, and leaching of bioactive compounds from fresh vegetables\textsuperscript{28}. Depending on the chemical and physical make-up, and nutritional properties of vegetables, heating can bring about a positive and negative effect\textsuperscript{15}. Different heating conditions (temperature and duration of heating) have different effects on the antioxidant properties of vegetables\textsuperscript{19} and food component viability. Lycopene bioavailability, for example, has been shown to improve by heating tomato in oil.\textsuperscript{31} However, Chen et al., (1985)\textsuperscript{29} found that boiling garlic at 100 °C for 20 min completely suppressed its antibacterial activities. Bordia et al., (1996)\textsuperscript{30} reported a dose-dependent inhibition of serum thromboxane B2 (TXB2) concentration in rats treated with aqueous extracts of raw garlic.

Onions belong to the same biological category as garlic, leeks and chives and to obtain maximum health benefits, raw onion should be used or moderately cooked. This study was carried out to investigate the effect of heat on the hypoglycemic property of Allium cepa, mostly onion is consumed after exposure to heat.

2 Materials and methods

2.1 Chemicals and Drugs

Sodium Citrates (C6H4(OH)(COONa)2H2O) (BDH chemicals LTD Pools England), Alloxan (monohydrate) LR, C6H6N2O2, H2O, (Qualikems Mumbai, INDIA). Metformin (Glucophage) Tablet. Insulin Injectable (The Rapid-acting type insulin). All the chemicals and drugs used were of analytical grade.

2.2 Collection of Plant Material

The bulbs of Allium cepa were bought from Abraka market, Ethiope East Local Government Area, Delta State, and identified and authenticated by in Department of Botany, Delta State University, Abraka, Nigeria were the voucher specimen was kept.

2.3 Preparation of Onion Extract

The bulb of Allium cepa was prepared by the method described by Azu et al., (2007)\textsuperscript{34}; 2000gm of fresh onions was washed and chopped into small pieces, blended, homogenized and soaked in 2 L of distilled water. The mixture was allowed to stand for 24 h with intermittent shaking. Following filtration, the filtrates were heated to dryness in a water bath and the weight of the crude extract determined. The extract was labeled “raw” and kept in refrigerator (4°C) thereafter. 2000 gm of another set of fresh onions were also washed; cut and boiled for 30 minutes, it was then blended and the same protocol for the raw was followed and the resultant extract labeled boiled”. The extract was later reconstituted in normal saline (0.85% NaCl) at a concentration of 1 g/ml before administration.

2.4 Animal
Forty Wistar male rats weighing 150-180 gm were used for this study. The animals were housed in the Animal facility of Department of Human Physiology, faculty of Basic Medical Sciences, Delta State University, (DELSU), Abraka, Nigeria. The animals were randomly divided into experimental and control groups and were housed in metabolic cages. They were maintained on standard animal feeds growers mash diet (a product of Top Feed, Sapele, Delta State, Nigeria) and water ad libitum.

Experimental procedures involving the animals and their care were conducted in conformity with international, national and institutional guidelines for the care of laboratory animals in biomedical research (NRC; 1996) and Permission was obtained from the Bioethics Committee for the Use of Animals for Research of the Faculty of Basic Medical Science, Delta State University Abraka, Nigeria (RBC/FBMS/DELSU/14/04).

2.5 Drug Preparations

2.5.1 Citrate Buffer and Alloxan

100 ml of distill water was used to dissolve 2 g of sodium citrate and 50 ml of the stock solution was used to dissolve 0.4 g of Alloxan monohydrate.

Five grams (5 g) of the metformin (Glucophage) tablet was dissolved in 100 ml of distilled water, to give a solution of anti-diabetic drugs to get the appropriate concentration that will be administered to the animals.

2.6 Induction of Experimental Diabetes Mellitus

Thirty five of the rats were fasted for about 18 - 24 hours with free access to water prior to the induction of diabetes. Before the induction, the body weight of each animal was recorded with the aid of an electronic weighing balance, and the Fasting Blood Glucose Level (FBGL) was also checked with the aid of a glucometer (Accu-Chek). The 40 rats were divided randomly into eight groups (Group 1 - Group 8) of five rats each. Group one was served as the positive control, hence the rats were not induced with diabetes and were not treated. Induction of diabetes was done on rats in Group two to Group eight by single intraperitoneal injections 40mg/kg of Alloxan monohydrate.

The successful induction was assessed 72 hours later using the glucometer to check the fasting blood glucose level (FBGL) after the injection of alloxan. Animals with blood glucose level of 50% and above beyond the pre-induction value was taken to be diabetic.

2.7 Animals Grouping and Treatment

Group 1 (n = 5) – Positive control group; rats were not induced and not treated within the period of the study.

Group 2 (n =5) – Negative control group; rats were induced diabetes with 40 mg/kg alloxan but not treated.

Group 3 (n = 5) – Diabetic Wistar rats were treated with 40 µg/kg Insulin subcutaneously.

Group 4 (n = 5) – Diabetic Wister rats were treated with 50 mg/kg of metformin diamet drugs orally.

Group 5 (n = 5) – Diabetic Wistar rats were treated with 600mg/kg Boiled Allium cepa juice orally.

Group 6 (n = 5) – Diabetic Wistar rats were treated with 400mg/kg Boiled Allium cepa juice orally.

Group 7 (n = 5) – Diabetic Wistar rats were treated with 600mg/kg Raw Allium cepa juice orally.

Group 8 (n = 5) – Diabetic Wistar rats were treated with 400mg/kg Raw Allium cepa juice orally.

2.8 Animal Sacrifice and Sample Collection

The treatment lasted for three weeks, at the end of which the animals were fasted for 12 – 18 hours, the body weight and the FBGL measured and then rats were sacrificed by cervical dislocation, and each rat was placed its dorsal surface, and a laparotomy were carried out to expose the internal organs, and blood was collected by cardiac puncture, using 5 ml syringe and 21G needle into plain blood sample containers. The blood samples were centrifuged at a rate of 4000 rpm for 10 minutes and the serum was collected and store in a refrigerator at 4 °C for analysis.

2.9 Statistical Analysis

All data collected are expressed as mean± SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by LSD’s Multiple Range Test (Fisher et al., 1956). The results were considered statistically significant if the P-value were 0.05 or less. The data were analyzed using Graphed Prism Versions San Diego, USA.

3 Results

The Table 1 shows the effect of Allium cepa Linn (raw and boiled juice extract), insulin and metformin on the body weight in normal and alloxan induced diabetic male Wistar rats after 21 days of treatment. Treatment after 21 days showed restoration of body weight in the treatment groups when compared to the negative control group.

Table 2 shows the effect of Allium cepa Linn (raw and boiled juice extract), Insulin, and Metformin Diamet drugs on the fasting blood glucose in normal and alloxan induced diabetic adult male Wistar rats.

The administration of Insulin at 40 µg/kg, Metformin at 50mg/kg and Allium cepa Linn juice of raw and boiled (400mg/kg and 600mg/kg) respectively reduced the blood glucose in diabetic
rats with the group treated with 400 mg/kg of the raw extract of Allium cepa showing most reduction in the blood glucose (table 3).

Table 1: Effect of Allium cepa Linn raw and boiled juice extract on body weight in normal and alloxan induced diabetic adult male Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (gm) (Mean±SEM)</th>
<th>Pre-treatment</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>initial</td>
<td>Base</td>
</tr>
<tr>
<td>Control</td>
<td>96.00±1.87</td>
<td>109.20±2.90</td>
<td>126.20±31.29</td>
</tr>
<tr>
<td>Negative control</td>
<td>144.56±3.58</td>
<td>133.58±2.52</td>
<td>129.25±2.76</td>
</tr>
<tr>
<td>Insulin 40µg/kg</td>
<td>151.48±2.29</td>
<td>141.72±2.28</td>
<td>140.94±2.09</td>
</tr>
<tr>
<td>Metformin 40mg/kg</td>
<td>122.00±4.64</td>
<td>101.60±1.03</td>
<td>105.00±1.87</td>
</tr>
<tr>
<td>Boiled 600mg/kg</td>
<td>121.52±8.06</td>
<td>122.00±2.00</td>
<td>105.00±6.52</td>
</tr>
<tr>
<td>Boiled 400mg/kg</td>
<td>119.94±8.16</td>
<td>114.40±4.02</td>
<td>110.60±6.94</td>
</tr>
<tr>
<td>Raw 600mg/kg</td>
<td>120.96±1.87</td>
<td>126.00±5.10</td>
<td>122.00±9.70</td>
</tr>
<tr>
<td>Raw 400mg/kg</td>
<td>96.00±1.87</td>
<td>126.00±5.10</td>
<td>122.00±9.70</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Number of animals, n=5); significantly different at #P when compared with negative control (p<0.05)

Table 2: Effect of Allium cepa Linn raw and boiled juice extract on fasting blood glucose in normal and alloxan induced diabetic adult male Wistar rats

<table>
<thead>
<tr>
<th>Fasting blood glucose level (mg/dl)</th>
<th>Pre-treatment</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>7 day</td>
</tr>
<tr>
<td>Control</td>
<td>70.80±3.54</td>
<td>78.00±1.67</td>
</tr>
<tr>
<td>Negative control</td>
<td>85.00±3.65</td>
<td>298.60±38.85</td>
</tr>
<tr>
<td>Insulin 40µg/kg</td>
<td>87.60±2.87</td>
<td>314.00±21.31</td>
</tr>
<tr>
<td>Metformin 40mg/kg</td>
<td>116.00±6.00</td>
<td>125.00±6.33</td>
</tr>
<tr>
<td>Boiled 600mg/kg</td>
<td>78.20±2.60</td>
<td>152.60±59.00</td>
</tr>
<tr>
<td>Boiled 400mg/kg</td>
<td>79.00±5.12</td>
<td>187.40±48.39</td>
</tr>
<tr>
<td>Raw 600mg/kg</td>
<td>75.60±3.84</td>
<td>162.00±26.38</td>
</tr>
<tr>
<td>Raw 400mg/kg</td>
<td>93.20±6.55</td>
<td>284.40±29.65</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Number of animals, n=5); significantly different at #P when compared with negative control (p<0.05); significantly different at *P when compared with insulin group (p<0.05); significantly different at +P when compared with insulin group (p<0.05)

4 Discussions

Hypoglycemic properties of various herbal preparations have been reported by several authors38-42.19 reported a dose-dependent reduction in blood glucose levels of diabetic rats after 6 weeks of treatment with Allium cepa. Comparing the effect of daily administration of Allium sativum and Allium cepa extract on alloxan-induced diabetic rats, Ojo et al.14, reported a significant serum glucose reduction with onion extract. This hypoglycemic property was attributed to the presence of allyl propyl disulphide and other sulphur containing amino acids contained in onions. These compounds are reported to mop-up reactive oxygen species and free radicals which mediate the toxic action of alloxan on pancreatic beta cells, thereby enhancing the release of insulin42. In a research study that evaluated the phytochemical composition of Allium sativum and Allium cepa and the effect of their cooked and raw extract on lipid profile and hepatic biochemical parameters in albino wistar rats, (Gazuwa et al. 2013)13 it was reported that the useful
active principles in Allium cepa are usually affected by temperature variations, and this could lead to the loss of the medicinal significance. However, reports showing how heat affects the hypoglycemic potency of some of these herbs have not being explored much. Onions is consumed mostly cooked in several parts of the world, and evidence base reports for and beneficial health use is what informed this research study. Results from this study showed that heated onions irrespective of the dose had reduced hypoglycemic potency [Raw: 600 mg kg\(^{-1}\) (30.9%), 400 mg kg\(^{-1}\) (57.2%); Boiled: 600 mg kg\(^{-1}\) (20.9%); 400 mg kg\(^{-1}\) (41.3%)]. This finding is clearly supported by the study carried out by Gazuwa et al. (2013)\(^4\), demonstrating that heating onions destroyed some heat sensitive bioactive compounds like the sulphur containing amino acids that are responsible for the hypoglycemic property of the vegetable.

Table 3: Percentage change in the plasma glucose level after 21 days of treatment

<table>
<thead>
<tr>
<th></th>
<th>Pre-induction</th>
<th>Post-induction</th>
<th>After 21 days treatment</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>70.80±3.54</td>
<td>78.00±1.67</td>
<td>102.40±12.34</td>
<td>-31.28</td>
</tr>
<tr>
<td>Negative control</td>
<td>85.00±3.65</td>
<td>298.60±38.85</td>
<td>600.00±0.00</td>
<td>-100.94</td>
</tr>
<tr>
<td>Insulin 40 µg/kg</td>
<td>87.60±2.87</td>
<td>314.00±21.31</td>
<td>148.40±7.56</td>
<td>52.74</td>
</tr>
<tr>
<td>Metformin 40 mg/kg</td>
<td>116.00±6.00</td>
<td>125.00±6.33</td>
<td>109.68±4.12</td>
<td>12.26</td>
</tr>
<tr>
<td>Boiled 600 mg/kg</td>
<td>78.20±2.60</td>
<td>152.60±59.00</td>
<td>120.72±10.68</td>
<td>20.89</td>
</tr>
<tr>
<td>Boiled 400 mg/kg</td>
<td>79.00±5.12</td>
<td>187.40±48.39</td>
<td>110.04±13.69</td>
<td>41.28</td>
</tr>
<tr>
<td>Raw 600 mg/kg</td>
<td>75.60±3.84</td>
<td>162.00±26.38</td>
<td>112.00±9.57</td>
<td>30.86</td>
</tr>
<tr>
<td>Raw 400 mg/kg</td>
<td>93.20±6.55</td>
<td>284.40±29.65</td>
<td>121.82±11.46</td>
<td>57.17</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. ANOVA followed by LSD’s multiple range tests. Values with P≤0.05 are considered significant.

5 Conclusions
The medicinal significance of onions has been reported by several authors and the bioactive chemical as well as phytochemical responsible for the various medicinal properties elucidated. Most of these compounds are however heat sensitive and can be easily destroyed by cooking, hence from the findings in this study, for management of hyperglycemia in diabetes; Allium cepa is best consumed raw.

6 Conflicts of Interests
No conflict of interest

7 Author’s contributions
This work was carried out in collaboration between all authors. Author OAE designed the study, wrote the protocol and interpreted the data. While author UAE and OIS managed the literature searches and produced the initial draft. Author EOL and ONR anchored the field study, gathered the initial data and performed preliminary data analysis. All authors read and approved the final manuscript.

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