Comparative Effect of Daily Administration of Allium sativum and Allium cepa Extracts on Alloxan Induced Diabetic Rats

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Abstract: Effective regulation of blood glucose level is the most important factor for reducing risks of diabetic complications and this must be done without any side effects or interference with normal physiological function of the body which is not usually achievable with the conventional diabetic drugs. This has resulted in the current shift to the use of herbal preparation which is presumed to be effective, cheap with no side effects. Most noted among these are onion and garlic. This present study was therefore conducted to compare the effects of daily administration of garlic and onion extracts on alloxan induced diabetic rat. Diabetes was induced in Female Wister rats weighing 150-200g by intraperitoneal injection of freshly prepared alloxan monohydrate (150 mg/kg body weight). Diabetes was confirmed after seven days in rats showing fasting blood glucose levels ≥ 200mg/dl. The diabetic rats were randomly allocated into three experimental groups which received garlic extract, onion extract or normal saline depending on the group. The extracts were administered orally for six weeks after which the animals were sacrificed and blood samples were collected for biochemical analyses. From the results obtained it can be concluded that the extracts of fresh Allium sativum and Allium cepa significantly reduced the serum glucose with Allium sativum extract proving to be more potent while no significant difference was shown in their nephro- and hepatoprotective activities.

Keyword: alloxan, diabetes, Garlic, herbal preparation, Onion

I. Introduction

The current prevalence of diabetes mellitus in the third world countries especially Africa has been linked to the spread of Western life styles like physical inactivity combined with diets that are high in calories; processed carbohydrates, saturated fats and insufficient dietary fiber [1, 2]. The estimated number of people with diabetes is expected to hit 380 million by 2025 and most of them will likely be found in Asia and Africa [3, 4].

Diabetes mellitus has been reported to be the major cause of blindness, kidney failure, lower-extremity amputation, cardiovascular diseases and premature mortality [5]. Effective control of blood glucose level is still the most important factor for reducing risks of diabetic complications [6] and this must be done without any side effects or any interference with normal physiological function of the body which is not usually achievable with the conventional diabetic drugs. This has resulted in the current shift to the use of herbal preparation which is presumed to be effective, cheap with fewer or no side effects [7]; most noted among these are ginger, onion and garlic which appear to be most effective and least toxic [8].

Allium cepa belongs to the family Liliaceae and is widely cultivated throughout the world [9], its most popular modern uses include regulation of blood pressure, blood glucose, blood cholesterol level [9, 10] and as antiseptic[11]. Active ingredient in A. cepa include phenolic compounds (flavonoids, anthocyanins, phenolic acids and flavonols), organosulphur compounds, vitamins and some minerals [12, 13, 14], these compounds mediate the pharmacological effects of A. cepa. Thus, phenolic acids, such as caffeic, chlorogenic, ferulic, sinapic, p-coumaric acids, vanillic, syringic and p-hydroxybenzoic appear to be active antioxidants [15, 16]. Its vitamins especially vitamin C have a protective function against oxidative damage and a powerful quencher of singlet oxygen (O2), hydroxyl (OH) and peroxyl (RO2) radicals[17,18]. Garlic bulb contains chemically active substances such as enzymes, amino acids, minerals and sulphur containing compounds such as alliin (S-allyl cysteine sulphoxide (SACS) and allicin (diallyl disulphide) which are responsible for garlic’s pungent odour and many of its medicinal effects [19]. Allicin which is associated with garlic action does not exist in garlic until it is crushed or cut where injury to the garlic bulb activates the enzyme allinase which metabolizes allin to allicin[20]. Garlic has been found to have antibacterial and antifungal activity, reduce aortic plaque deposits[21],
inhibits vascular calcification in human patients with high blood cholesterol[22], reduce hyperlipidemia[23,24] and hyperglycaemia[25]. The primary purpose of this research was to compare biochemical effects of Allium sativum and Allium cepa extracts in alloxan induced diabetic rats.

II. Materials And Methods

2.1 Collection of Plant Samples
Plant samples were obtained from Masaka market in Nasarawa State and identified in Biological Sciences Department, Bingham University karu, Nasarawa State, Nigeria.

2.2 Preparation of Allium sativum (Garlic) and Allium cepa(onion)
The same method was used to prepare garlic and onion extract from their respective bulbs as follow: fresh healthy bulbs of garlic or onion weighing 50g were washed and peeled; the peeled bulbs were then cut into small pieces and homogenized in 70 ml cold, sterile 0.9% NaCl in the presence of some crushed ice. The homogenization was carried out in a blender at high speed using 30-second bursts for a total of 10 minutes. The homogenized mixture was filtered 3 times through cheesecloth, the filtrate was centrifuged at 2000 RCF for 10 minutes and the clear supernatant obtained was diluted to100 ml with normal saline. The concentration of this garlic or onion preparation was considered to be 500 mg/ml on the basis of the weight of the starting material (50g/100 ml). The aqueous extract of the garlic or onion was stored at 4 °C until use.

2.3 Experimental Animal
Female wister rats weighing 150-200g were purchased from Bingham University animal house Nasarawa state. The rats were approved for the experiment by the local committee and were housed in individual plastic cages with stainless steel at room temperature (25±3°C) at 12 hr dark-light cycles. All the rats were allowed free access to the same diets and water and acclimatized for 14 days before the treatment.

2.4 Preparation of alloxan
Two grams of crystalline alloxan monohydrates were dissolved in 50mls of normal saline (0.9% NaCl solution) to yield a concentration of 40mg/ml.

2.5 Induction of diabetes
The rats were divided into two groups before the induction of diabetes: non-diabetic control group and experimental group (to be induced with alloxan) with the weights of all the rats taken before alloxan administration. Diabetes was induced in the experimental rats after overnight fasting (12hrs) by intraperitoneal administration of 150mg of alloxan per kilogram body weight of rat (150mg/kg body weight).

After the induction, all the rats were allowed free access to the same feed and water and the alloxan-induced rats were carefully examined for the next 24hrs for evidence of allergic reactions, behavioural changes and convulsion.

After one week, blood was collected from the tail vein and diabetes was confirmed in alloxan treated rat with fasting blood glucose levels greater than 200mg/dl.

2.6 Experimental Design
The control and experiment animals were divided into different groups and treated accordingly:

- **Group 1** (non-diabetic group): normal control (receive 1ml normal saline through direct stomach intubation every day).
- **Group 2** (Diabetic control): receive 1ml normal saline through direct stomach intubation every day.
- **Group 3** (Garlic group): Diabetic rats receiving 400mg garlic extract per kilogram body weight daily.
- **Group 4** (Onion group): Diabetic rats receiving 400mg onion extract per kilogram body weight daily.

2.7 Preparation of samples for biochemical analysis
After six weeks of treatment with the different extracts, the body weights of all the rats were taken again and were allowed to fast for 12hr before they were sacrifice under sodium pentobarbitone anaesthesia. Whole blood was collected via cardiac puncture using sterile syringes and needles and emptied into plain bottles; this was allowed to clot for about two hours. The clotted blood was centrifuged at 3,500rpm for 30mins to recover the serum from clotted blood. Serum was separated with sterile syringes and needles and stored frozen until it is used for biochemical analysis.
2.8 Assays

The blood glucose in a protein free serum was determined as described by Sood [26]. Urea, creatinine and total bilirubin concentrations were determined by the methods of Patton and Crouch [27] Henry et al.[28] and Pearlman and Lee[29], respectively. Total cholesterol was measured by the procedure described by Allain et al.,[30]. Serum aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were estimated with the Randox reagent kit using 2, 4-dinitrophenylhydrazine as substrate according to the method described by Reitman and Frankel[31]. Protein content was determined by the method of Lowry et al. [32] while Alkaline phosphatase was assessed as described by Principato et al.[33]. All the assays were carried out at the Department of Biochemistry, Bingham University Karu Nasarawa state, Nigeria.

III. Result And Discussion

The administration of garlic extracts to the diabetic rats significantly reduced serum glucose compared to the diabetic group that did not receive the same treatment, although these reductions were not enough to bring it down to the normal rats’ serum glucose levels (Table 1). This effect of garlic has been reported for both alloxan and streptozotocin-induced diabetic rats[25,34,35,36], this ability to reduce serum glucose level is associated with the presence of -S-allyl cysteine sulphoxide, a sulphur-containing amino acid which has been shown to possess the potential to reduce the blood glucose in rats almost to the same level as insulin[37,38]. These active constituents of garlic extract are believed to act as hypoglycaemic agent by increasing either the pancreatic secretion of insulin from the beta cell or its release from bound insulin[2,39,40], interferes with intestinal glucose absorption[41] and peripheral glucose utilization[42,43] and lastly by stimulating the few surviving β-cells to produce insulin or regenerates β-cells of the islets, since β-cells have been shown to have a potential for regeneration[41, 44,45]. Onion extract also significantly reduce the serum glucose level .This has also been reported before by Eyo et al.[2] .This action may be attributed to allyl propyl disulphide and other sulphur compounds in onions[2,46] which may act by mopping up the Reactive Oxygen Species (ROS) and free radicals which mediate the toxic action of alloxan on pancreatic beta cells[47,48,49,50].The best reduction in blood glucose level was however observed with garlic extract (70.23%) compare to onion(63.35%) while normal saline administered to the diabetic rats did not affect the glucose levels.

Table 1: Effect of Allium cepa and Allium sativum on Blood Glucose Level of Diabetic Rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>DAY 1 (mg/dl)</th>
<th>DAY 28 (mg/dl)</th>
<th>% Change In Glucose Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL CONTROL</td>
<td>77.00 ± 7.35</td>
<td>79.00 ± 6.00</td>
<td>+2.60</td>
</tr>
<tr>
<td>DIABETIC CONTROL</td>
<td>245.50 ± 4.91</td>
<td>338.75 ± 7.42</td>
<td>+37.98</td>
</tr>
<tr>
<td>ONION (400mg/kg)</td>
<td>231.25 ± 3.40</td>
<td>84.75 ± 1.85</td>
<td>-63.35</td>
</tr>
<tr>
<td>GARLIC (400mg/kg)</td>
<td>374.50 ± 9.29</td>
<td>111.50 ± 10.29</td>
<td>-70.23</td>
</tr>
</tbody>
</table>

All Values Are Expressed As Mean ± Standard Deviation of Four Observations

As shown in Table 2, alloxan induction produced significant increase in the hepatic marker enzymes (ALT, AST and ALP). The increase in these hepatic marker enzymes (ALT, AST, and ALP) levels is as a result of these enzymes leaking from liver cells cytosol into bloodstream[51] since the toxic action of alloxan is mediated through the generation of free radicals[47,48] which eventually damage the liver cells leading to the leakage of these enzymes which are located in the cell cytoplasm once the cellular membrane integrity is compromised; the serum concentrations of these enzymes are usually proportional to the damage[51,52]. In particular, the elevation of ALT an enzyme found mainly in the liver is an indication of liver damage. Significant reduction in the activities of these enzymes in garlic and onion extracts treated diabetic rats is an indication of their hepatoprotective activities [53, 54]. These protective effects of onion and garlic extracts may be associated with their inherent-antioxidant properties as reported by Rahman [49] which is believe to accelerate the regenerative capacity of the hepatocytes, cause stabilization of the cell membrane of the hepatocytes, and ultimately protect the liver cells against deleterious agents and free radical mediated toxic damages. For example, onion contains sulphur-containing compounds of which their oxidized thiols can trap electrons from other systems[35,55,56,57].Generally, from the results(TABLE 2),there is no significant difference between the hepatoprotective effects of garlic and onion in contrast to the observation of Micheal et al.,[58] and Tatfeng and Enitan [59] who reported that garlic protects liver from damages better than onion.

Table 2: Effect of Allium cepa and Allium sativum on the Liver of the Treated Diabetic Rats

<table>
<thead>
<tr>
<th>BIOCHEMICAL PARAMETER</th>
<th>AST (U/I)</th>
<th>ALT(U/I)</th>
<th>ALP(U/I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL CONTROL</td>
<td>35.50 ± 0.58</td>
<td>20.85 ± 0.98</td>
<td>22.26 ± 0.64</td>
</tr>
<tr>
<td>DIABETIC CONTROL</td>
<td>80.00 ± 5.77</td>
<td>37.65 ± 6.73</td>
<td>63.81 ± 8.90</td>
</tr>
<tr>
<td>ONION(400mg/kg)</td>
<td>40.50 ± 0.58</td>
<td>24.00 ± 1.15</td>
<td>15.40 ± 2.53</td>
</tr>
<tr>
<td>GARLIC (400mg/kg)</td>
<td>40.00 ± 0.30</td>
<td>23.50 ± 1.73</td>
<td>26.95 ± 0.63</td>
</tr>
</tbody>
</table>

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All Values Are Expressed As Mean ± Standard Deviation of Four Observations.

Values with different superscript in the same column are significantly different at p< 0.05

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>UREA (mg/dl)</th>
<th>CREATININE (mg/dl)</th>
<th>CHOLESTEROL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL CONTROL</td>
<td>26.32 ± 1.50</td>
<td>0.45 ± 0.06</td>
<td>51.29 ± 0.33</td>
</tr>
<tr>
<td>DIABETIC CONTROL</td>
<td>103.57 ± 9.21</td>
<td>1.36 ± 0.16</td>
<td>128.70 ± 9.28</td>
</tr>
<tr>
<td>ONION(400mg/kg)</td>
<td>29.88 ± 1.79</td>
<td>0.67 ± 0.04</td>
<td>56.17 ± 0.77</td>
</tr>
<tr>
<td>GARLIC (400mg/kg)</td>
<td>29.00 ± 0.50</td>
<td>0.81 ± 0.01</td>
<td>55.10 ± 2.70</td>
</tr>
</tbody>
</table>

All Values Are Expressed As Mean ± Standard Deviation of Four Observations.

Values with different superscript in the same column are significantly different at p< 0.05

### IV. Conclusion

From the results obtained it can be concluded that the extracts of fresh Allium sativum and Allium cepa significantly reduced serum glucose and can also protect liver and kidney in diabetic patients although the extract of garlic seem to be more effective in reducing the blood glucose.

### Reference

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