Anti-Diabetic Effects of Ethanol Leaf Extract of Onions (Allium Cepa) On Alloxan-Induced Diabetic Wistar Albino Rats

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Abstract

Diabetes is a chronic disease characterised by high blood glucose level and abnormal metabolism of carbohydrates, protein and fat. The condition is characterised by persistent hyperglycaemia. Allium cepa leaf is a functional food used in traditional medicine for the treatment of diabetes mellitus. The use of plants especially vegetables as antidiabetic remedies have added interest of joining two basic diabetes mellitus control factors: food and medication. The ethanol extract of Allium cepa leaf was investigated for antidiabetic effects using alloxan-induced diabetic wistar albino rats. Wistar albino rats were randomly divided into six groups; Group A rats were non-diabetic control. Diabetes was induced in groups B, C, D, E and F by single intraperitoneal injection of alloxan (150mg/kg body weight). Group B were not treated and served as negative control group. Group C were treated with glibenclamide (5mg/kg body weight), thus served as postive control group. Groups D, E and F were treated with 200, 300 and 400mg/kg body weight of the extract respectively for a period of two weeks through intraperitoneal route. The effect of treatment with the doses of the extract and standard drug were studied on blood glucose level, total serum cholesterol and body weight. Allium Cepa extract produced a dose-dependent significant reduction in the blood glucose level when compared with that of the control group. Significant total serum cholesterol reduction was observed at 300 and 400mg/kg. An observed decrease in body weight of the negative control group was recorded and significant increase for all other groups. The findings from this study indicate that the crude extract of Allium cepa leaf caused a significant hypoglycaemic and hypocholesterolemic activity in alloxan-induced diabetic rats thus, validates its use in ethno-medicine for the control of diabetes mellitus.

KEY WORDS: Diabetes mellitus, Allium cepa, Alloxan, Blood glucose, Glibenclamide.
Introduction

Diabetes Mellitus (DM) is a group of metabolic disorders associated with disturbances in the metabolism of fuel molecules due to absolute deficiency of insulin, insufficient insulin secretion and/or its secretion [1]. It is a disorder that affects the body’s ability to make or use insulin. Insulin is a hormone produced in the pancreas that helps transport glucose (blood sugar) from the bloodstream into the cells so they can break it down and use it for fuel. People cannot live without insulin [2]. It is also a widespread endocrine disorder that is associated with considerable morbidity and mortality and is found in all population throughout the world [3].

Despite the presence of anti-diabetic drugs in the pharmaceutical market, the treatment of diabetes with medicinal plants is often successful. Herbal medicine and plant components with insignificant toxicity and less or no side effect are notable therapeutic options for the treatment of this disease around the world [4]. The most common herbal active ingredients used in treating diabetes are flavonoids, tannins, phenols and alkaloids [5]. The existence of these compounds implies the importance of the anti-diabetic properties of these plants [4].

*Allium cepa* is one of the recognised medicinal plants known to possess several medicinal properties including lowering of blood pressure, antiseptic, hypoglycaemic and hypocholesterolemic activity [6]. In the rural communities, many people depend solely on medicinal plants for the treatment of diabetes due to its easy accessibility, affordability and availability even when the efficacy of the herbal remedies has not been established [6].

Dietary therapy is unarguably the best treatment for diabetes. The diabetic diet should be carefully monitored to minimize the load placed on the blood glucose regulating mechanism. The use of plants, especially vegetables, by the population as antidiabetic remedies has added interest of joining two basic diabetes mellitus control factors: food and medication [7]. This research is thus geared towards finding a medicinal plant that will not only increase the energy content of diabetics but also lower glycaemic index properties for the management of diabetic pressures in our society.

Results
The ethanol leaf extract of *Allium cepa* was concentrated in a rotary evaporator and gave a percentage yield of 57g (10.64%).

The qualitative phytochemical screening of crude ethanol leaf extract of *Allium cepa* as showed in Table 1 revealed the presence of cardiac glycosides, phenolic group, saponins, tannins, flavonoids and alkaloids while anthracene and Cyanogenic glycosides were not detected in the sample.

**Table 1: Qualitative phytochemical result of Allium cepa ethanol leaf extract.**

<table>
<thead>
<tr>
<th>Test</th>
<th><em>Allium cepa</em> leave extract</th>
</tr>
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<tbody>
<tr>
<td>Anthracene glycosides</td>
<td>--</td>
</tr>
<tr>
<td>Saponins</td>
<td>+++</td>
</tr>
<tr>
<td>Tannins</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Cyanogenic Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+++</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic group (Tyrosine Millions method)</td>
<td>++</td>
</tr>
</tbody>
</table>

*KEY: - = Absent Negative result + = Positive Result*

There was no mortality or any signs of behavioural changes or toxicity observed after oral administration of crude ethanol leaf extract of *Allium cepa* in the three groups of the mice that received 10,100,1000 mg/kg body weight at the end of the first experiment. Further increased doses of 1900, 2600 and 5000mg/kg body weight of the extract showed no signs of behavioural changes. The results showed that the extract was safe up to the dose of 5000mg/kg body weight as showed in Table 2.
Table 2: Acute toxicity testing of ethanol leaf extract of Allium cepa leaf with mice. As described by [10].

<table>
<thead>
<tr>
<th>Stage one</th>
<th>Dose (mg/kg)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0/3</td>
</tr>
<tr>
<td>Stage two</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1600</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>2900</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>0/1</td>
</tr>
</tbody>
</table>

The results of administration of the standard drug (glibenclamide) and crude ethanol leaf extract of *Allium cepa* showed that the standard drug and the extracts at graded doses of 200mg/kg, 300mg/kg and 400mg/kg have the ability to lower the fasting blood sugar level in alloxan-induced diabetic wistar albino rats. The increasing dosage (200, 300 and 400mg/kg) of *Allium cepa* ethanol leaf extracts produced a dose dependent significant (P<0.05) reductions in the blood glucose levels of diabetic rats after two weeks of treatment when compared with that of the control rats as can be seen in (Figure 1). *Allium cepa* ethanol leaf extract at 200mg/kg reduced fasting blood glucose levels by 42% (426.2±49.06 to 246.4±68.81). At 300mg/kg it reduced fasting blood glucose levels by 48% (404.5±81.30 to 208.7±27.43). Whereas at a dose of 400mg/kg body weight showed 53% (394.8±77.09 to 213.8±32.47) reduction in the fasting blood sugar level on the fourteenth day of treatment. Glibenclamide at 5mg/kg reduced fasting blood glucose by 52%( 414.7±84.20 to198.2±34.34). The most effective percentage reduction in blood glucose level was observed at 400mg/kg.
Figure 1: Blood glucose profile of treatment with glibenclamide, 200mg/kg, 300mg/kg and 400mg/kg body weight of the ethanol leaf extract of Allium cepa.

The results of the total serum cholesterol assay in this study showed that the dose of the crude extract at 300mg/kg and 400mg/kg bodyweight showed a reduction. This reduction is statistically significant (P<0.05) when compared to that of the Diabetic untreated (Figure 2). Allium cepa at 300mg/kg body weight reduced total serum cholesterol by 28% (82.00±9.092 to 59.33±12.72), at 400mg/kg it reduced it by 14% (82.00±9.092 to 70.50±14.50). Glibenclamide at 5mg/kg reduced total serum cholesterol by 19.3% (82.00±9.092 to 66.20±17.84) after two weeks of treatment. The most effective percentage reduction in total serum cholesterol was observed at 300mg/kg with 28%.
Figure 2: Effect of ethanol leaf extract of Allium cepa on total serum cholesterol.

The weight of the animals for the group that received glibenclamide (standard drug) and the graded doses of crude ethanol leaf extract of *Allium cepa* at a dose of 200mg/kg, 300mg/kg and 400mg/kg body weight increased while the weight of the diabetic untreated group showed a reduction. This is shown in the Figure 3.
Figure 3: Effect of ethanol leaf extract of Allium cepa on body weight of the experimental and control groups before and after two weeks of the experiment

Discussion

Diabetes Mellitus is a complex disorder characterised by high blood glucose levels due to the inability of the body cells to utilise glucose properly [11]. Results obtained from the qualitative phytochemical screening of the ethanol leaf extract showed the presence of phytochemicals such as saponins, tannins, flavonoids, alkaloids, phenols and the absence of anthracine and cyanogenic glycosides. The results obtained were in agreement with the phytochemical analysis of Allium cepa (bulb) and Allium sativum carried out by [15] with the exception of cardiac glycosides.

Secondary metabolites have been reported to have antihyperglycaemic activity. Saponins extracted from Citrullus colocynthis fruit have been reported to cause marked hypoglycaemic effect in alloxan-induced diabetic rabbits [16]. Steroidal saponins isolated from B. aegyptiaca exhibited prominent antidiabetic activity in streptozotocin-induced diabetic mice [17]. Saponins isolated from the leaves of A. centicosus injected to mice (100,200mg/kg intraperitoneally) decreased experimental hyperglycaemia induced by injecting of adrenaline, glucose and alloxan, without affecting the levels of blood sugar in untreated mice [18]. An unsaturated triterpene acid isolated from an
ethanolic extract of *B. satorum* mart root bark produced a hypoglycaemic effect in alloxan –induced diabetic rats [19]. It increased glucose uptake and glycogen synthesis in isolated rat diaphragm and plasma insulin level [19]. It appears that this effect was mediated by an insulin secretagogue effect in pancreatic B- cells. Senegin II, a triterpenoidal glycoside isolated from rhizomes of *P. senega* has been reported to have anti-diabetic effects on mice [20]. Glycoside of leucopelargonidin isolated from the bark of *F. bengalensis* demonstrated significant hypoglycaemic, hypolipidemic and serum insulin raising effects in moderately diabetic rats [21]. The most common herbal active ingredients used in treating diabetes are flavonoids, tannins, phenols and alkaloids [5]. The existence of these compounds implies the importance of the anti-diabetic properties of this plants [4].

The median lethal dose (LD₅₀) results showed that *Allium cepa* leaf is safe for consumption and can be classified as being non-toxic. Its use can therefore be encouraged since it has also been found to contain some biologically active phytochemicals.

Results for the blood glucose level showed that on the 4th day of treatment, the rats that received 200mg/kg showed 26% reduction in their glucose level while on the fourteenth day of treatment it showed 42% reduction in their glucose level. This reduction was shown to be statistically significant (P<0.05) when compared with diabetic (negative) control animals that were not treated. The dose levels of the extract, 300mg/kg and 400mg/kg showed 26% and 20% reduction in their glucose levels respectively on the fourth day of treatment while on the fourteenth day of treatment; they showed 48% and 53% reduction in their glucose levels respectively relative to the diabetic (negative) control group.

The increasing dosage (200, 300 and 400mg/kg) of *Allium cepa* ethanol crude extract produced dose-dependent significant (P< 0.05) reduction in the blood glucose level of diabetic rats after two (2) weeks of treatment when compared with that of diabetic (negative) control rats. The most effective percentage reduction in blood glucose level was observed at 400mg/kg. These observations were in line with the findings of [22], [23] and [24]). [25] reported that ethylene extracts of *Allium cepa* bulb (containing quercetin) significantly controls the blood glucose level by inhibiting the activity of alpha glucosidase (involved in the absorption of glucose in intestine). The active ingredient
allyl propyl disulphide in *Allium cepa* may have antidiabetic properties as reported by previous studies [24]. The hypoglycaemic effect of *Allium cepa* observed in this study could be as a result of the phytochemicals acting singly or in synergy on the beta cells of the pancreases. The pharmacological potentials of these phytochemicals have been reported. Flavonoids have been reported to be active in many medicinal plants as antioxidants that protect organs toxicity due to agents such as alloxan [26]. Saponins possess anti-hyperglycaemic activity by inhibiting liver glycogenesis and might have contributed to the anti-diabetic effect of *Allium cepa* extract. It is also suspected that onion extracts, like glibenclamide, may induce hypoglycaemia by stimulating insulin release and action, thereby enhancing cellular uptake and utilization of glucose in rats. It is possible that onions extracts may act by undetermined ways apart from stimulating insulin production from the pancreatic islets since these would have been severely damaged by alloxan. The exact mechanism of the hypoglycaemic effects of onions extracts remains unclear; therefore, further studies are required to unravel the pathway of its hypoglycaemic action and to shed more light on the hypoglycaemic constituents of the plants. It is however evident from this research that onions extracts studied contains hypoglycaemic agents capable of lowering blood glucose level in Alloxan- induced diabetic rats.

Hypercholesterolemia has been reported to occur in Alloxan-induced diabetic rats [27]. The lowering of serum total cholesterol level in the treated animals clearly demonstrated the presence of hypolipidemic agent in the *Allium cepa* extracts. The ability of plant extracts to manage dyslipidemia is a potential beneficial effect on cardiovascular risk factors which is a major cause of death in *Diabetes mellitus* [28]. The lowering of total serum cholesterol of *Allium cepa* ethanol leaf extract is in line with the work of [28], and [29]. The ethanol extract of *Allium cepa* at a dose of 300mg/kg body and 400mg/kg weight showed a significant reduction (p<0.05) in the serum total cholesterol when compared to the diabetic untreated rats. A significant total serum cholesterol reduction was also observed in the group that were treated with the standard drug (Glibenclamide). The marked hyperlipidaemia that characterised the diabetic state may be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots [30]. The hypolidaemic effect of *Allium cepa* may be connected to its active ingredient allyl propyl disulphide [22]
The results obtained for the body weight of the experimental animals before and at the end of the research showed a significantly increase in body weight of animals that were administered 200mg/kg, 300mg/kg, 400mg/kg of the *Allium cepa* leaf extract. A significant increase was also recorded for the non-diabetic control group and the diabetic (positive) control group but a significant decrease was recorded for the diabetic (negative) control group in comparison to the pre-treatment period. The observed decrease in body weight of the untreated group as seen in (Figure 3) could be due to the selective destruction of pancreatic beta cells of the islets of Langerhans (insulin producing cells) by alloxan. This leads to insulin deficiency and decrease in peripheral glucose uptake, utilisation and increase gluconeogenesis. These cause increased degradation of structural proteins thereby affecting the body weight of the experimental animals in that group [1]. The observed increased in the body weight in the groups that were treated with 200mg/kg, 300mg/kg and 400mg/kg could be a function of antidiabetic effect of the *Allium cepa* bioactive components. The *Allium cepa* ethanol leaf extract ability to reduce hyperglycaemia and improve glucose metabolism may lead to improved peripheral glucose uptake and utilisation, glycogen synthesis and decrease gluconeogenesis. These spare structural proteins from degradation (muscle wasting) and help maintain the weight. [1].

**Materials.**

**Plant**

The *Allium cepa* leaves used for the experiment was bought from Barkin Ladi Market in Barkin Ladi L.G.A of Plateau State, Nigeria. The plants were identified by Mr. O.E. Agyeno in the Department of Botany, University of Jos, Plateau State. A voucher specimen with number JUHN21000345 was deposited in the herbarium unit of the department.

**Experimental Animals.**

A total of thirty-six (36) adult male wistar albino rats weighing 80 to 150g and twelve (12) mice were used for the experiment. The experimental animals were purchased from Chris Animal Farm, G.R.A. Awka. They were housed six (6) rats per cage at the experimental Animal House of Applied Biochemistry Department, Nnamdi Azikiwe University, Awka, Anambra State. They were acclimatized for two weeks under standard laboratory conditions and were maintained on water and Guinea growers mash pellet (Vital Feed Grand Cereals Nigeria Ltd, Jos, Nigeria) that was
obtained from Eke Awka Market, Awka, Anambra State.

**Methods**

**Preparation of ethanol leaf extract of Allium cepa.**

The leaves of *Allium cepa* were properly washed with distilled water and dried at room temperature for three weeks. The dried leaves were then pulverised using corona manual grinding machine. The powdered samples of *Allium cepa* was weighed and exactly 1475g was extracted in 5 litres of 80% ethanol for 24 hours with occasional stirring, sieved and filtered using filter paper (Whatman number 1). The filtrate was then concentrated using a rotary evaporator at 60°C and appeared as a dark brown gel solid. The extracts were kept in a labelled glass container and stored in a refrigerator until when required for reconstitution and administration.

**Phytochemical Screening of Secondary metabolites ( Constituents)**

The qualitative phytochemical screening of the ethanol leaf extract of *Allium cepa* was carried out using standard procedures as outlined by [8], [9].

**Acute toxicity and Median Lethal Dose (LD	extsuperscript{50}) test of ethanol leaf extract of Allium cepa.**

The median Lethal Dose (LD	extsuperscript{50}) was determined using Wistar albino mice as described by the modified method of [10]. Test animals were divided into six (6) groups. The first 3 groups which contain 3 animals each were given 10mg/kg, 100mg/kg and 1000mg/kg body weight of the ethanol extract of *Allium Cepa* leaves. The *Allium Cepa* extract was administered orally and was monitored for 24 hours. The last 3 groups which contain one animal each per group were then given 1600mg/kg, 2900mg/kg and 5000mg/kg body weight of the ethanol extract of *Allium Cepa* leaves and were observed for 24 hours.

**Induction of Diabetes.**

Alloxan was prepared and induced by adopting the method of [11]. All rats, except for the normal control group were intraperitoneally injected with 150mg/kg body weight of the prepared alloxan dissolved in normal saline solution. The blood glucose levels of the rats were checked before the administration of alloxan using one touch glucometer (Fine touch, USA) and test strips. The rats were then fasted for 16 hours, but with free access to water after which they received an intraperitoneal injection of alloxan 150mg/kg body weight. The rats were orally given 20ml each of 10% glucose solution after 2 hours to prevent
hypoglycaemia. The animals were allowed free access to food and water *libitum* after alloxan administration. After 48 hours of the alloxan administration, blood was collected orbito-rectally and their glucose levels were checked using one touch glucometer and test strips. Diabetes was confirmed to have been induced if the glucose level was observed to be far much higher than normal (above 140mg/dl).

**Experimental Design**

This study was carried out on alloxan – induced diabetic rats for two (2) weeks. A total of thirty-six (36) Wistar albino rats were used for the experiment. The albino rats were randomly divided into six (6) groups with six (6) rats in each group. The extract and the reference drug were administered intraperitoneally to the animals.

Group A – Normal (non-diabetic control)

Group B – Diabetic (negative) control group

Group C – Diabetic (positive) control – this group received 5mg/kg body weight of glibenclamide.

Group D – This group received 200mg/kg body weight of the extract.

Group E – This group received 300mg/kg body weight of the extract.

Group F – This group received 400mg/kg body weight of the extract.

The weights of the animals were carefully monitored and determined before the induction and throughout the duration of the experiment.

**Biochemical Assay**

**Blood glucose level determination**

Determination of the blood glucose level was done by the glucose-oxidase principle [12] using the one touch instrument and results were reported as mg/dl [13].

**Determination of total serum cholesterol.**

The cholesterol of the serum was oxidised to tetraene derivative by ferric ions derived from ferric perchlorate using four different test tubes that were marked test, control, standard and blank. The absorbance was measured (using spectrophotometer) at 590nm wavelength and compared with that of a pure solution of cholesterol [14].

**Weight determination of the experimental animals.**

The initial weight of experimental animals (wistar albino rats and mice) were weighed...
using a weighing scale. A large plastic bowl which was transparent was placed on the weighing scale and was set to zero. The bowl was then taken off and the animal was placed gently into the bowl and was placed back on the scales for the weight reading to be taken. Thereafter, the final weight was taken after 14 days of treatment.

**Statistical Analysis**

The Data obtained from the experiments were analysed using Statistical Package for Service solutions (SPSS) software for windows version 21 (SPSS Inc. Chicago, Illinois, USA). All the data were expressed as mean ± SD. The limit of significance was set at P<0.05. Data obtained were subjected to test of significance using ANOVA to determine if significant differences exist between the mean of the test and control.

**Conclusion**

The results obtained from this study indicated that *Allium cepa* ethanol leaf extract at various doses administered to alloxan - induced diabetic rats changes certain biochemical parameters such as blood glucose and total serum cholesterol level to near normal level. This demonstrated that *Allium cepa* leaf possess significant hypoglycaemic and hypocholesterolemic effects and can be used in the development of *Allium cepa* leaf based anti – diabetic nutraceutical for the management of diabetes and other related disease

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