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An Evaluation of the Hypoglycemic, Antioxidant and Hepatoprotective Potentials of Onion (Allium cepa L.) on Alloxan-induced Diabetic Rabbits


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Abstract: Diabetes mellitus is a chronic disorder of carbohydrate metabolism whose prevalence is raising globally, especially the resource-starved countries such as Nigeria. Since antiquity, diabetes has been treated with plant medicines. Several investigations have confirmed the efficacy of many of these traditional preparations, some of which have proven efficacy. In the present study, the hypoglycemic, antioxidant and hepatoprotective effects of Allium cepa (A. cepa) aqueous extracts on alloxan-induced diabetic rabbits was investigated. Diabetes mellitus was induced in 15 adult male rabbits, using 200 mg kg⁻¹ of alloxan monohydrate as a single intraperitoneal injection. These alloxan-diabetic rabbits were then divided into three groups; one group was administered aqueous extract of A. cepa 100 mg Kg⁻¹ b.wt. orally daily for 30 days, another group received A. Cepa 300 mg Kg⁻¹ b.wt. orally daily for 30 days and the last group of diabetic rabbits received peanut oil (the vehicle) instead of A. cepa to serve as the diabetic control. There were also five rabbits which received neither alloxan nor A. cepa (the negative control group). All the liver histological derangements caused by diabetes were attenuated in the A. cepa-treated group. Increasing dosages of A. cepa aqueous extract produced a dose-dependent significant reduction in the blood glucose levels. Additionally, A. cepa remarkably improved the reduction of antioxidant parameters-Superoxide dismutase, catalase (SOD), catalase (CAT) Glutathione Peroxidase (GPx), Reduced Glutathione (GSH) and increased malondialdehyde (MDA), a product of lipid peroxidation. It is concluded based on these findings that A. cepa may be effective in ameliorating diabetic’s related hepatotoxicity and alterations of biochemical parameters.

Key words: Allium cepa, diabetes mellitus, hypoglycemia, hepatotoxicity, antioxidants

INTRODUCTION

Diabetes Mellitus (DM) is an endocrine disorder which is characterized by chronic hyperglycemia (high blood sugar) due to disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (WHO, 1999). Diabetes mellitus is the most common metabolic condition that affects more than 100 million people worldwide which represents about 6% of the world population (WHO/Accadia, 1992; ADA, 2005). More worrisome is the fact incidence the disorder is increasing rapidly and it is estimated that by the year 2030, this number will double (ADA, 2005). Erasto et al. (2005) asserted that DM is a common and very prevalent disease affecting the citizens of both developed and developing countries. Several factors are incriminated in the rising incidence of DM worldwide. Some of these factors are the increasing proportion of the aging population, consumption of calorie rich diet, obesity and sedentary life style (Vats et al., 2002).

The chronic hyperglycemia of DM is associated with long term complications and poses huge social and financial burdens on countries ill-equipped to meet them. These complications include renal failure, blindness or diabetic cataract, poor metabolic control and increased risk of cardiovascular disease including atherosclerosis and advance-glycation end (AGE) products (Zimmet et al., 2001). The secondary complications in DM are due mainly to sustained hyperglycemia and increased oxidative stress resulting from excessive productive or reduced scavenging of free radicals (Baynes, 1991; Bayraktutan, 2002).

Allium cepa (onion), also known as the bulb onion, common onion and garden onion, is the most widely cultivated species of the genus Allium (Fritsch and

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Freisen, 2002). It has a globose bulb that is an underground part of the stem, it is biennial and perennial and it is widely distributed in the temperate regions. *Allium cepa* (*A. cepa*) is used commonly in foodstuff and as a traditional remedy in the treatment of a variety of disorders. The pharmacological evidence for the use of *A. cepa* as an anti-asthmatic, anti-hypertensive, anti-hyperglycemic, anti-hyperlipidemic and anti-tumor agent has been reported (Augusti, 1996; Stajner and Varga, 2003).

Active ingredients in *A. cepa* include phenolic compounds (flavonoids, anthocyanins, phenolic acids and flavonols), organosulfur compounds, vitamins and some minerals (Teyssier et al., 2001; Kamal and Daoud, 2002; Campos et al., 2003; Gabler et al., 2003; Ismail et al., 2003; Wang et al., 2005; Elhassaneen and Sanad, 2009). These compounds may 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 (Larson, 1988; Ibrahim et al., 2004). Its vitamins, especially vitamin C have a protective function against oxidative damage and a powerful quencher of singlet oxygen (O$_2^*$), hydroxyl (OH) and peroxyl (RO$_2^*$) radicals (Niki, 1991; Saatli et al., 2009).

Herbal products are commonly utilized in the management of disease in nearly every culture and society on earth. Resort to folkloric medication is particularly prominent in Africa where traditional beliefs induce people to use medicinal plants whenever they have health problems. Further, the cost of administering modern treatment including antidiabetic drugs is beyond the reach of most people in the low income group and those living in the rural areas, hence the use of plants for the treatment of common diseases such as diabetes are very common. It is in realization of these facts that the WHO (1980), expert committee on diabetes recommended that traditional methods of management of diabetes should be further investigated.

It is partly in response to the above charge that investigate in the present study the potentials of *Allium cepa* as a hypoglycemic, antioxidant and hepatoprotective agent in alloxan-induced diabetic rabbits.

**MATERIALS AND METHOD**

**Animals:** A total of twenty adult Rabbits (10 females and 10 males) were obtained from a breeding stock maintained in the animal house of the college of health sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomosho, Nigeria and housed at animal facility of the department of Anatomy, Ladoke Akintola University of Technology (LAUTECH), Ogbomosho, Nigeria. The rabbits were maintained under standard natural photoperiodic condition.

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 (National Research Council, 1996).

**Plant extract** *Allium cepa:* Twenty fresh mature *A. cepa* fruits weighing 200 g were bought from Sabo market Ogbomosho, Oyo state Nigeria on 12th December, 2010. The botanical identification and authentication of the plant sample was done at the Herbarium Section, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Nigeria (Voucher No. 20).

Aqueous extract of AC fruit was obtained using the method described by Azu et al. (2007).

**Acute oral toxicity study of Allium cepa extract:** The acute oral toxicity study for *Allium cepa* extract was conducted using the Organization for Economic Cooperation and Development (OECD, 2000) Guidance Document on Humane End points that should reduce the overall suffering of animals used in this type of toxicity test. The test used was the limit dose test of the up and down procedure.

**Chemicals:** Alloxan$^\text{6}$ (Sigma, St. Louis, MO, USA) was obtained from a chemical shop in Lagos Nigeria and was dissolved in 0.1 M cold citrate buffer, pH 4.5 (Lenzen, 2008).

**Induction of diabetes:** Alloxan monohydrate was used to induce diabetes mellitus in normoglycemic rabbits. Animals were allowed to fast for 12 h and were injected intraperitoneally with freshly prepared alloxan monohydrate in normal saline in a dose of 200 mg kg$^{-1}$ b. wt. (Federiuk et al., 2004). Blood glucose levels of these rabbits were estimated 24 h after alloxan administration using One Touch Ultra Mini Glucometer (Life Scan Inc. Milpitas, CA, USA). Animals with blood glucose equal or more than 200 mg dL$^{-1}$ were declared diabetic and were used in the experimental groups (Lenzen, 2008). Twenty five hour after induction of experimental diabetes, the experimental protocol was started.

**Animals grouping and treatment:** Twenty rabbits weighing between 1,500 and 1,800 g were randomly allocated into four groups:
Normal control animals received 5.0 mL kg⁻¹ b.wt. sterile water intraperitoneally (i.p.). Diabetic control group of rabbits received 200 mg kg⁻¹ b.wt. of alloxan monohydrate i.p. as a single dose. This dosage is known to induce diabetes in rabbits (Foderiuk et al., 2004). The animals were started on peanut oil (the vehicle) 5 mL kg⁻¹ b.wt. orally daily after 24 h for 30 days.

Diabetic with low dose A. cepa group of animals were administered alloxan monohydrate 200 mg kg⁻¹ b.wt., i.p. as a single dose; the animals were started after 24 h on aqueous extract of A. cepa 100 mg kg⁻¹ b.wt. per oral daily for 30 days.

Diabetic with high dose A. cepa group of rabbits received alloxan monohydrate 200 mg kg⁻¹ b.wt., i.p. as a single dose. Then animals were started after 24 h on aqueous extract of A. cepa 300 mg kg⁻¹ b.wt. per oral daily for 30 days.

Prior to injection of sterile water or alloxan, blood was taken from the auricular vein of the rabbit to determine the basal blood glucose level. Blood of the animals was similarly sampled for glucose concentration at the end of the experimental period.

Animal sacrifice and sample collection: After blood sampling for glucose concentration the animals were sacrificed. Each rabbit was at the time of sacrifice was first weighed and then anaesthetised by placing it in a closed jar containing cotton wool soaked with chloroform anaesthesia. The abdominal cavity was opened up through a midline abdominal incision to expose the liver. Then the liver was excised and trimmed all of fat. The liver weight of each animal was evaluated with an electronic analytical and precision balance (BA 210S, d = 0.0001 g Sartorius GA, Goettingen, Germany). The liver volume was measured by water displacement method.

A portion of the median lobe of the liver was dissected and fixed in fixed in 10% formal saline for histological examination. The remaining parts of the liver were frozen quickly in dry ice and stored at -25°C for biochemical analysis.

Histological procedures and analysis: This was done as described in our earlier studies (Saalu et al., 2007; Saalu et al., 2008). Photomicrographs were taken with a JVC colour video digital camera (JVC, China) mounted on an Olympus light microscope (Olympus UK Ltd, Essex, UK).

Assay of superoxide dismutase (SOD) activity: Superoxide dismutase activity was measured according to the method of Winterbourn et al. (1975) as described by Rukmini et al. (2004). It was expressed as u mg⁻¹ protein.

Assay of glutathione peroxidase (GPx) activity: Glutathione peroxidase activity was measured by the method described by Rotruck et al. (1973). The absorbance of the product was read at 430 nm and expressed as nmol mg⁻¹ protein.

Assay of liver non-enzymatic antioxidants
Assay of liver reduced glutathione (GSH) concentration: GSH was determined by the method of Ellman (1959). The absorbance was read at 412 nm, expressed as nmol mg⁻¹ protein.

Estimation of lipid peroxidation (Malondialdehyde): Lipid peroxidation in the liver tissue was estimated colorimetrically by thiobarbituric acid reactive substances TBARS method of Duggle and Aust (1978). Concentration was calculated using the molar absorbptive of malondialdehyde which is 1.56 × 10² M⁻¹ cm⁻¹ and expressed as nmol mg⁻¹ protein.

Statistical analysis: All data were expressed as Mean±SD of number of experiments (n = 3). The level of homogeneity among the groups was tested using Analysis of Variance (ANOVA) as done by Snedecor and Cochran (1980). Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). A value of p<0.05 was considered to indicate a significant difference between groups (Duncan, 1957).

RESULTS

Acute oral toxicity studies: There were no deaths of rabbits dosed 3000 mg kg⁻¹ b.wt. of the plants extract both within the short and long outcome of the limit dose test of Up and Down method (Table 1). The LD₅₀ was calculated to be greater than 3000 mg kg⁻¹ b.wt. orally.

Blood glucose levels: The increasing dosage (100 and 300 mg kg⁻¹) of A. cepa aqueous extracts produced dose-dependent significant (p<0.05) reductions in the blood glucose levels of diabetic rabbits after 30 days of treatment when compared with that of the control rabbits (Fig. 1). A. cepa at 100 mg kg⁻¹ reduced fasting blood glucose levels by 53.3% (300, 2±11.2 to 140±3.4) and 300 mg kg⁻¹ it reduced fasting blood glucose levels by 73.3% (300, 2±11.2 to 80±4.1). Peanut oil 5 mg kg⁻¹ which was used as a vehicle for A. cepa had no effect on the fasting blood glucose.
Fig. 1: Effect of *A. cepa* on blood glucose of rabbits

![Blood glucose levels for normal and diabetic rabbits treated with *A. cepa*](image)

Fig. 2: Effect of *A. cepa* on the levels of SOD in the liver of rabbits

![Liver SOD levels for normal and diabetic rabbits treated with *A. cepa*](image)

Table 1: Results of acute toxicity test for *Allium cepa* (AC) extract (up and down procedure) in rabbits

<table>
<thead>
<tr>
<th>Serial no</th>
<th>Test animal</th>
<th>Dose (mg kg⁻¹)</th>
<th>(48h)</th>
<th>(14 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 REP</td>
<td>2000</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>2 LEP</td>
<td>2000</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>3 TC</td>
<td>2000</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>4 RLT</td>
<td>2000</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>5 Intact</td>
<td>2000</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

S = Survival, REP = Right ear piercing, LEP = Left ear piercing, TC = Tail cut, RDC = Right leg tagged, Intact = Intact rabbit

Fig. 3: Effect of *A. cepa* on levels of CAT in liver of rabbits

![Liver CAT levels for normal and diabetic rabbits treated with *A. cepa*](image)

Fig. 4: Effect of *A. cepa* on levels of liver GPX in rabbits

![Liver GPX levels for normal and diabetic rabbits treated with *A. cepa*](image)

dosage regimes of *A. cepa*, the levels came back to near normal values. It was further observed that the higher dose of *A. cepa* provided showed a better ability in reducing the liver oxidative stress as compared to the lower dose.

**Liver content of glutathione (GSH) and malondialdehyde (MDA):** There was a notable reduction in GSH content in diabetic control group of animals. Administration of both doses of *A. cepa* significantly elevated the liver content of GSH compared to animals that were given alloxan without a follow up plant extract treatment (Fig. 5). Co-administration of alloxan and *A. cepa* exhibited a remarkable reduction in the liver MDA level compared to alloxan-alone treated rabbits.

As shown in Fig. 6, diabetic control rabbits showed significantly elevated liver content of lipid peroxides (products of lipid peroxidation) expressed as MDA when compared to control animals.
Like was the case with liver antioxidative enzymes, the beneficial changes in GSH and MDA were dose-dependent, the higher dose showing better potentials.

**Liver histopathological results:** The histopathological examination of diabetic rabbits showed marked distortion and degeneration of the liver parenchyma. The liver also showed dilated and congested portal vessels (Fig. 7).

There was a more organized cytoarchitecture of the liver in the group that received alloxan with low dose of *A. ceca* as compared with untreated diabetic group (Fig. 8). Furthermore, in the group where the diabetes rabbits were treated with high dose of *A. ceca* extract, the cyto-architecture appeared well restored with visible central veins surrounded by hepatocytes and well arranged hepatic ducts (Fig. 9).

![Fig. 5: Effect of *A. ceca* on the levels pf liver GSH in rabbits](image1)

![Fig. 6: Effect of *A. ceca* on the liver contents of MDA in rabbits](image2)

![Fig. 7: Selection showed diabetic control. (H and E x40). CV: Central vein, ILBD: Interlobular bile duct, SD: Sinusoids and BD: Bile duct](image3)
**DISCUSSION**

Recent studies have shown that many chronic diseases initiated and propagated at least in part by oxidative stress mediated through reactive oxygen species (Halliwell, 2001; Klaunig and Kamendulis, 2004; Stocker and Keaney, 2004; Dalle-Donne et al., 2006; Saalu, 2010; Saalu et al., 2010). Diabetes mellitus, the most common
metabolic disorder is multifactorial in causation. Of particular interest in the pathogenesis of diabetes mellitus is the correlation between oxidative stress and development of diabetes (Baynes, 1991; Bayraktutan, 2002; Abdel-Hamid et al., 2008). It has indeed been asserted that the major concern in diabetes is oxidative stress (Khaki et al., 2010).

Herbal products are commonly utilized in the management of diseases in nearly every culture and society on earth. However, only a few of these plant products have been scientifically evaluated. Allium cepa (onion) known to contain antioxidative bioflavonoids is evaluated in this study for its capacity to reduce blood sugar, moderate liver oxidative stress and attenuate the alterations in liver cytoarchitecture usually associated with diabetes. We are encouraged to carry out this study because few previous reports investigating the potentials of A. cepa assess all these three broad but complementary parameters.

This study demonstrated a raised blood sugar in diabetic alloxanized diabetic rabbit's models which was reduced by A. cepa in a dose dependent manner, with the higher percentage reduction at the higher dose. The elevated blood glucose in diabetes was also the finding in several previous reports (Mathew and Augusti, 1975; Hamme et al., 1991; Sharpe et al., 1998; Tukunou et al., 1998; Zhou and Sato, 2008). Studies have found that Allium cepa (onions) has blood sugar lowering effects (Sharma et al., 1977; Sheela and Augusti, 1992). The molecular mechanism by which A. cepa mediate its antihyperglycemic and antioxidative effects has not been properly elucidated. Andallu et al. (2001) reported the active compounds of onion are mainly, sulfur-containing compounds-allyl propyl disulfide (APDS). It has been postulated that these active ingredients lower glucose levels by competing with insulin (which is also a disulfide) for insulin-inactivating sites in the liver (Kumari et al., 1995) resulting in an increase of free insulin. There are also reports that A. cepa could lower blood sugar by facilitating better glycogen storage (Guo et al., 2002) and improve oxidative status by increasing glutathione peroxidase (Helen et al., 1999).

Klans-Dieter (1983), earlier explained that onion contains sulfur-containing compounds such as dialkyl disulfides and their oxidized thiols which can trap electrons from other systems. Onion oil containing these compounds has been reported to have an antioxidative effect against the oxidative damage caused by nicotine in experimental animals (Helen et al., 2000). It is therefore plausible to infer that these antioxidative constituents of A. cepa may have provided the protection against oxidative stress and hepatotoxicity in alloxan-induced diabetic rabbits evidenced in the present study.

In conclusion, the present investigation shows that aqueous extract of A. cepa possess antihyperglycemic effect, antioxidant activity and ultimately hepatoprotective potentials. It is therefore recommended that further studies be carried out to determine the probable place of this nutraceutical in diabetes management.

REFERENCES


