International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 8 (1): 21-29, 2012
ISSN 1811-7775 / DOI: 10.3923/ijp.2012.21.29

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

[1]O.S. Ogunmodede, [1]L.C. Saalu, [1]B. Ogunlade, [1]G.G. Akunna and [2]A.O. Oyewopo
[1]Department of Anatomy, Lagos State University,
College of Medicine (LASUCOM) Ikeja, Lagos, Nigeria
[2]Department of Anatomy, College of Health Sciences, University of Ilorin, Ilorin, Nigeria

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/Acadia, 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

Corresponding Author: Dr. L.C. Saalu, Department of Anatomy, Lagos State University College of Medicine (LASUCOM) Ikeja, Lagos, Nigeria Tel: 08033200876

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, antocyanins, phenolic acids and flavonols), organosulphur 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; Saalu *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 administrating 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® (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 intraperitonially 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 intraperitonially (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 (Federiuk *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 anaesthesized by placing it in a closed jar containing cotton wool sucked 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- Sartoriusen 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% formol-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 liver enzymatic antioxidants

Assay of catalase (CAT) activity: Catalse activity was measured according to the method of Aebi (1983) as modified by Akunna *et al*. (2011). Activity of enzyme was expressed as units mg^{-1} protein.

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 Buege and Aust (1978). Concentration was calculated using the molar absorptive of malondialdehyde which is 1.56×10^{5} 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 = 5). 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 140.1±3.4) and 300 mg kg^{-1} it reduced fasting blood glucose levels by 73.3% (300.2±11.2 to 80.4±1.2). Peanut oil 5 mg kg^{-1} which was used as a vehicle for *A. cepa* had no effect on the fasting blood glucose.

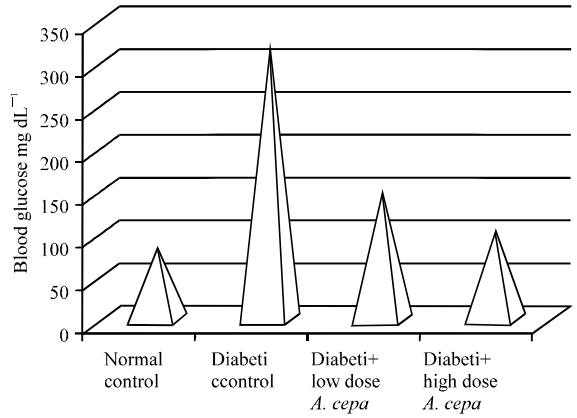

Fig. 1: Effect of *A. cepa* on blood glucose of rabbits

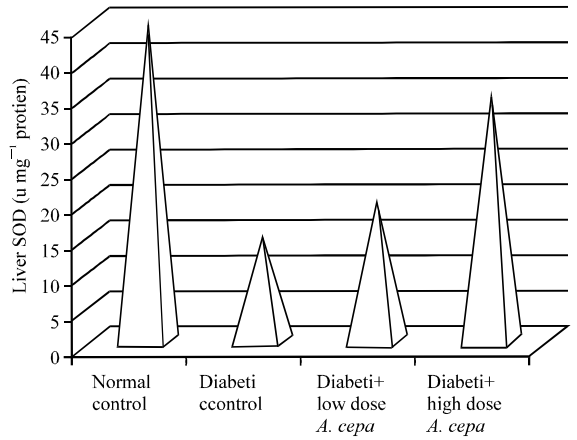

Fig. 2: Effect of *A. cepa* on the levels of SOD in the liver of rabbits

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

TEST animal dose of ac short term results long term results			
Serial no identity	mg kg^{-1}	(48 h)	(14 days)
1 REP	2000	S	S
2 LEP	2000	S	S
3 TC	2000	S	S
4 RLT	2000	S	S
5 I	2000	S	S

S = Survival; REP = Right ear pierced; LEP = Left ear pierced; TC = Tail cut; RDC = Right leg tagged; I = Intact rabbit

Changes in the liver oxidative status

Activities of liver enzymes-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gpx): Figure 2 to 4 show changes in the activities of SOD, CAT and Gpx in liver of normal and treated rabbits. The activities of SOD, CAT and Gpx levels in liver decreased in diabetic control rabbits when compared the control values (15.2±0.5 vs. 42.1±0.3 u mg^{-1} protein; 3.9±0.1 vs. 18.5±1.2 u mg^{-1} protein; 0.25±0.01 vs. 0.7±0.03 nmol mg^{-1} protein, respectively). After treatment with the two

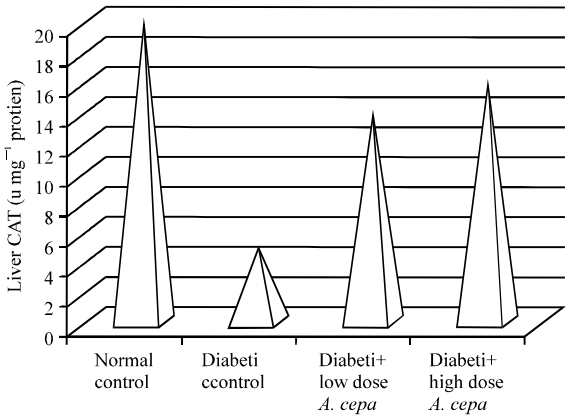

Fig. 3: Effect of *A. cepa* on levels of CAT in liver of rabbits

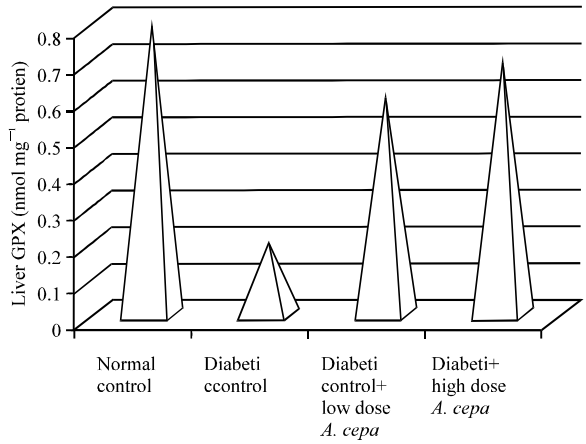

Fig. 4: Effect of *A. cepa* on levels of liver GPX in rabbits

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 receive alloxan with low dose of *A. cepa* as compared with untreated diabetic group (Fig. 8). Furthermore, in the group where the diabetes rabbits were treated with high dose of *A. cepa* extract, the cyto-architecture appeared well restored with visible central veins surrounded by hepatocytes and well arranged hepatic ducts (Fig. 9).

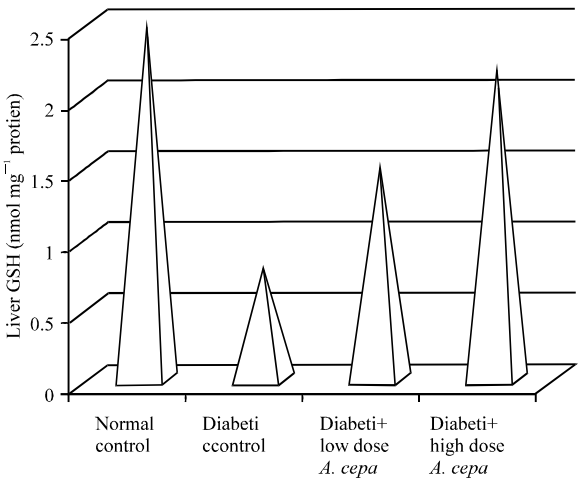

Fig. 5: Effect of *A. cepa* on the levels pf liver GSH in rabbits

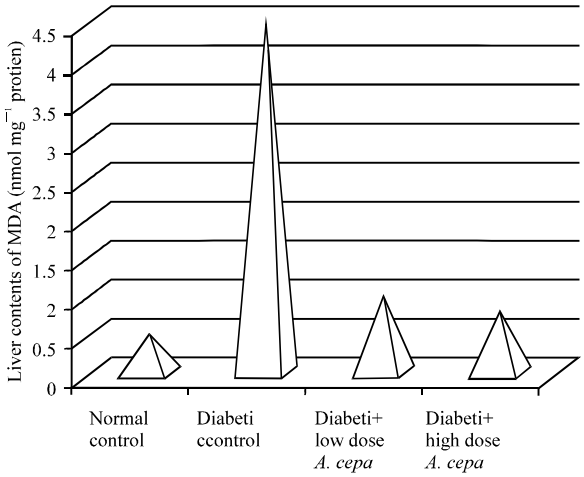

Fig. 6: Effect of *A. cepa* on the liver contents of MDA in rabbits

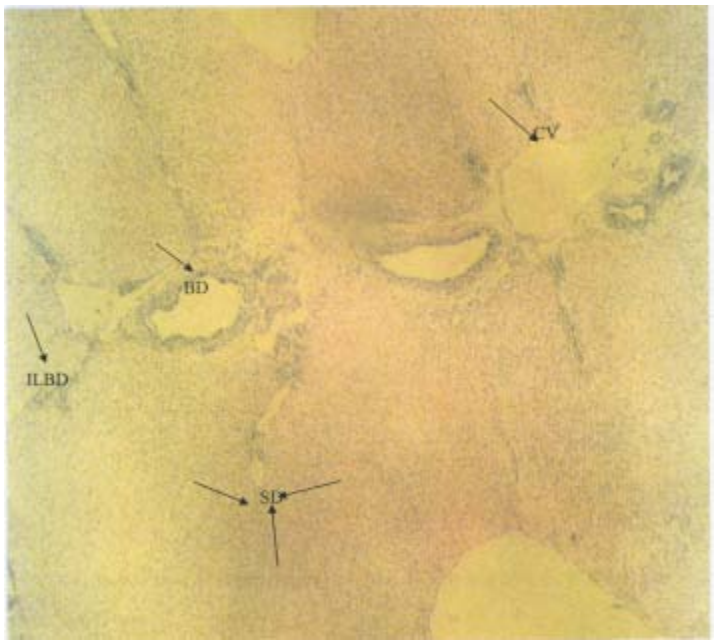

Fig. 7: Selection showed diabetic control. (H and E x40). CV: Central vein, ILBD: Interlobular bile duct, SD: Sinusoids and BD: Bile duct

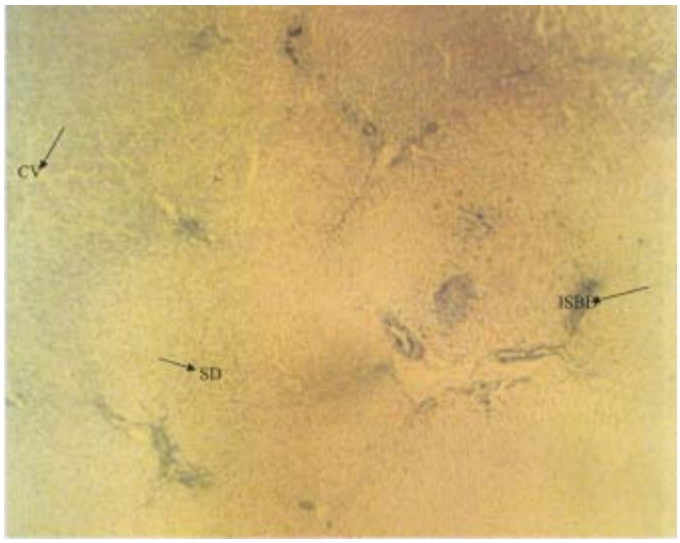

Fig. 8: Section showed slightly improved histology with low dose. (H and E x40). CV: Central vein, SD: Sinusoids, ISBD: Interlobular septum and bile duct

Fig. 9: Section showed improved histology with high dose of onion. (H and E x40). CV: Central vein, BPV: Branches of portal vein, SD: Sinusoids

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 alloxinized 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; Tukuncu *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 antihyperglycocemic 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).

Klanns-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 there 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 antihypergylcemic 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

ADA, 2005. Total Prevalence of Diabetes and Pre-Diabetes. American Diabetes Association, USA., pp: 15.

Abdel-Hamid, N.M., L.M. Faddah, M.A. Al-Rehany and A.A. Awad, 2008. Oxidative stress in the liver of diabetic rats treated with a combination of sildenafil citrate and a free radical scavenger. Asian J. Biochem., 3: 32-37.

Aebi, H.E., 1983. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H.U. (Ed.). Verlag Chemie, Weinheim, 273-286.

Akunna, G.G., L.C. Saalu, O.S. Ogunmodede, B. Ogunlade, G.A. Adefolaju and A.J. Bello, 2011. The effects of two Nigerian made perfume on the liver of adult Wistar rat. J. Med. Sci., 11: 220-225.

Andallu, B., A.V. Suryakantham, B.L. Srikanthi and G.K. Reddy, 2001. Effect of mulberry (*Morus indica* L.) therapy on plasma and erythrocyte membrane lipids in patients with type 2 diabetes. Clin. Chim. Acta, 314: 47-53.

Augusti, K.T., 1996. Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). Indian J. Exp. Biol., 34: 634-640.

Azu, N.C., R.A. Onyeagba, O. Nworie and J. Kalu, 2007. Antibacterial activity of *Allium cepa* (Onions) and *Zingiber officinale* (Ginger) on *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from high vaginal swab. Internet J. Trop. Med., 3: 234-237.

Baynes, J.W., 1991. Role of oxidative stress in development of complications in diabetes. Diabetes, 40: 405-412.

Bayraktutan, U., 2002. Free radicals, diabetes and endothelial dysfunction. Diabetes Obes. Metab., 4: 224-238.

Buege, J.A. and S.D. Aust, 1978. Microsomal Lipid Peroxidation. In: Methods in Enzymology, Fleischer, S. and L. Packer (Eds.). Academic Press, New York, USA., pp: 302-330.

Campos, K.E., Y.S. Diniz, A.C. Cataneo, L.A. Faine, M.J. Alves and E.L. Novelli, 2003. Hypoglycaemic and antioxidant effects of onion, *Allium cepa*: Dietary onion addition, antioxidant activity and hypoglycaemic effects on diabetic rats. Int. J. Food Sci. Nutr., 54: 241-246.

Dalle-Donne, I., R. Rossi, R. Colombo, D. Giustarini and A. Milzani, 2006. Biomarkers of oxidative damage in human disease. Clin. Chem., 52: 601-623.

Duncan, B.D., 1957. Multiple range tests for correlated and heteroscedastic means. Biometrics, 13: 164-176.

Elhassaneen, Y.A. and M.I. Sanad, 2009. Phenolics selenium, Vitamin C, amino acids and pungency levels and antioxidant activities of two egyptian onion varieties. Am. J. Food Technol., 4: 241-254.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82: 70-77.

Erasto, P., P.O. Adebola, D.S. Grierson and A.J. Afolayan, 2005. An ethanobotanical study of plants used for the treatment of diabetes in the eastern cape province, South Africa. Afr. J. Biotechnol., 4: 1458-1460.

Federiuk, I.F., H.M. Casey, M.J. Quinn, M.D. Wood and W.K. Ward, 2004. Introduction of type-I diabetes mellitus in laboratory rats by use of alloxan: Route of administration, pitfalls and insulin treatment. Comp Med., 54: 252-257.

Fritsch, R.M. and N. Freisen, 2002. Evolution, Domestication and Taxonomy. In: Allium Crop Science: Recent Advances, Rabinowitch, H.D. and L. Currah (Eds.). CABI Publishing, Wallingford, UK., ISBN-13: 9780851995106, pp: 5-30.

Gabler, N.K., E. Ostrowska, R.B. Jones, M. Imsic and M. Jois *et al*., 2003. Consumption of brown onion (*Allium cepa*) cultivars reduce the risk factors of cardiovascular disease. Proceedings of the Australian Postharvest Horticulture Conference, October 1-3, 2003, Brisbane, Australia, pp: 178-179.

Guo, B.C., J. Zimniak, S.K. Srivastava, S.P. Singh, P. Zimniak and S.V. Singh, 2002. Critical role of allyl groups and disulfide chain in induction of Pi class glutathione transferase in mouse tissues in vivo by diallyl disulfide, a naturally occurring chemopreventive agent in garlic. Carcinogenesis, 23: 1661-1665.

Halliwell, B., 2001. Role of free radicals in the neurodegenerative diseases: Therapeutic implications for antioxidant treatment. Drugs Aging, 18: 685-716.

Hamme, H.R., S. Martins, K. Federlin, K. Geisen and M. Brownee, 1991. Aminoguanidine treatment inhibits the development of experimental diabetes retinopathy. Proc. Natl. Acad. Sci., 88: 11555-11558.

Helen, A., C.R. Rajasree, K. Krishnakumar, K.T. Augusti and P.L. Vijayammal, 1999. Antioxidant role of oils isolated from garlic (*Allium sativum* Linn) and onion (*Allium cepa* Linn) on nicotine-induced lipid peroxidation. Vet. Hum. Toxicol., 41: 316-319.

Helen, A., K. Krishnakumar, P.L. Vijayammal and K.T. Augusti, 2000. Antioxidant effect of onion oil (*Allium cepa*. Linn) on the damages induced by nicotine in rats as compared to alpha-tocopherol. Toxicol. Lett., 116: 61-68.

Ibrahim, S.M., M.S. Mostafa, Y.A. Elhassaneen and M.A. El-Soadany, 2004. Dietary phytochemicals as chemopreventive for liver cancer. Bull. Pharma. Sci., 27: 87-94.

Ismail, A.M., A.A. Sedki and A.G. Abdallah, 2003. Influence of black seed, garlic and onion supplementation on reproductive performance in rabbits. Egypt J. Agric. Res., 81: 1193-1207.

Kamal, A.M. and J.R. Daoud, 2002. Effect of onion and/or garlic as feed additives on blood, tissue constituents and growth performance in Muscovy ducks. Vet. Med. J., 51: 161-175.

Khaki, A., F. Fathi Azad, H.R. Ahmadi Ashtiani, S.H. Rezazadeh, H. Rastegar and A.M. Imani, 2010. Compartments of quercetin and *Allium cepa* (onion) on blood glucose in diabetic rats. J. Med. Plans, 9: 107-112.

Klanns-Dieter, A., 1983. Sulfur Contend Free Radicals. In: Radioprotectors and Anticarcinogens, Nygaard, O.F. and M.G. Simic (Eds.). Academic Press, New York, USA., pp: 23-42.

Klaunig, J.E. and L.M. Kamendulis, 2004. The role of oxidative stress in carcinogenesis. Annu. Rev. Pharmacol., 44: 239-267.

Kumari, K., B.C. Mathew and K.T. Augusti, 1995. Antidiabetic and hypolipidaemic effects of S-methyl cysteine sulfoxide, isolated from *Allium cepa* L. Indian J. Biochem. Biophys., 32: 49-54.

Larson, R.A., 1988. The antioxidants of higher plants. Phytochemistry, 27: 969-978.

Lenzen, S., 2008. The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia, 51: 216-226.

Mathew, P.T and K.T. Augusti, 1975. Hypoglycaemic effects of onion, *Allium cepa* linn on diabetes mellitus-a preliminary report. Indian J. Physiol. Pharmacol., 19: 213-217.

National Research Council, 1996. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, DC., USA., ISBN-10: 0-309-05377-3.

Niki, E., 1991. Action of ascorbic acid as a scavenger of active and stable oxygen radicals. Am. J. Clin. Nutr., 54: 1119S-1124S.

OECD, 2000. Guidance document on acute oral toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No. 24.

Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoekstra, 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science, 179: 588-590.

Rukmini, M.S., B. D'Souza and V. D'Souza, 2004. Superoxide dismutase and catalase activities and their correlation with malondialdehyde in Scizophrenic patients. Indian J. Clin. Biochem., 19: 114-118.

Saalu, L.C., 2010. The incriminating role of reactive oxygen species in idiopathic male infertility: An evidence based evaluation. Pak. J. Biol. Sci., 13: 413-422.

Saalu, L.C., A.A. Osinubi, P.I. Jewo, A.O. Oyewopo and G.O. Ajayi, 2010. An evaluation of influence of *Citrus paradisi* seed extract on doxorubicin-induced testicular oxidative stress and impaired spermatogenesis. Asian J. Scient. Res., 3: 51-61.

Saalu, L.C., G.O. Ajayi, A.A. Adeneye, I.O. Imosemi and A.A. Osinubi, 2009. Ethanol seed extract of graprfruit (*Citrus paradisi*) as an effective attenuator of doxorubicin-induced oxidative stress in the rat heart. Int. J. Cancer Res., 5: 44-52.

Saalu, L.C., P.I. Jewo, I.O. Fadeyibi and S.O. Ikuerowo, 2008. The effect of unilateral varicocele on the contralateral testicular histo-morphology and function in *Rattus norvegicus*. J. Medical Sci., 8: 654-659.

Saalu, L.C., T. Kpela, L.A.J. Shittu and O.A. Ashiru, 2007. Grapefruit seed extract moderates morphologic, functional and biochemical evidences of epidoxorubicin-induced testicular toxicity. J. Med. Sci., 7: 650-654.

Sharma, K.K., R.K. Gupta, S. Gupta and K.C. Samuel, 1977. Antihyperglycemic effect of onion: Effect on fasting blood sugar and induced hyperglycemia in man. Indian J. Med. Res., 65: 422-429.

Sharpe, P.C., K.M. Yue, M.A. Catterwood, D. Mcmarter and E.R. Tribble, 1998. The effects of glucose induced oxidative stress on growth and extracellular matrix gene expression of vascular smooth muscle cells. Diabetologia, 41: 1210-1219.

Sheela, C.G. and K.T. Augusti, 1992. Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. Indian J. Exp. Biol., 30: 523-526.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Method. 7th Edn., Amess Iowa State University, USA., Pages: 215.

Stajner, D. and I.S. Varga, 2003. An evaluation of the antioxidant abilities of *Allium* species. Acta Biol. Szegediensis, 47: 103-106.

Stocker, R. and J.F. Jr. Keaney, 2004. Role of oxidative modifications in atherosclerosis. Physiol. Rev., 84: 1381-1478.

Teyssier, C., M.J. Amiot, N. Mondy, J. Auger, R. Kahane and M.H. Siess, 2001. Effect of onion consumption by rats on hepatic drug-metabolizing enzymes. Food Chem. Toxicol., 39: 981-987.

Tukuncu, N.B., M. Bcyraktar and K. Varli, 1998. Reversal of defective nerve conductions with vitamin E supplementation in type 2 diabetes: A preliminary study.. Diabetes Care, 21: 1915-1918.

Vats, V., J.K. Grover and S.S. Rathi, 2002. Evaluation of antihyperglycemic and hypoglycemic effect of *Trigonella foenumgraecum* L. *Ocimum sanctum* L. and *Pterocarpus marsupium* L. in normal and alloxanized diabetic rats. J. Ethnopharmacol., 79: 95-100.

WHO, 1980. The World Health Organization committee on diabetes mellitus: Second report. Technical Report Series 646. World Health Organization, Geneva.

WHO, 1999. Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation Part 1: Diagnosis and classification of diabetes mellitus. Department of Non-Communicable Disease Surveillance, World Health Organization, Geneva, Switzerland. http://www.staff.ncl.ac.uk/philip.home/who_dmg.pdf.

WHO/Acadia, 1992. Report of the international journe. Diabetes: World Health Organization, Geneva, Switzerland, October 14, 1992.

Wang, B.S., J.H. Chen, Y.C. Liang and P.D. Duh, 2005. Effects of Welsh onion on oxidation of low-density lipoprotein and nitric oxide production in macrophage cell line RAW 264.7. Food Chem., 91: 147-155.

Winterbourn, C.C., R.E. Hawkins, M. Brian and R.W. Carrell, 1975. The estimation of red cell superoxide dismutase activity. J. Lab. Clin. Med., 85: 337-341.

Zhou, W. and K. Sato, 2008. Physiological vulnerability to diet induced obesity in inbred alloxan-induced diabetes-susceptible mice. J. Boil. Sci., 8: 421-425.

Zimmet, P., K.G.M.M. Alberti and J. Shaw, 2001. Global and societal implications of the diabetes epidemic. Nature, 414: 782-787.