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Research Article

PLASMA GLUCOSE LEVEL AND ANTIOXIDANT VITAMINS C AND E IN AN ALLOXAN-INDUCED DIABETIC MELLITUS RATS

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ABSTRACT

In this present study, 12 albino rats of different sexes, grouped into two (control and test) groups of six each were used. Alloxan monohydrate was used to induce diabetes in the test group after which normal feeding was maintained for the two groups of rats. At the end of administration period, weight were taken, the animals were fasted overnight, decapitated under chloroform and blood collected by cardiac puncture and separated. The plasma obtained was used for estimating FPG and antioxidant vitamins C and E status. Investigation reveals that administration of Alloxan (to the Test group B) significantly increased the FPG to 152.55±4.63mg/dl as against 73.12±7.5mg/dl in non-diabetic control (group A). Antioxidant vitamin C of alloxan induced diabetic group (B) was found to be 0.77±0.08mg/dl as against 1.56±0.24mg/dl in control (group A). Also vitamin E was 1.02±0.10mg/dl in control compared to 0.65±0.07mg/dl in the alloxan induced diabetic group. Hence from the result there is a significant increase ($p<0.05$) in FPG and a significant decrease ($p<0.05$) in Antioxidant vitamins C and E which correspond to previous works. Therefore antioxidant supplementation and antidiabetic agents and drugs is encouraged in diabetic situation to reduce positive complication.

Key Words: *Alloxan, Diabetes Mellitus, Blood Glucose, Antioxidant Vitamins*

INTRODUCTION

Diabetes mellitus is a multifactorial disorder or disease characterized by hyperglyceamia, glucouria, lipoprotein abnormalitis (Scoppota *et. al*., 2001) and altered intermediary metabolism of major food substances. According to Vivek, (2010) it is a major degenerative disease in the world today affecting at least 15 million people with many complications, including retinopathy, neuropathy, angiopathy and many others. The first clinical description of the disease was made by causes in about 1000 AD and the name diabetes introduced by Aratacus about the same time. It's debilitating and life threatening associated with impaired nutrient homeostasis and has increasing incidences throughout the world (WHO, 1980 , Gill 1990, Manes and Famworth 1995)

Diabetes increases oxidative stress in tissues of both humans and animals and this increase might play a role in the development of diabetic complications (Amstrong *et.al*., 1992, Kowluru *et.al*., 2000). Possible sources of oxidative stress in diabetes mellitus (DM) include generation of reactive oxygen species (ROS) by auto-oxidation of glucose, decreased tissue concentration and impaired activities of antioxidant defence enzymes (Kowluru 2000, Kern *et.al*., 1994). High levels of free radicals with concurrent decline of antioxidant defence machanism may lead to the damage of cellular organelles and enzymes (Gwarzo *et.al.*, 2010). This however can result lipid perioxidation and development of insulin resistance, which may consequently promote the development of complications of diabetes mellitus (Demozay *et.al*., 2008). Several studies have reported that the basal metabolic rate is increased in diabetic condition (Avesani *et.al*., 2001). This increase is a reflection of increased metabolic demands in diabetes mellitus.

Statistics shows that Indians today, has the highest incidence of diabetes in the world and are more genetically susceptible and the World Health Organization (WHO) predicts that by the end of 2010 the number of diabetic persons will go up by 40 million and 74 million of 2025 (Pilloi 2006).

Alloxan was used to induce experimental diabetes by selectively destroying pancreatic β-cells. Alloxan is taken up by pancreatic β-cells and subsequently generates reactive oxygen species (ROS),which contributes to DNA fregmentation and evoke other deleterious changes in the cell (Gwarzo *et.al*.,2010) and cell death (Heikkila, 1976). Free radicals when generated in large quantities are believed to be etiogenesis of several disorders (Dallatu *et al*., 2009) including artherosclerosis, carcinogenesis, neurodegerative diseases and many pathological effects. The first line of body defence against free radicals damage are the antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GDX) and catalase (CAT).

It has been advocated that hyperglyceamia should be treated very well as to avoid these complication due to or associated with diabetes mellitus.

Oxidative stress contributes to impairment of islets cell function (Haskin *et al*., 2004),insulin resistance, micro and macro vascular disease and may increase their requirement for micronutrient with antioxidant effects. Antioxidant supplementation or food reach in antioxidant maybe used in reducing Oxidative damage by these free radicals and active oxygen species can protect the body against lipid peroxidation (Gulcin *et al*., 2002). However, antioxidant such as retinol, β-carotene and vitamin C may diminish the oxidative process by inactivation of free radical. Several observational, nested, case control and prospective studies have suggested a protective effect of dietary fruit intake (Sebastian *et.al*.,2006). In contrast, negative evidence has been found in large clinical trials of the effect, at pharmacologic doses of a single antioxidant vitamins such as vit E or β-carotene on the risk of type 2 diabetes or associated complication. However, no definitive conclusion about a protective effect of multiple antioxidant long–term supplementation, at nutritional doses on fasting glucose has been drawn thus this study tries to elucidates prospective association between fasting plasma glucose and antioxidant vitamins in an alloxan-induced diabetic rats.

MATERIALS AND METHODS

Materials

Chemicals and Reagents

All chemicals and reagents used in this work were of analytical grade. They were purchased from reputable manufacturers and used without further purification and in line with the manufacturers directives.

Animals

The experiment is performed on twelve (12) adult albino rats of different sexes weighing between 180-200g obtained from the Department of Physiology imo state University, Owerri and were kept in the animal house of the same department. The animals were housed in wooden cages obtained from relief market in Owerri, Imo State and ad-libitum pattern of feeding was maintained for both the control and test rats.

The animals were left in these environment for two weeks to acclimatize, the cages were cleaned daily and water and feed changed while maintaining sterility, weights were taken after two weeks of acclimatization and were randomly assigned into two groups of six (6) rats in each group.

Weighing of Animals

The animals were weighed using bathroom weighing balance. The

Weighing balance was zeroed and kept on a flat surface. The animals were carried into the circular container placed on the balance, made to stay quite and the weight quickly taken and recorded; this was done daily before and after induction of diabetes.

Labelling of Animals

The animals were labeled thus,

Group A (Non-diabetic rats)– the rats here were not induced and the receive normal feeds (palletized feed) and serve as control group

Group B (Diabetic rats)- the rats here were induced with alloxan for diabetics. The also receive normal feeds (palletized) and serve as test group.

Preparation of Chemical Alloxan

Alloxan monohydrate purchased from BDH chemicals was of analytical grade. 0.2g was weighed from the stock with electronic weighing balance and dissolved in 6mls of distilled water to prepare a concentration of 150mg, which was based on experimental design.

Experimental Induction of Diabetes

All animals having been fed after two weeks on normal diet were experimentally (the test group) induced for diabetes with alloxan by intraperitoneal injection with concentration of 150mg/ml. Administration was done at a dose of 150mg/kg and based on the body weight of animals.

Experimental Design

In the experiment, a total of 12 rats were used which was divided into two groups of six (6) in each group, weights of the animals were taken before and after induction of diabetes with alloxan.

The animals were grouped into two of A and B. Group A animals (control group) received normal daily diet and were not induced while those in group B were induced with alloxan intraperitonelly and were continued to be fed with normal diets. Both the two groups were fed normally for more seven days and were sacrificed for blood collection.

Blood Collection

At the end of 21 days of handling and administration period, the animals where fasted overnight after which they were painlessly sacrificed by decapitation under chloroform anaesthesia and the blood (6mls) collected by cardiac puncture was dispensed into anticoagulant bottles and was centrifuged for 5min at 2500 rpm. The plasma obtained was used for the estimation of glucose level and antioxidant vitamins C and E status.

Methodology

Method of Chemical Analytes Measurement

Glucose oxidase method was used for FBG estimation. the glucose oxidase reagent Q-kit (phenol free) of Hi-tech diagnostic laboratory was used. Also the estimation of vitamin C was based on Tietz, 2006 method of reduction reaction while that of vitamin E was a reduction reaction method given by Qualfe *et.al,1979*. All the value obtained were corrected and expressed in mg/dl.

Statistical Analysis

Values obtained were expressed as mean±S.D, statistical significance was determined using the students t-test and values with $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

Results

The data obtained was analysed and presented based on the formulated hypothesis of 0.05 significant level using the students t-test distribution.

Analysis

Table 1 shows the mean and standard deviation of the initial, final and change in the body weights(g) of the control and alloxan induced diabetic group. The body weight increased significantly ($P<0.05$) in diabetic group when compared with the control group.

Also table 2 shows the mean and standard deviation of the estimated three parameters (FPG, Vitamin C and E). A clear look shows a significant 1increase of FPG ($P<0.05$) in alloxan-induced diabetic rats (group B) in comparison with the control rats (group A).

Table 1: The mean and standard deviation of body weights of the two groups of rats before and after induction of diabetics with Alloxan.

	Body Weight (g)		
Group	**Initial**	**Final**	**Change**
A (control group)	**191.66±8.97**	**195.00±7.63**	**3.33±4.71**
B (test group)	**191.30±7.45**	**198.33±3.40**	**5.00±3.33***

**Significantly different when compared with the control group.(P<0.05)*

Table 2: The mean and standard deviation of fasting plasma glucose, fasting plasma Vitamin C and E status in the two groups of rats (n=6) after the three weeks of administration.

Parameters (Mg/dl)	**Control group (n = 6)**	**Alloxan induced group (n = 6)**	**P-value**
Glucose	**73.12±7.95**	**152.55±4.63***	**P<0.05**
Vitamin C	**1.56±0.24**	**0.77±0.08***	**P<0.05**
Vitamin E	**1.02±0.10**	**0.65±0.07***	**P<0.05**

** All are significantly different from control group (P<0.05)*

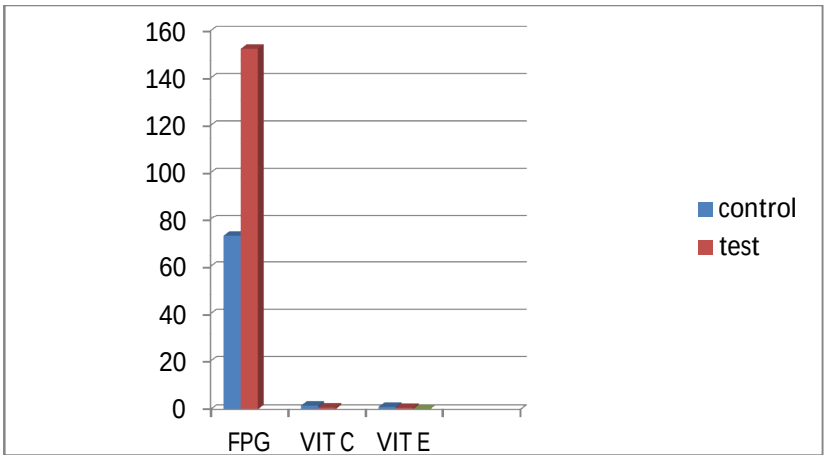

Figure 1: Graphical representation of data in Table 2

There is also a significant decrease (P<0.05) in plasma vitamin C and E in diabetic rats (group B) when compared with the control rats (group A).

DISCUSSION

Increased Oxidative stress has been proposed to be one of the major causes of hyperglyceamia in induced diabetic complication, and hyperglyceamia in an organism stimulates the production of reactive oxygen species (ROS) from variety of sources (Dallatu *et al*., 2009).

In this present study, attempt is made to establish a correlation between diabetes and oxidative stress. Following the induction of diabetes with alloxan by its intraperetonael injection (concentration is based on the body weights of the animals) into the experimental animals, destruction of pancreatic beta cells occurs via generation o f oxidative stress. According to Vivek (2010), besides the impairment of islet cells by alloxan, alloxan also has a significant effect on the body weight of the animals.

However, from the result obtained (FPG-73.12 in control and 152.55 in test) it can be deduced that increased hyperglyceamia causes a significant decrease (p<0.05) in the body in the body. Antioxidant vitamins (i.e., Vit C-1.56 in control, 0.65 in test and Vit E- 1.02 in control , 0.65 in test) which are among the first line of body defence against free radical generated during oxidative stress were all reduced significantly. Nwanjo (2006), confirms that diabetes leads to oxidative stress resulting to oxidative damage caused by reactive oxygen species that promote lipid perioxidation. He further stated that its treatment protects the cell through attenuation of lipid perioxidation while decreasing the production of free radical derivatives.

In several studies relating to alloxan induced diabetes, Sabastian *et.al.(,2006),* firmly stated that antioxidant supplementation does not effect fasting plasma glucose in the supplementation with antioxidant vitamins and minerals.In his work, evidence was given to show that supplementation with antioxidant vitamins will restore the vitamins level though reducing diabetic complications but will not affect the plasma glucose level.

Generally, impaired glucose metabolism leads to oxidative stress (Ceriello *et al*., 1992) and protein glycation produces free radicals (Wolf *et al*., 1991). Therefore the elevation of plasma glucose level (FPG-152.55) in alloxan induced diabetic rats recorded in this work, along with a significant decrease (P<0.05) in Antioxidant vitamins C and E could be probably be associated with oxidative stress and /or decreased antioxidant defence potentials as documented in the works done by Mohdi, (2002), or could be at least in part, result from inactivation of the enzyme by H_2O_2 or glycation which are known to occur in diabetes.

Conclusion

in the cause of this study, results obtained has given a little or More evidence that diabetes patients are always at the risk of exhibiting abnormalities of carbohydrates and protein metabolism as a result of insulin deficiency following the damage of pancreatic islet cell by alloxan. This carbohydrate imbalance and islet cell damage always leads to generation of oxidative stress which must be prevented if life is contemplated. Its therefore advocated that as much as diabetes mellitus is not curable, the use of antidiabetic drugs or plants extract and antioxidant supplementation at a non-toxic dose should be used. This will in a great measure, not only reduce glucose, but will reduce the risk of developing the much well known complications associated with diabetes. Administration of antioxidant will also stimulate cell survival through strengthening of the defence system.

REFERENCES

Ather, M.A.,(1979),The induction of diabetes in laboratory animals for research purposes. *English Medical Journal* 43(6):128-132

Armsrong, D., Abdella, N., Salman,A., Miller,N., Rehama,E.A.J(1992). Relationship of lipid perioxidation and Diabetes conplications. *Journal of diabetic complications 6(3):116-122.*

Avesani,C.M, Cuparri,L., Silva,A.C., Sigulem,D.M., Sesso,R., Cendoroglo,M., Draibe,S.A., (2001). Resting energy expenditure in predialysis patients: *Nephrological Dialysis transplant. 16;556-560*

Burtis,C.N., Ashwood,E.R.,Bruns,D.E.,(2008). Vitamin C in Tietz fundamental of clinbical chemistry, 6th edition,Elsevier inc. new dehli, 480-491.

Carl,A.B., Edward,R.A., David,E.B., (2006). Tietz: fundamental of clinical chemistry. 6th Edition, Sounders publishers New Dehli. *Pg 380-586, 478-492.*

Cerriella,A, Quatraro,A., and Glugliano,D.,(1992). New insight on non-enzymatic glycosylation may lead to therapeutic approaches for the prevention of diabetic con[plications. *Diabetic medicine 9;297-299.*

Dallatu,M.K, Anaja,P.O, Bilbis,L.S., Mojiminiyi,F.O.B., (2009). Antioxidant micronutrients potentials in strengthening the antioxidant defence in Alloxan Induced Diabetic rats. *Nigeria journal of pharmaceutical science, 8(1), 189-194.*

Demozey,D, Mas,J.C., Rochi,S, Van,O.E., (2008), FADLH Reverses the deleterious actions nof oxidative stress induced by lipid perioxidation product 4-hydroxynonenal on insulin signaling in 3T3-Li Adepocytes diabetes *57;1216-1226.*

Gill,G.V, (1990), Diabetes- Some recent advancement. *Medicine digest 8:5-29*

Gulcin,I.M., Oktey,O.I, Kufreroiglu, Aslam, A, (2002). Determination of Antioxidant activity of *Lichen cetracina islandika. Arch. Journal of ethnopharmacology79: 325-329.*

Gwarzo,M.Y, Nwachuku,V.A, Lateef, A.O, (2010). Prevention of Alloxan induced Diabetes in rats by Vitamin A supplementation. *Asian journal of Animal Science, 4: 190-196*

Haskin,K.J, Power,K, Bradly, (2004). Role of Oxidative Stress in the regeneration of islets cells. *Journal of Investigative medicine 52:45-49.*

Heikilla,R.N, Winston, B., Cohen G, (1976). Alloxan Induced diabetes evidence for hydroxyl radical as a cytotoxic intermediate. *Biochemistry Pharmacology 25:1085-1092.*

Helmet,S, (1991). Oxidative Stress from basic research to Clinical Application. *American Journal of Medicine 315-365.*

Kern,T.S, Kowluru,R., Engerman, R.C, (1994). Abnormalities of retina Metabolism in Diabetes or Galactoseamia :A.T Pases in Glutathione. *Investigative Opthamology. 35:2962-2967.*

Kowluru,R.A, Engerman,R.L, Kern,T.S, (2000). Diabetes-induced Metabolic Abnormalities in Myocarduim;Effects of Antioxidant Therapy. *Free Radical Research 35:67-74.*

Manes,R.J., and Famdworth, N.R., (1995). Antidiabetic plants and their Active constituents. *Phytomedicine 2(2):37-39.*

Ness,O.A.,Tememoto,H., Tobe,K., Mori, Y., (1996) Antioxidant activity of *Ficus glomerata Indian journal of plant biotechnology 17(2): 180-192.*

Nwanjo,H.U., Oze,G.O., (2007). Oxidative inbalance and non-enzymatic antioxidant studies in pulmonary tuberculosis infected subjects: carcinogenic potential. *Pakistan Journal of Nutrition.6(6) 590-592.*

Nwanjo,H.U., (2005). Diabetes Mellitus in Basic clinical Endocrinology. 1st Edition, Hacyn Publishers Owerri, Nig *Pg 82-87.*

Nwanjo,H.U., (2006). Antioxidant Activity of exudates from *Aloe barbadensis leaves* in diabetic rats. *Journal of Biochemistry, 18(2):77-81.*

Pillai,M.,(2006) Could you be a diabetic; In Readers digest New Delhi. *Living Media Press Ltd, India*

Quaife,M.L., Scrimshan,N.S, Lowry,O.H., (1949). A Micro Nutrition method for essay of total tocopherol in bllod serum. *Journal of Biological Chemistry 180: 1229-1236.*

Sabastian,C, Aline,C., Sandrine,B, Anne-Clair,V, Luc,D., Pilar,G., Saege,H., (2006). Antioxidant Supplementation does not affect fasting plasma glucose in a supplementation with antioxidant vitamins and minarals(su,vi,Max) study in France: Association with dietary intake and plasma concentration *84(2), 395-406.*

Scoppola,A., Monteechi,F.R., Mezinger,G, Lala,A, (2001). Urinary mevalonate excreation rate in diabetes: Role of metabolic control. *Artherosclerosis 156:357-361.*

Sies,H., (1991). Oxidative Stress, Oxidants and Antioxidants. *New York Academy Press 108.*
Tietz,N.W.,(2008) Ascorbic acid in fundamental of clinical chemistry, 6th edition saunders company, britian, 318-319.
Tyrberg,E., Barger,Z.F., Banmin,E.H., (2001). Species differences in susceptibility of transplanted and cultured pancreatic islets to the beta cell toxin alloxan. *Journal of clinical Endocrinology 54:254-260.*
Upston ,P.I., Ugoshen,K., Martha,O.R.,(1991) Vitamin C improves basal metabolic rate in an alloxan-induced diabetic rats. *Journal of free radicals* 88: 128-132.
Vivek,K.S., Suresh K., Hitesh,J.P., Shivakumar,H, (2010). Hypoglyceamic activity of *Ficus glomerata* in alloxan induced diabetic rats. *International journal Pharmaceutical Sciences 1(2) 976-981*
Wolf,S.P., Jiang,Z.Y., Shunt,J.V., (1991). Protain glycation and oxidative stress in diabetes mellitus and aging. *Journal of free radical biology medicine. 10: 339-352.*
World Health Organization, Department of Comminicable Disease and Surveillance (1999). Definition, Diagnosis and Classification of Diabetes Mellitus and its complications.
WHO Expert Committee on Diabetes Mellitus (1980). Second Report. *Technical Series 646.*