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Effects of alpha lipoic acid on blood lipids, renal indices, antioxidant enzymes, insulin and glucose level in streptozotocin-diabetic rats

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Abstract

This study investigated the effects of ALA on blood lipids, renal indices, antioxidant activities, glycemic control, and insulin level in streptozotocin (STZ)-induced diabetic in laboratory rats. The rats used were either administered alpha lipoic acid (ALA) (test group) or distilled water (control group) orally for four weeks after being confirmed diabetic. Timed fasting blood glucose (FBG) and oral glucose tolerance test (OGTT) measurements were performed on days 0, 7, and 14. Serum collected from all control and diabetic rats at the end of the experiment were analysed for insulin, lipid profile, antioxidant, and lipid peroxidation indicators. Administration of ALA lowers the FBG in STZ-induced diabetic rats in spite of producing no significant effect on insulin level. ALA significantly improves on the lipid profile and renal functions in diabetic rats by reducing the low density lipoprotein (LDL), very low density lipoprotein (VLDL), and increased creatinine levels respectively. It also increased the activities of endogenous superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), thus increasing body antioxidant capacity. Oral administration of ALA improves glucose regulation, increased the activities of endogenous antioxidants in combating oxidative stress under diabetic condition in experimental rats.

Keywords: Alpha lipoic acid; diabetes; antioxidants; glucose; insulin.

Introduction

Diabetes is one of the metabolic disorders that has remained a major health concern globally. The worldwide prevalence is about 2% while it averages 2.5% in Nigeria (Nwankwo *et al.*, 2010). Diabetes mellitus (type 2) is an immune mediated and idiopathic form of beta-cells dysfunction which lead to absolute or relative deficiency in insulin secretion and insulin action. It is associated with deterioration of glycemic control with resultant hyperglycemia and progressive derangement in carbohydrate, lipid, and protein metabolism. There is increasing evidence from both clinical and experimental studies to show that increased generation of reactive oxygen species (ROS) also known as free radicals and glycation of body proteins are responsible for the various secondary complications experienced in diabetes (Bashan *et al.*, 2009; Robertson, 2004).

Attenuation of oxidative stress by a way of reducing the generation and activities of these ROS is widely acknowledged as a potential therapeutic intervention for management of diabetes (Camió *et al.*, 2006; Venditti *et al.*, 2007). Administration of exogenous antioxidants is known to boost body antioxidant system, decrease the availability and activities of ROS thereby offering protection against oxidative destruction of pancreas cells (Ihara *et al.*, 1999).

There is increasing focus on alpha lipoic acid (ALA) as a good source of exogenous antioxidant. ALA is a naturally occurring dithiol compound, an important cofactor in metabolic activities. ALA significantly reduced a variety of reactive oxygen species such as peroxy nitrite (Trujillo and Radi, 2002), nitric oxide (Vriesman *et al.*, 1997), hydroxyl radical, superoxide anion (Suzuki *et al.*, 1991), peroxy radical (Kagan *et al.*, 1992), and hydrogen peroxide (Scott *et al.*, 1994). Furthermore, ALA chelates metal ions

and regenerates other endogenous antioxidants such as vitamin C, vitamin E, and glutathione (Bast and Haenen, 2003; Petersen *et al.*, 2008). We had earlier reported that in experimental rat model of hepatotoxicity and nephrotoxicity, ALA exerts strong oxidative protection in the liver and kidney against free radical induced cellular damage (Morakinyo *et al.*, 2012). Other studies on the use of ALA as a complementary therapeutic approach have also reported positive effects on insulin sensitivity (Evans *et al.*, 2002; Kamenova, 2006) and diabetic complications (Chang *et al.*, 2007; Zeigler *et al.*, 1999). In the present study, we have investigated the effects of ALA on glycemic control, insulin sensitivity, beta-cells functions, and oxidative balance in (STZ)-induced diabetic rats.

Materials and Methods

Animal grouping and treatment

Male Sprague-Dawley rats of about 15 weeks old were used for this study. They were obtained from the Animal House, College of Medicine, University of Lagos. The animals were housed in plastic cages and acclimatized to the laboratory environment for a week under room temperature of $24 \pm 20^\circ\text{C}$, humidity 50%–64%, 12 h light, and 12 h dark. Clean tap water and rat pellet were provided *ad libitum*. All experimental protocols adopted in this study including animals handling were in compliance with United States National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985).

Animals were randomly divided into four groups of six rats each. Group 1 (NC) served as the normal control group treated with 0.5 ml of distilled water, Group 2 (NA) served as non-diabetic group treated with ALA, Group 3 (DC) served as diabetic control treated with 0.5 ml of distilled water, while Group 4 (DA) served as diabetic group treated with ALA throughout the experimental period. Daily treatment with either distilled water or ALA was done using oral dosing needle for a period of two consecutive weeks for all groups. The treatment of animals began 24 h after the rats were confirmed diabetic and this was considered as 1st day of treatment.

Induction of experimental diabetes

Diabetes was induced in overnight fasted rats by a single intra-peritoneal injection of STZ

(45 mg/kg body weight) in a freshly prepared 0.1 M sodium citrate buffer (pH 4.5). The control rats received an equivalent amount of citrate buffer. Five days after STZ administration, the development of hyperglycemia in rats were confirmed by measuring the fasting blood glucose (FBG) level (16 h) using the tail vein blood with help of a portable glucometer (Accu-Chek, Roche, Germany). Rats showing moderate diabetes with glycosuria and hyperglycemia (blood glucose levels of 250–450 mg/dl) were used for the experiment.

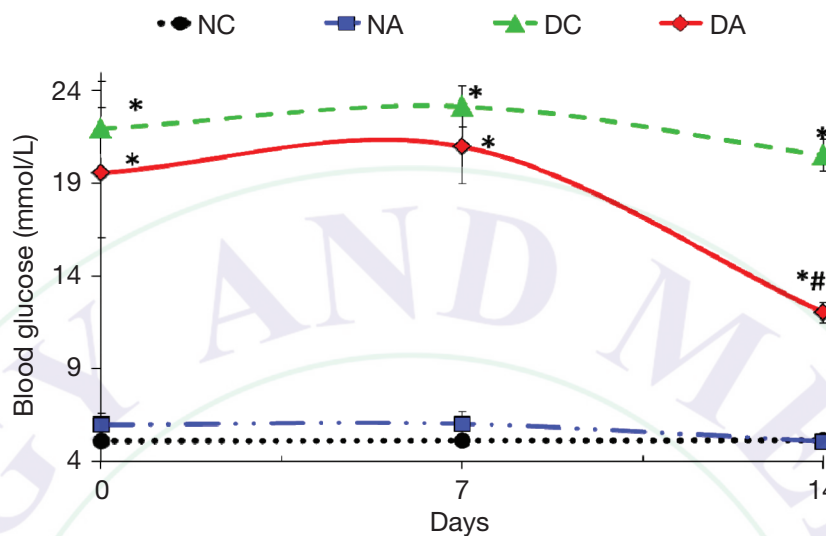
Blood glucose and glucose tolerance test

The normal and STZ-induced diabetic rats were fasted overnight before blood samples were collected from the tail vein for blood glucose measurement. The FBG was measured at days 0, 7, 14, and oral glucose tolerance test (OGTT) was also performed to evaluate the change in glucose concentration with an oral glucose load (Figure 1). For this, experimental rats were fasted overnight for 16 h and subsequently challenged with a glucose load of 2 g/kg body weight. Blood glucose levels were determined at 0 h (pre-glucose treatment) and at 30, 60, 90, 120, and 180 min (post-glucose treatment). The area under the glucose curve (AUGC/AUC) was calculated using the trapezoidal rule. The glucose levels were measured using a complete blood glucose monitoring system (Accu-Chek glucometer, Roche, Germany). AUGC/AUC was measured using the trapezoidal rule. Only positive excursions from the baseline were included in the calculations.

Serum insulin and biochemical profile

After 14 days of treatment, the rats were fasted overnight and sacrificed by cervical dislocation, and blood samples were drawn from the rat's heart. The blood samples were centrifuged at 3500 rpm for 20 min to separate blood serum. The serum insulin was estimated using the enzyme linked immunoassay kit (ELISA). The biochemical data include total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), blood urea nitrogen (BUN), creatinine, total protein, albumin, and alkaline phosphatase (ALP) which were measured by an automatic blood chemical analyzer BT 2000 plus, Germany. Creatinine, urea, total bilirubin, and albumin were estimated using commercially available kits (Randox Laboratories, Kearneysville, West Virginia, USA).

Figure 1: Fasting blood glucose readings in level in diabetic + ALA treated rat, diabetic untreated rat, normal rat treated with ALA compared with control (values are mean \pm SEM; $n = 6$).



N = Normal, C = Control, A = ALA, D = Diabetic. Values are significant at * $P < 0.05$ when compared to NC and # $P < 0.05$ when compared to DC.

Antioxidant activities and lipid peroxidation

Oxidative analyses of the plasma were carried out using previously described standard methods (Morakinyo *et al.*, 2012). The most abundant individual aldehyde resulting from lipid peroxidation breakdown in biological systems, malondialdehyde (MDA) was estimated with the method of Uchiyama and Mihara (1978). The reduced glutathione (GSH) content of the liver homogenate was determined using the method described by Van Doorn *et al.* (1978) while the activity of the superoxide dismutase (SOD) enzyme was determined according to the method described by Sun and Zigman (1978). Catalase (CAT) activity was determined by measuring the exponential disappearance of H_2O_2 at 240nm and expressed in units/mg of protein as described by Aebi (1984). Absorbance was recorded using Shimadzu recording spectrophotometer (UV 160) in all measurements.

Statistical analysis

Data were expressed as mean \pm standard error of mean (SEM) and analysed using the ANOVA followed by SNK post-hoc test. $P < 0.05$ was accepted as significant. All the analyses were carried out using the GraphPad InStat Version 3.05 for Windows Vista, GraphPad Software, San Diego, California, USA.

Results

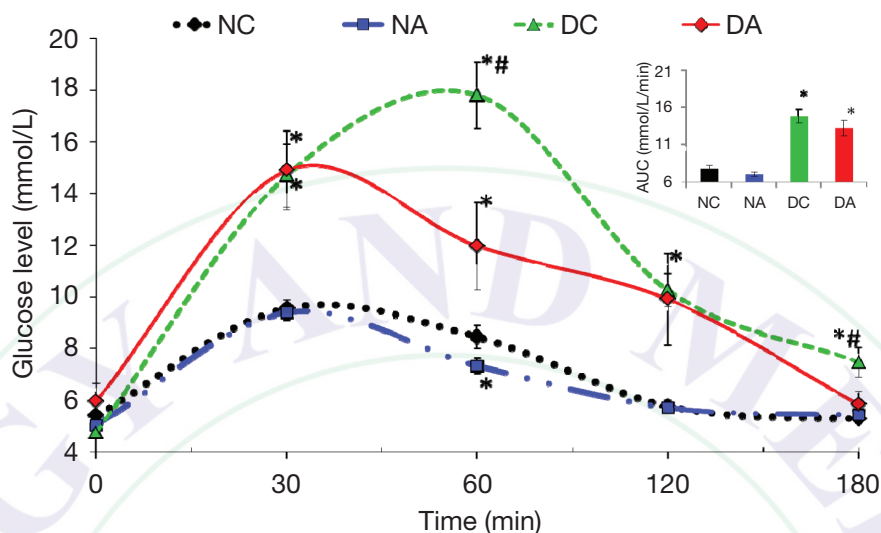
Effects of ALA on FBG and glucose tolerance

Figure 2 depicts the result of the OGTT in experimental animals. FBG level was higher in both diabetic groups of rats when compared to that in control rats. Significant elevations in the glucose level after the oral glucose load were noted in diabetic control rats at all-time points. Diabetic rats treated with ALA was however showed markedly lower glucose level at 60 min of the OGTT period. The incremental AUGC/AUC was significantly greater in STZ-diabetic rats compared with normal control rats. The AUGC/AUC was decreased in diabetic rats treated with ALA but not significantly different when compared with diabetic control. The diabetic control rats did not return to baseline glucose levels after the 180 min OGTT period while all other experimental groups returned to baseline on or before 180 min OGTT period.

Effects of ALA on renal functions

As shown in Table 1, there was no significant change ($P > 0.05$) in bilirubin, urea, creatinine, and albumin levels in normal rats treated with ALA. In STZ-diabetic rats, there was a significant increase in the plasma level of bilirubin, urea, and albumin, an indication of renal impairment. These parameters were however,

Figure 2: Oral glucose tolerance test in control and experimental animals with the area under curve (AUC) for the OGTT (values are mean ± SEM; n = 6).



N = Normal, C = Control, A = ALA, D = Diabetic. Values are significant at *P < 0.05 when compared to NC and #P < 0.05 when compared to DC.

Table 1: Plasma levels of bilirubin, urea, creatinine, and albumin in control and experimental animals (values are mean ± SEM; n = 6). N = Normal, C = Control, A = ALA, D = Diabetic.

Group	NC	NA	DC	DA
Bilirubin (mg/dl)	0.59 ± 0.03	0.57 ± 0.11	1.22 ± 0.26*	0.36 ± 0.05#
Albumin (g/dl)	3.75 ± 0.19	3.97 ± 0.13	6.02 ± 0.46	4.67 ± 0.32#
Urea (mg/dl)	59.18 ± 2.30	60.9 ± 2.15	75.73 ± 2.12*	70.98 ± 2.91
Creatinine (mg/dl)	1.91 ± 0.08	1.83 ± 0.17	0.92 ± 0.08*	1.37 ± 0.08#

Values are significant at *P < 0.05 when compared to NC. Values are significant at #P < 0.05 when compared to DC.

significantly reversed by administration of ALA when compared to the untreated diabetic group. However, ALA was not able to restore the level of these parameters back to that obtained in control group.

Effect of ALA on serum lipid indices and insulin level in diabetic rats

Table 2 shows the effect of ALA on the levels of TG, TC, HDL, LDL, and VLDL as well as insulin in normal and diabetic rats. In normal rats treated with ALA, the levels of all lipid parameters were almost similar to the control group. Lipid profile was impaired in STZ-diabetic rats the levels of TC, TG, LDL, and VLDL were significantly (P < 0.05) increased while the level of HDL was significantly (P < 0.05) reduced compared with their respective control group. However, treatment with ALA significantly (P < 0.05) increased

the level of HDL, while LDL was significantly (P < 0.05) reduced. In addition, ALA treatment significantly (P < 0.05) decrease in TC, TG, and VLDL levels in STZ-diabetic rats when compared with diabetic control rats.

Serum insulin values of normal rats treated with ALA was not significantly (P > 0.05) different from the normal control rats. Serum insulin level in the STZ-diabetic rats showed significantly (P < 0.05) lower values than normal control rats. The insulin level of diabetic rats treated with ALA however showed no significant difference compared with diabetic control rats.

Effects of ALA on antioxidant enzymes and lipid peroxidation

Table 3 shows the effect of ALA on antioxidant enzymes activities and lipid peroxidation level in normal and diabetic rats. In normal rats treated

Table 2: Serum levels of TG, TC, HDL, LDL, VLDL, and insulin in control and experimental animals (values are mean \pm SEM; $n = 6$). N = Normal, C = Control, A = ALA, D = Diabetic.

Group	NC	NA	DC	DA
TG (mg/dl)	63.71 \pm 2.20	72.12 \pm 3.12	114.46 \pm 4.71*	85.06 \pm 5.77#
TC (mg/dl)	114.77 \pm 6.12	122.92 \pm 10.49	158.32 \pm 6.56*	142.9 \pm 5.74
HDL (mg/dl)	62.62 \pm 2.88	59.23 \pm 3.32	44.03 \pm 2.36*	57.89 \pm 2.16#
LDL (mg/dl)	52.45 \pm 2.15	55.47 \pm 4.31	88.64 \pm 5.60*	72.52 \pm 6.62#
VLDL (mg/dl)	6.17 \pm 0.51	6.55 \pm 0.60	9.40 \pm 0.67*	7.91 \pm 0.62
Insulin (g/ml)	3.64 \pm 0.48	3.08 \pm 0.19	1.41 \pm 0.17*	1.55 \pm 0.12

Values are significant at * $P < 0.05$ when compared to NC.

Values are significant at # $P < 0.05$ when compared to DC.

Table 3: Antioxidant activities and lipid peroxidation level in control and experimental animals (values are mean \pm SEM; $n = 6$). N = Normal, C = Control, A = ALA, D = Diabetic.

Group	SOD (mmol/ml)	CAT (mmol/ml)	GSH (mol/ml)	MDA (nmol/ml)
NC	1.41 \pm 0.07	1.27 \pm 0.08	1.50 \pm 0.09	0.63 \pm 0.04
NA	1.38 \pm 0.06	1.33 \pm 0.05	1.50 \pm 0.08	0.62 \pm 0.06
DC	0.59 \pm 0.10*	0.70 \pm 0.06	0.71 \pm 0.06*	1.33 \pm 0.07*
DA	1.04 \pm 0.06#	1.02 \pm 0.05	1.10 \pm 0.08#	0.92 \pm 0.12#

Values are significant at * $P < 0.05$ when compared to NC.

Values are significant at # $P < 0.05$ when compared to DC.

with ALA, activities of SOD, CAT, and GSH were almost similar to the control rats. However, activities of SOD, CAT, and GSH in STZ-diabetic rats were significantly ($P < 0.05$) decreased compared with control rats. Meanwhile, ALA treatment in diabetic rats significantly ($P < 0.05$) increased the activities of all measured antioxidants when compared with diabetic control rats. In addition, MDA level in the normal rats treated with ALA was not different from the normal control group. Compared with normal rats, MDA level was significantly ($P < 0.05$) higher in the STZ-diabetic rats. However, treatment with ALA significantly ($P < 0.05$) decreased MDA level of STZ-diabetic rats when compared with diabetic control rats.

Discussion

Diabetes incidence and prevalence is on the increase worldwide (Shaw *et al.*, 2010). Type 2 diabetes accounts for well over 90% of diabetes in Sub-Saharan Africa with the attendant burden on both the individual and the government. Most interventional approaches employed in the management of type 2 diabetes are targeted at reducing hepatic glucose production, improving insulin sensitivity, lowering peripheral insulin resistance, and slowing

down carbohydrate absorption from the intestine (Kimmel and Inzucchi, 2005). Meanwhile, most of the complications associated with diabetes have been linked to generation of free radicals (Nishikawa *et al.*, 2000). Therefore, it is important to manage the ROS generated under diabetic conditions while targeting glucose control. ALA, a naturally occurring dithiol compound synthesized enzymatically in the mitochondrion from octanoic acid has been suggested as a good antioxidant compound that has the ability to not only mop up the ROS but also with anti-inflammatory potentials (Han *et al.*, 1997). It is absorbed intact from some dietary sources such as liver, brewer's yeast, potatoes, and red meat.

Oral administration of ALA for a period of four weeks significantly reduced the FBG in the STZ-diabetic rats. This gave an indication of possible effects on the blood glucose metabolic pathway. A closer look at the serum insulin production revealed that ALA does not affect the production of insulin after the destruction of the beta-cells by STZ. Thus there was no significant difference in the serum insulin level between the untreated diabetic group and treated diabetic group with ALA. Most authors who have reported on glycemic control potential of ALA have linked it with its ability to recruit glucose transporter-4 to plasma membranes similar to

the insulin-stimulated glucose uptake (Packer *et al.*, 2001; Singh and Jialal, 2008). The AUGC/AUC for untreated diabetic and ALA treated diabetic rats were significantly higher compared with normal control rat. This showed that it took the diabetic animals a longer time to substantially clear the sudden increase in blood glucose as a result of the glucose load. Although there was a reduction in the AUGC/AUC recorded for diabetic rat treated with ALA compared with untreated diabetic group, this reduction was however, not statistically significant. It is possible however, that prolonged administration of ALA beyond the 14 days duration allowed in this study may produce a significant decrease in the FBG that will be strong enough to significantly reduce the AUGC/AUC.

Further study on possible influence of ALA on serum lipid profile revealed a significant increase in HDL with a concurrent significant reduction in TC, TG, LDL, and VLDL (bad cholesterol). Lipid impairment is one of the known effects of diabetes which further complicates glucose metabolism. Diabetic rats treated with ALA showed significant increase in the activities of SOD, CAT, and GSH compared with the untreated diabetic group (Table 3). ALA antioxidant and anti-inflammatory effect have been previously reported (Han *et al.*, 1997; Lykkesfeldt *et al.*, 1998). It has been shown to have rapid gastrointestinal uptake and rapid clearance by uptake into the tissue. Its antioxidant properties have been reported to be via indirect increase in intracellular and hepatic ascorbate level (Lykkesfeldt *et al.*, 1998; Michels *et al.*, 2003), and maintenance of GSH level (Kilic *et al.*, 1998).

From the present study, activities of the endogenous antioxidants SOD, CAT, and GSH were significantly increased in diabetic rats administered ALA compared to the untreated diabetic group. It therefore appears that ALA administration promotes increased production of endogenous antioxidants; in addition to being an established antioxidant (Shay *et al.*, 2009). This helps in combating and reducing peroxidation of lipids under diabetic conditions. ALA has been previously reported to mediate induction of GSH through transcription factor Nrf2 thus maintaining GSH availability for combating oxidative stress (Kilic *et al.*, 1998; Moini *et al.*, 2002). We have earlier reported similar antioxidant and protective effect of ALA on carbontetrachloride

induced liver and kidney damage in rat (Morakinyo *et al.*, 2012). This antioxidant property of ALA has been exploited in the management of diabetic neuropathy, cataract model (Kilic *et al.*, 1998), and rodent model of cerebral ischemia (Wolz and Kriegstein, 1996).

Further study on the effect of ALA administration on kidney functions in STZ-induced diabetic rats showed a significant increase in the level of plasma creatinine compared to the untreated diabetic group, while urea level remain relatively unchanged. Plasma creatinine and urea are established biomarkers of glomerular filtration rate and most importantly, plasma creatinine is a more sensitive index of measuring kidney function than urea (Perrone *et al.*, 1992). The significant increase recorded in creatinine level in ALA-treated diabetic group indicates a significant improvement in renal functions. Kagan *et al.* (1992) reported that cisplatin-induced decrease in renal function, measured by BUN, serum creatinine level, and renal tubular injury scores was attenuated by ALA treatment in mice. The increased plasma bilirubin and albumin level in diabetic rats were also significantly reduced by administration of ALA in the present study.

Conclusion

Our findings indicated that oral administration of ALA to STZ-induced diabetic rats improved body handling of blood glucose and lipid profile. It also enhanced antioxidant activities with reduction in tissue damage as shown by a significant reduction in MDA level. This may be of importance in preventing various complications and tissue damage usually experienced in diabetic condition.

Ethical Approval

The study was approved by the Research Ethics Committee, College of Medicine, University of Lagos, Lagos, Nigeria.

Conflict of Interests

We declare that there is no conflict of interest whatsoever concerning this research work.

Authors' Contributions

All authors contributed equally to this work.

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